Al-Rafidain Dental Journal

rden.mosuljournals.com

Impact of Azithromycin on Gingival Enlargement

Israa M. Mustafaa [1]*, Maha T. ALsafar [2], Abdul sattar S. Mahmoud [3]

[1] Department of pharmacy /AI-jumhory teaching hospital/Nineveh health
[2] Department of Dental Basic Science, College of Dentistry, University of Mosul
[3] Department of maxillofacial Surgery, College of Dentistry, University of Mosul

Article information

Received: 25, June, 2021
Accepted: 25 July, 2021
Available online: 10 September, 2022

Keywords
Pathological Gingival Enlargement.
Azithromycin.
Immunohistochemistry.

***Correspondence:**

E-mail:
israa.dep52@student.uomosul.edu.iq

Abstract

Aims: This study was aimed to evaluate the effect of azithromycin in reducing pathological gingival enlargement. **Materials and methods:** twelve adult New Zealand male rabbits weighing (900gm-1250mg) and almost the same in age and circumstance were chosen in this study. All animals with pathological gingival enlargement were clinically examined by using a periodontal probe and measuring their gingival sulcus depth. Then, six of them were slaughtered and considered the control group and the other six were experimental group, and at the end of study a specimens of oral mucosa were taken for immunohistochemical examinations. **Results:** Clinical examination shows there is no reduction in gingival enlargement in the control group while this enlargement decreased in the experimental group after use of azithromycin. Immunohistochemical analysis shows mild matrix metalloproteinase-1(MMP1) expression in the control group and moderate to strong MMP1 expression in sub-epithelial of gingiva in the experimental group. **Conclusion:** This study establishes that azithromycin is clinically and immunohistochemically effective in the reduction of pathological gingival enlargement.

الخلاصة

الأهداف : تم التخطيط لهذه الدراسة لمعرفة تأثير أزيثروميسين في الحد من تضخم اللثة المرضي. **المواد وطرائق العمل:** تم اختيار اثني عشر من ذكور الأرانب النيوزيلندية البالغ وزنها (٩٠٠ جرام - ١٢٥٠ مجم) وتقريباً نفس العمر والظروف في هذه الدراسة. جميع الحيوانات لديها تضخم لثة مرضي تم فحصها سريريا باستخدام مسبار اللثة وقياس عمق التلم اللثوي ، ثم ذبح ستة منها واعتبرت مجموعة ضابطة والستة الأخرى مجموعة تجريبية. **النتائج:** أظهر الفحص السريري عدم وجود انخفاض في تضخم اللثة في المجموعة الضابطة بينما انخفض هذا التوسيع في المجموعة التجريبية بعد استخدام أزيثروميسين. يُظهر التحليل الكيميائي المناعي تعبيرًا معتدلًا عن بروتين Matrix metalloproteinase-1 (MMP1) في المجموعة الضابطة وتعبير MMP1 المعتدل إلى القوي في الظهارية الفرعية للثة في المجموعة التجريبية. **الاستنتاجات:** تشير هذه الدراسة إلى أن أزيثروميسين فعال سريريًا في الحد من تضخم اللثة المرضي.

DOI: 10.33899/rdenj.2021.130646.1113

INTRODUCTION

Pathological gingival enlargement is associated with multiple factors including inflammation, drug use, neoplasia, hormonal disturbances, pregnancy, puberty and ascorbic acid (vitamin C) deficiency [(1)]. In some cases, the cause of pathological gingival overgrowth is unknown.

Disorders affecting the gingival fibroblasts or the enzymes responsible for the catabolism of the extracellular matrix (proteoglycanases, neutral proteases and collagenases) lead to a metabolic imbalance that favors the development of pathological gingival enlargement [(2)].

Several studies revealed the presence of some temporary improvement of the pathological gingival enlargement and periodontal condition with surgical and non-surgical periodontal therapy [(3)]. The enlarged gingiva due to the drug facilitates the retention of bacterial plaque and predisposes the person to dental caries, and periodontal disease [(4)]. Azithromycin is an effective therapeutic tool in the management of pathological gingival enlargement as it is conservative, well tolerated, and rapidly effective with minimal side effects [(5)].

