Increased E-Cadherin Expression in Gingiva after Exposure *Lipopolysaccharide Porphyromonas Gingivalis* (LPS Pg) with Curcumin Application

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Abstract

The initial line of defense against pathogens is the gingival epithelium, which functions as a mechanical barrier. A molecule called E-cadherin, or E-cad, is involved in intercellular adhesion, which is crucial for preserving barrier function in the human epithelium, including the gingiva. To further understand the modifications in E-cad expression in human gingiva after exposure to Lipopolysaccharide Porphyromonas gingivalis (LPS Pg), curcumin was used.

This research is a laboratory experimental research post-test only designed regularly alive using white mice as experimental animals (Rattus norvegicus) Wistar strain male. Exposure to LPS Pg on the gingiva caused a decrease in E-Cadherin expression on day 1, but increased insignificantly on day 7 and day 14. Administration of curcumin increased E-Cadherin expression significantly on day 1, day 7, and 14. The effect of curcumin on increasing E-Cadherin expression showed the best results on day 14.

Curcumin has been proven to increase E-cadherin expression significantly. Giving 1% curcumin to the gingival epithelium that experiences inflammation after exposure to LPS Pg can increase the defense epithelial barrier so that the progression of periodontal disease can be slowed and reduced.

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Introduction

Due to its extremely high frequency, periodontitis-an inflammatory disease of the periodontal tissue-remains a concern in many developing nations, including Indonesia. This is not due to technological developments and advances in the field of dentistry both in Indonesia and throughout the world. Microorganisms on the surface of teeth cause periodontitis, an infection that can lead to tooth loss by forming an irreversible subgingival biofilm.[1] Periodontitis tends to have no symptoms so sufferers are unaware of it and is generally found in an advanced condition. The chronic nature and rarely causing symptoms are the cause of the high prevalence and severity of periodontitis.

Innate and adaptive responses to inflammation interact to cause chronic inflammation of the periodontal tissue, which is the hallmark of chronic periodontitis. The main trigger for chronic periodontitis is the presence of a collection of pathogenic microorganisms that have the potential to cause destructive inflammation. Systemic health status and environmental factors also play a role in chronic periodontitis. Overall, the occurrence of inflammation in periodontal tissue and the host supports a chronic inflammatory state in periodontal tissue, which causes injury and causes bone resorption and tooth loss.[2]

The most coronal part of the junctional epithelium facing the biofilm is divided by the second or third layer of DAT cells, which are directly linked to the tooth. This leads to the onset of periodontal disease. After the junctional epithelium divides, neutrophils gather within the epithelium due to the release of cytokines and chemokines, including interleukin (IL)-8, and the destruction of epithelial barriers by neutrophil-segregated proteins. Therefore, regulating the

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epithelium barrier and preventing neutrophil activity could be effective therapeutic strategies for gingival epithelium inflammation.[3]

By creating cell-cell junction complexes that facilitate intercellular communication and act as a mechanical barrier to prevent pathogenic organisms from entering the body, epithelial cells also trigger the release of antimicrobial peptides and inflammatory cytokines. Multi-protein collagen junctional complexes, which are symmetric structures formed between collagen strands, are essential for maintaining the structural and functional integrity of tissues. Typically, epithelial cells are joined using DESmosomes, gap junctions, tight junctions, and adhesion junctions. Conversely, in clinically sound parietal tissue, the junctional epithelium is rarely joined by gap junctions and demotics and has large intracellular gaps.[3]

The initial line of defense against bacterial attempts is the gingival epithelium, which functions as a mechanical barrier. The molecule known as e-cadherin, or E-cad, is involved in adherens junctions, which are responsible for cell adhesion. These adherens junctions are crucial for maintaining barrier function in the human epithelium, including the gingiva.[4] According to recent studies, periodontitis and gingivitis cause the gingival epithelium to express less E-cad. Moreover, research has demonstrated that some inflammatory mediators, such as ROS and tumor necrosis factor-a (TNF-α), decrease the expression of δ-cad in human peripheral blood cells.[4]