Despite their pharmacological diversity, the three major drugs causing pathological gingival enlargement, namely; anticonvulsants, calcium channel blockers, and immunosuppressants have similar mechanisms of action on gingival connective tissue, by their effect on synthesis of extra cellular matrix component, also causing imbalance collagenase expression that effect on collagen apoptosis and leading to collagen accumulation [(6)].

An appraisal of the various investigations into the pathogenesis of drug-induced gingival enlargement supports the hypothesis that it is multifactorial. Plaque scores and gingival inflammation appear to exacerbate the expression of drug-induced gingival enlargement, irrespective of the initiating drug [(7)]. The severity of pathological gingival enlargement in patients taking medications correlates well with poor plaque control and is commensurate with the degree of plaque-induced inflammation [(8)].

The prevalence of this gingival overgrowth varies between drugs from 6 to 15% for nifedipine, about 50% for phenytoin, and is from 25% to 30% in adult patients and >70% in children for cyclosporine [(9)].

Azithromycin, a macrolide antibiotic, affects collagen synthesis and cytokine production in human gingival fibroblasts (hGFs) [(10)]. Azithromycin has a long half-life and a wide antibacterial spectrum.

MATERIALS AND METHODS

New Zealand adult male twelve (12) rabbits weighing (900gm-1250mg) their age (8-12months) are examined by a veterinary physician to check the general health and condition of the animals. They were allowed to adapt for one week before

the experiments in a special Animal House. They were housed in rodent plastic cages (90 × 60 × 45) cm with wire mesh covers at (22 ±2)°C, 12hr light /12 hr. dark cycle and received fresh hay, fresh fruits, and vegetables daily and reverse-osmosis water ad libitum. This study was done in accordance with the guidelines of the institutional animal research ethics committee.

All animals have pathological gingival enlargement. They were clinically examined by using a periodontal probe and measuring their gingival sulcus depth then Six of them were sacrificed, and specimens of oral mucosa were taken for immunohistochemical examinations and considered as the control group; The remaining animals, which are six, received azithromycin drug at a dose of 62.5mg/kg/day [(11)] orally for 5days and considered as experimental group. After twenty four hours following the last treatment(day6), all animals of the experimental group were clinically examined to assess their gingival sulcus depth using a periodontal probe, then they were sacrificed and oral mucosa (specimen) were excised from their gingiva and placed in 10% buffered formalin for immunohistochemical examination**.**

Immunohistochemical (IHC) analysis:

IHC staining was done to analyze the

RESULTS

Results of clinical examination

By using a periodontal probe for clinical examination, there was a pathological gingival enlargement in the control group while this enlargement decreased in the experimental group as a result of azithromycin administration.

Statistical analysis by using an independent Mann-Whitney Test showed a highly significant difference between both groups (p=0.001). The mean of gingival sulcus depth in the control group was (6±1.12) while in the experimental group was (1.34±0.11) as seen in (Table 1) and (Figure 1).

Table (1) Clinical examination of gingival sulcus depth:

Group	Gingival sulcus depth	P_value
Control	6±1.12	0.001
Experimental	1.34±0.11	

Values set as Mean±SD. P-value ≤ 0.05

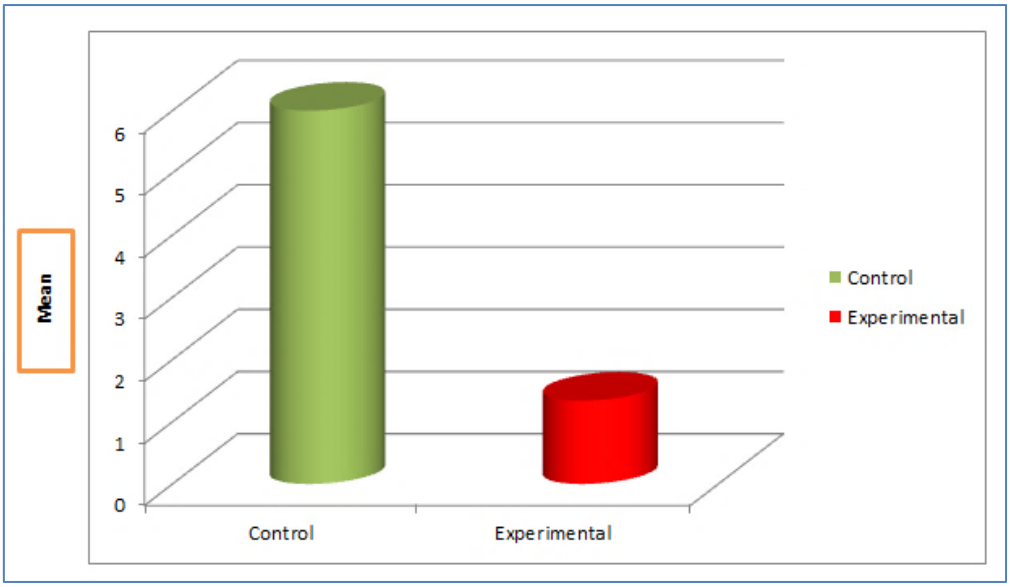

Figure (1): Clinical examination of gingival sulcus depth in both groups

Results of immunohistochemical (IHC) examination:

In IHC analysis for MMP1 of rabbit's oral mucosa of control group showed negative to mild positive reaction of MMP1 expression to immunohistochemistry in the fibroblasts and collagen fibers of connective tissue of submucosa (Figure 2).

While rabbit's oral mucosa of the experimental group shows moderate to strong positive reaction of MMP1 expression to immunohistochemistry in the fibroblasts and collagen fibers of connective tissue of submucosa (Figure 3).

In statistical analysis, the nonparametric Mann-Whitney Test was used to analyze MMP1 expression in Immunohistochemical examination in the fibroblasts and collagen fibers of connective tissue of submucosa in rabbits oral mucosa in both groups .There was a statistically significant difference in MMP1 expression between the two groups (P-value=0.000) in control group mean scores was (1.00±0.08) while in experimental group was (2.6±0.5) (table2).

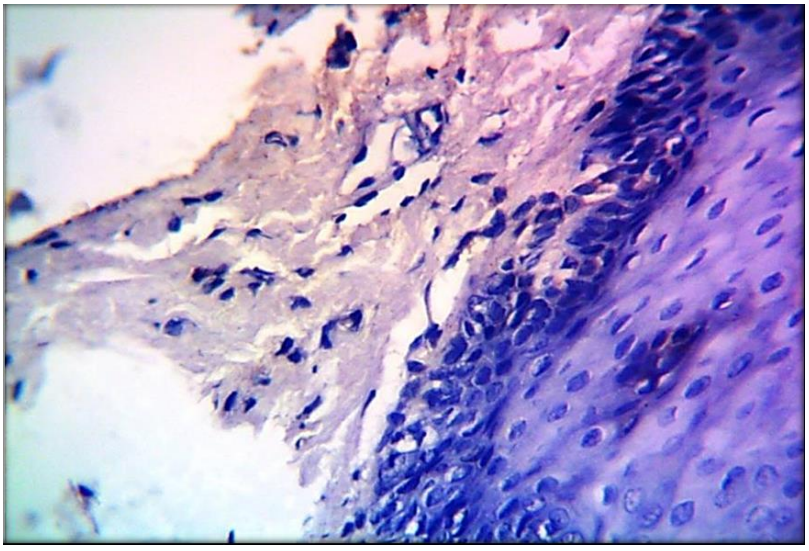

Figure (2): Photomicrograph of control group of rabbit oral mucosa shows negative reaction of MMP1 expression to immunohistochemistry in the fibroblasts of connective tissue of submucosa. IHC for MMP1, 400X.