E-cadherin is a glycoprotein located in cell membranes, with a major role in connecting epithelial cells via adherens junctions.[5] In the junctional gingival epithelium E-cadherin is responsible for maintaining its structural integrity and preventing bacterial invasion and a reduction in E-cadherin molecules has been shown in inflamed gingival tissue, thereby causing increased epithelial permeability for periodontological bacteria and the development of periodontitis.[5]

Through the creation of epithelial cellular connections, this condition plays a significant role in epithelial cohesiveness. Additionally, a decrease in cell count improves epithelial cell motility and local invasion ability. Tumor formation and the epithelial-mesenchymal transition (EMT).[6] As a structural integrity of the Oral Gingival Epithelium (OGE), high E-cadherin expression is required. Because of its adhesive activity in the development of adherens junctions, which maintains the function of the epithelial barrier, e-cadherin also plays a crucial role for other junctional complexes such tight junctions, gap junctions, and desmosomes.[7]

As epithelial cells lose their cellular connections during periodontitis, downregulation of E-cadherin favors EMT processes. These findings are consistent with prior research that shown downregulation of E-cadherin expression in inflamed periodontal tissue in vivo studies.[8] Based on the description above, E-Cadherin is important in the gingival defense mechanism against bacterial invasion. If E-Cadherin is damaged or degraded, the AJ structure will also be damaged and result in a disintegrated *epithelial barrier*, damage to the gingival epithelium, and deeper periodontal tissue damage (periodontitis). This idea arises because damage to the gingival epithelial defense occurs in the inflammatory process. Based on this description, this research aimed to clarify changes in E-cad expression in human gingiva after exposure to *Lipopolysaccharide Porphyromonas gingivalis* (LPS Pg) by administering curcumin.

Materials and methods

This research is a laboratory experimental research post-test *only design* regularly alive using white mice as experimental animals (*Rattus norvegicus*) male Wistar strain, 12 weeks old, 200–250 gram body weight, from the Biochemistry Laboratory, Airlangga University, Surabaya, Faculty of Medicine.

Results

The gingiva's E-Cadherin expression levels in the control group were measured, LPS Pg treatment group, and LPS Pg curcumin treatment group can be seen in Table 1 and Figure 1, 2.

A Shapiro-Wilk normality test for δ-Cadherin exposure in all groups revealed that the data was not normally distributed ($p<0.05$). Significant differences ($p<0.05$) were seen in all groups when the Kruskall-Wallis test was administered. After that, a study is conducted to

determine whether groups differ significantly from the multiple comparisons Mann-Whitney test.

Group	Mean (cell/View)	SD (Cell/View)
K	2.70^{c}	0.89
K1	0.40^{a}	0.25
K2	1.06^{ab}	0.43
K3	2.07^{c}	0.48
K4	2.50^{c}	0.56
P1	1.26^{b}	0.43
P2	2.21^{c}	0.91
P3	3.84^{d}	0.63

Table 1. Mean and Standard Deviation of E-Cadherin Expression in Gingiva.
Note: * significant at a=0.05 (Kruskal-Wallis test).

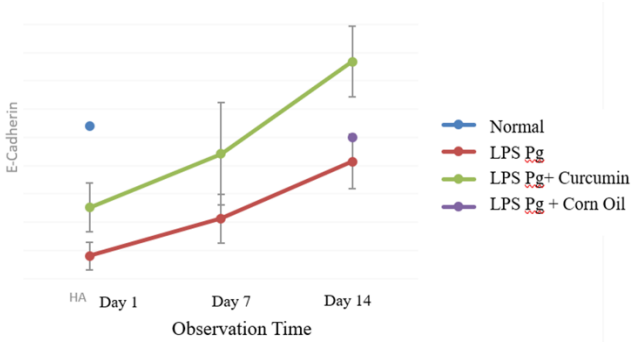

Figure 1. Expression of E-Cadherin in the gingiva in each treatment group.