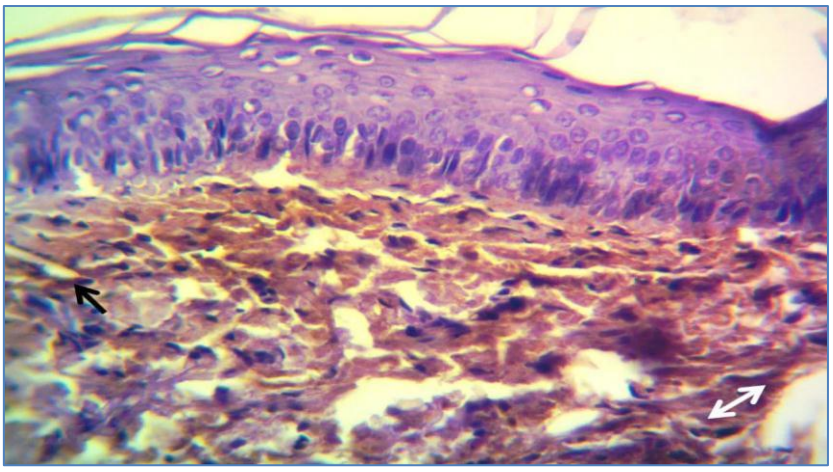

Figure 3: Photomicrograph of rabbit oral mucosa of experimental group shows strong positive reaction of MMP1 expression to immunohistochemistry in the fibroblasts (→) and collagen fibers (↔) of connective tissue of submucosa. IHC for MMP1, 400X.

Table (2): Statistical results of MMP1 expression to immunohistochemistry in fibroblasts and collagen fibers of connective tissue of submucosa in rabbit's oral mucosa of both groups at end of study:

Intensity of MMP-1 expression	Mean ±SD	Sig.(P-value)
Control	1.00±0.08	0.000
Experimental	2.6±0.5	

Values set as Mean ± SD. P-value ≤ 0.05

DISCUSSION

Pathological gingival enlargement should be treated in the presence of gingival inflammation or caries. Gingival enlargement affects speech, function, comfort and/or aesthetics. Treatment varies according to the degree of enlargement in each particular patient and should be individualized on the basis of etiology and predisposing factors.

Different treatment options can be suggested to manage pathological gingival enlargement; they can be categorized as nonsurgical approach and surgical approach or both of them for severe cases of pathological gingival enlargement as well as drug withdrawal [12]. The nonsurgical approach is aimed at reducing the inflammatory component in gingival tissue. The surgical approach eliminates the fibrotic component of the gingival tissue when it is severe and persists after nonsurgical therapy [13].

The enlargement of gingival connective tissue is characterized by the excessive accumulation of extracellular matrix (ECM), particularly the collagenous components [14]. Gingival fibrosis mainly results from the imbalance between collagen synthesis and degradation [15]. Inflammatory changes in the gingival tissue can enhance the imbalance and thus aggravate gingival enlargement [16]. Pathological gingival enlargement may be associated with abnormal cytokine balances, which lead to changes in ECM metabolism in the gingival connective tissue [17]; affects the expression of matrix metalloproteinase-1 (MMP-1, also known as interstitial collagenase). Pathological gingival enlargement may be significantly due to inhibited expression of the MMP-1 in gingival fibroblasts [18], whereas other in vitro studies suggested that pathological gingival enlargement did not have a significant effect on expression of the MMP-1 in gingival fibroblasts [19].

This study was in agreement with Kim *et al.* reported that azithromycin had a significant effect on the fibroblastic part of pathological gingival enlargement, and that azithromycin increased reduced matrix-metalloproteinase (MMP)-1 activities [20].

Also Nagano *et al*. study was in agreement with this study indicates that azithromycin increased the expression of MMP1. This suggests that the action of MMP-1 produced by gingival fibroblasts on collagen promoted its decomposition, thereby inducing periodontal tissue remodeling [21].

An immunohistochemical study indicates that the MMP-1-positive cells decrease in pathological gingival enlargement [22], which lends further support to this postulation.

In the current study, there were significant statistical differences in the expression of MMP-1-reaction among the control and experimental groups (p=0.00). However, the intensity of MMP-1 immunoreactivity were different among the two groups. The intensity of MMP-1 in the control group were weaker compared to the experimental group. These results indicated that Azithromycin alleviated gingival fibrous overgrowth by enhancing MMP-1, which was consistent with the observation in cardiac fibroblasts by Yang et al.

Azithromycin administration leads to amplifying the gene expression of IL-6 and IL-8, which are involved in neutrophil migration in the inflamed gingival region [23]. This contributes to the amelioration of inflammation during the early stage. This suggests the existence of a mechanism by which the periodontal sulcus depth is reduced as a result of the remodeling of the gingival connective tissue due to the increased gene expression of MMP 1[24].

Azithromycin increases IL-8 production in human gingival fibroblasts more than other macrolide antibiotics (i.e., erythromycin, josamycin), resulting in further promotion of neutrophil migration during the inflammatory response [21].

This study was in agreement with Shamekh and Graz reported that azithromycin improved pathological gingival enlargement, and continued research indicated that said effect was due to the promotion of phagocytosis of fibroblasts by azithromycin [25].

The effect of azithromycin on pathological gingival enlargement revealed that a 5-day course of azithromycin reduces the degree of gingival overgrowth [26].

It has been shown that pathological gingival enlargement may be reduced or even prevented by improved oral hygiene, periodontal prophylaxis and careful control of dental plaque [27]. Prophylaxis includes oral hygiene instructions with frequent and correct brushing of teeth, and use of floss and rinses (e.g. chlorhexidine) in order to reduce gingival inflammation.

CONCLUSION

This study establishes that azithromycin is clinically and immunohistochemically effective in the reduction of pathological gingival enlargement.

REFERENCES

1. Aboelsaad, N., El-Shinnawi, U. and Bakr, A., 2013. Azithromycin treatment of drug induced gingival hyperplasia in renal transplant patients. The International Arabic Journal of Antimicrobial Agents, 3 (3).
2. Abushama, A.A. and Ramadan, A.M., 2021. Transforming growth factor-β profile in cyclosporine- A induced gingival enlargement in renal transplant patients. The Saudi Dental Journal, 33(2), p.85.
3. Adegun, O.K., 2021. SBAs and EMQs for Human Disease (Medicine) in Dentistry. Oxford University Press, USA.
4. Alassiri, S., 2021. Utility of active MMP-8 as a test biomarker in periodontal and peri-implant diseases: diagnosis, prevention and treatment outcomes.
5. Castelino, R.L., Buch, S.A. and Laxmana, A.R., 2021. Nifedipine-induced gingival enlargement: A case report with review. Current Medical Issues, 19(1), p.54.
6. De Jesus, A.O., 2018. Comparação da eficácia e segurança do laser cirúrgico de diodo com o eletrocautério no tratamento da hiperplasia fibrosa inflamatória.
7. Doyle, C.J., Fitzsimmons, T.R., Marchant, C., Dharmapatni, A.A.S.S.K., Hirsch, R. and Bartold, P.M., 2015. Azithromycin suppresses P. gingivalis LPS-induced pro-

inflammatory cytokine and chemokine production by human gingival fibroblasts in vitro. Clinical oral investigations, 19(2), pp.221-227.