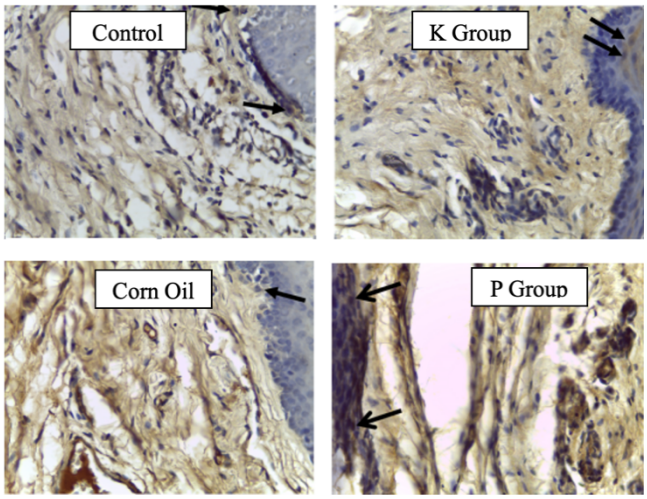

Figure 2. Results of immunohistochemical examination for E-Cadherin. Black arrows indicate cells expressing E-Cadherin (examination by light microscope at 400X magnification).

From Test*Mann-Whitney test,* found in the LPS Pg group from day 1 to day 7 there was a significant decrease, but not significant on day 7 and day 14. In the LPS Pg and curcumin group there was a significant increase on day 1, as well as day 7 to day 14.

Based on the analysis above, it can be concluded that exposure to Pg LPS on the gingiva caused a decrease in E-Cadherin expression on day 1, but it increased insignificantly on day 7 and day 14. Administration of curcumin began to increase E-Cadherin expression significantly on day 1, day 7 and 14. The effect of curcumin on increasing E-Cadherin expression showed the best results on day 14.

Discussion

Epithelial cells interact with the external environment in a few different places, including the digestive system, oral cavity, and airways. They function as a barrier against insults that are damaging to health, whether they be microbial, chemical, or physical. Periodontitis is the most common oral disease in humans and the main cause of tooth loss. Peridontitis is a persistent tongue inflammation. The gingival epithelium acts as a structural barrier between the surrounding tissue and the external environment, serving as the first line of defense against foreign pathogens. Phenodial cleft-to-cleft adhesion disruption, or "leaky gum," is associated with the onset and progression of periodontal disease.[9]

Saliva acts as a chemical barrier because it contains antimicrobial proteins, mucin, and secretory immunoglobulin A in the oral cavity. It also acts as a physical barrier because of the cellular-to-cellular connection structure. There is a significant rate of cell turnover in the gingival sulcus. both during SE (6–12 days) and JE (4–6 days). These are advantageous settings because they facilitate the quick repair of tissue components and cells that have been harmed by microbial assaults. Persistent interactions between pathogens in the subgingival biofilm and nearby gingival epithelial cells as the biofilm develops cause an inflammatory response in the gingiva. Microbes release several metabolic chemicals that are hazardous to tissues, such as propionic and butyric acids. Moreover, bacteria release the protein N-formyl-methionyl-leucil-phenylalanin, which is a potent chemoattractant for leukocytes. Neutrophils migrate chemotactically from blood vessels in the connective tissue to the biofilm via intercellular

spaces in the JE, as JE is more durable than SE. These things cause gingivitis in its early stages.[9]

The experimental animals in this study were wistar rats, which were given Lipopolisacaharid Porphyromonas gingivalis (LPS Pg) twice a day as a gingivitis model. Because wistar rats are an animal model for gingivitis, they were used in this investigation. Gingival tissue was taken to see the expression of variables related to the inflammatory process using the immunohistochemical method.

According to this study, adhesion between epithelial cells is a crucial factor in the formation of epithelial barrier connections, which is facilitated by E-cadherin. In human and mouse gingivitis specimens, e-cadherin expression was lower in inflammatory tissue than in normal tissue. Comparable outcomes for E-cadherin mRNA levels were noted in a study by Li Y et al. (2021). These results imply that the breakdown of epithelial connections and the induction of inflammatory responses are linked to decreased expression of E-cadherin.[10] Additionally, Due to its essential role in maintaining connections between epithelial cells, e-cadherin plays a part in epithelial defense against pathogens. Consequently, a breakdown of the protective barrier and epithelial cell death are suggested by the downregulation of E-cadherin expression. As a result, the gingival epithelial barrier becomes dysfunctional, which promotes the spread of infection and inflammation.[10,11]