8. Du Bois, A.H., 2013. The effect of azithromycin on acute and chronic inflammation in an in vivo experimental model (Doctoral dissertation).
9. Feng, J., Li, X., Zhu, S., Xie, Y., Du, J., Ge, H., Bai, Y., Liu, Y. and Guo, L., 2020. Photobiomodulation with 808-nm diode laser enhances gingival wound healing by promoting migration of human gingival mesenchymal stem cells via ROS/JNK/NF-κB/MMP-1 pathway. Lasers in Medical Science, 35, pp.1831-1839.
10. Hosokawa, Y., Hosokawa, I., Ozaki, K. and Matsuo, T., 2021. The Polymethoxy Flavonoid Sudachitin Inhibits Interleukin-1β-Induced Inflammatory Mediator Production in Human Periodontal Ligament Cells. BioMed Research International, 2021.
11. Prieto-Ventura, V.E., Farris III, A.B., Nagaraju, G.P., Bassel, E., Lawal, T.O. and Kim, H.S., 2013. ■ Dr. Constantin Cope Medical Student Research Award Establishment of human metastatic colorectal cancer model in rabbit liver. Pilot study. Journal of Vascular and Interventional Radiology, 24(4), p.S88.
12. Howard, K.C., Gonzalez, O.A. and Garneau-Tsodikova, S., 2021. Porphyromonas gingivalis: where do we stand in our battle against this oral pathogen? RSC Medicinal Chemistry.
13. Kharitonova, M., Vankov, P., Abdrakhmanov, A., Mamaeva, E., Yakovleva, G. and Ilinskaya, O., 2021. The composition of microbial communities in inflammatory periodontal diseases in young adults Tatars. AIMS microbiology, 7(1), p.59.
14. Kim, J.Y., Park, S.H., Cho, K.S., Kim, H.J., Lee, C.K., Park, K.K., Choi, S.H. and Chung, W.Y., 2008. Mechanism of azithromycin treatment on gingival overgrowth. Journal of dental research, 87(11), pp.1075-1079.
15. Kim, Y.G., Lee, S.M., Bae, S., Park, T., Kim, H., Jang, Y., Moon, K., Kim, H., Lee, K., Park, J. and Byun, J.S., 2021. Effect of Aging on Homeostasis in the Soft Tissue of the Periodontium: A Narrative Review. Journal of personalized medicine, 11(1), p.58.
16. Malek, R., El Houari, B. and Kissa, J., 2019. Periodontal management of cyclosporin A-induced gingival overgrowth: A nonsurgical approach. Case reports in dentistry, 2019.
17. Migneault, F. and Hébert, M.J., 2021. Autophagy, tissue repair, and fibrosis: A delicate balance. Matrix biology.
18. Nagano, T., Yamaguchi, T., Kajiyama, S., Suzuki, T., Matsushima, Y., Yashima, A., Shirakawa, S. and Gomi, K., 2021. Effect of Azithromycin on Proinflammatory Cytokine Production in Gingival Fibroblasts and the Remodeling of Periodontal Tissue. Journal of Clinical Medicine, 10(1), p.99.

19. Navarrete, M., Oñate, H., Loyola, K. and Olivares, P., 2020. Effect of Periodontal Debridement Plus Systemic Azithromycin v/s Periodontal Debridement as Unique Therapy in Subjects with Periodontitis: A Randomized Controlled Clinical Trial.
20. Orozco-Páez, J., Méndez-Rodríguez, M.A., Rodríguez-Cavallo, E., Díaz-Caballero, A. and Méndez-Cuadro, D., 2021. Protein carbonylation associated with nickel liberation in orthodontic gingival overgrowth. Archives of Oral Biology, 125, p.105103.
21. Oztug, N.A.K., Ramachandra, S.S., Lacin, C.C., Alali, A. and Carr, A., 2021. Regenerative Approaches in Periodontics. In Regenerative Approaches in Dentistry (pp. 103-131). Springer, Cham.
22. Petit, C., Anadon-Rosinach, V., Rettig, L., Schmidt-Mutter, C., Tuzin, N., Davideau, J.L. and Huck, O., 2021. Influence of psychological stress on non-surgical periodontal treatment outcomes in patients with severe chronic periodontitis. Journal of Periodontology, 92(2), pp.186-195.
23. Prameswari, N., Brahmanta, A. and Revianti, S., 2020. The Effect of Stichopus hermanii to TLR-4 in Mediating Periodontal Ligament Remodeling during Orthodontic Relapse. Systematic Reviews in Pharmacy, 11(3), pp.667-673.
24. Rowińska, I., Szyperska-Ślaska, A., Zariczny, P., Pasławski, R., Kramkowski, K. and Kowalczyk, P., 2021. The Influence of Diet on Oxidative Stress and Inflammation Induced by Bacterial Biofilms in the Human Oral Cavity. Materials, 14(6), p.1444.
25. Shamekh, A. and Graz, M.U., Common Drugs and Complications in dental medicine–A Systematic Review.
26. Teshome, A., Girma, B. and Aniley, Z., 2020. The efficacy of azithromycin on cyclosporine-induced gingival enlargement: Systematic review and meta-analysis. Journal of Oral Biology and Craniofacial Research, 10(2), pp.214-219.
27. Zariczny, P., Pasławski, R., Kramkowski, K. and Kowalczyk, P., 2021. The Influence of Diet on Oxidative Stress and Inflammation Induced by Bacterial Biofilms in the Human Oral Cavity. Materials 2021, 14, 1444.