The primary structural protein of adherens junctions is called e-cad. Moreover, E-cadherin plays a crucial role in preserving the function of the epithelial barrier since it is necessary for the formation of adherens junctions as well as other junctional complexes due to its adhesive activity.[11] The structural stability of the oral epithelium, especially the JE, depends on e-cadherin. Intercellular connection permeability is increased by E-cadherin dissociation and significantly decreased by E-cadherin expression in JE inflammation.[12]

In this study, it was found that the Pg LPS treatment group with curcumin administration increased E-Cadherin expression compared to the Pg LPS treatment group. The gingival epithelium of patients with periodontitis, in specifically the gingival pocket epithelium, has been shown to express E-cad less frequently. Katz et al. demonstrated that P. gingivalis downregulated the expression of E-cad in Madin-Darby canine kidney cells. The investigation also revealed that a proteinase originating from P. gingivalis was probably responsible for the degradation of E-cad. Nevertheless, additional research has demonstrated that chronic inflammatory gingival epithelial lesions also exhibit a decrease in E-cad mRNA. This implies that gingival discomfort may not be the only mechanism contributing to the decrease of E-cad in the gingival epithelium.[4]

Abe-Yutori et al. (2017) found that P. gingivalis LPS reduced E-cad mRNA and protein in epithelial cells without causing cytotoxicity. Gingipains contamination in P. gingivalis-LPS was not investigated in that investigation. Nonetheless, it is thought that the extraction of P. gingivalis LPS at 65°C with 90% phenol will likely inactivate gingivitis. As a result, P. gingivalis can weaken the connection of epithelial cells by endotoxins as well as gingivitis.[4] In this study, it appeared that the first day after LPS Pg injection, there was a decrease in E-Cadherin.

All epithelial cells express E-cadherin members, and Ca2+-dependent cell-cell adhesion is influenced by E-cadherin expression. In the absence of Ca2+, cadherin is rapidly degraded by proteolysis due to its high Ca2+ sensitivity, the calcium-dependent membrane protein E-cad, which maintains periodontal health when calcium is sufficient, and is necessary for building bone and muscle.[13] A transmembrane receptor called e-cadherin aids in preserving the integrity of epithelial cell interactions, which are critical for the function of the epithelial tissue barrier. A common EMT (epithelial-mesenchymal transition) event that triggers a cascade of signaling molecules and significant cytoskeletal remodeling is thought to be the loss of E-cadherin.[14]

A curcumin concentration of 1% was used in this research, based on previous research, namely Kuo*et al*, 2012 which gave 1% curcumin to mice made obese with liver disorders, decreased the expression of MCP-1, IL-6, and TNF-α considerably in comparison to the group that did not receive curcumin. When probing indications, 1% curcumin subgingival irrigation helps lessen gingival inflammation and bleeding.[15] According to research by Augustina et al. (2017), using 1% curcumin as an irrigation produced positive effects for healing by lessening the depth of remaining pockets and lowering gingival inflammatory indicators (BOP and

redness). Due to its ability to prevent NF-κB activation triggered by P. gingivalis LPS, curcumin influences the expression of MMP-7 and NF-κB. Curcumin prevents the IκB kinase inhibitory factor from being phosphorylated, which prevents the factor's translocation to NF-κB in the cell nucleus. This lowers NF-κB activity and reduces the expression of proinflammatory cytokines.[15]

Periodontitis can be avoided by treating gingivitis appropriately. The goal of therapy for someone who already has periodontitis is to halt the disease's progression because even with treatment, the periodontal tissue will not recover to a healthy state. Because periodontitis is permanent and has a wide-ranging effect, effective prevention of its progression is therefore more important than treatment.

Conclusion

In research that has been conducted, curcumin has been proven to significantly increase E-cadherin expression. Applying 1% curcumin to inflamed gingival epithelium following exposure to LPS Pg can strengthen the protective epithelial barrier, slowing and reducing the advancement of periodontal disease.

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Declaration of Interest

The authors report no conflict of interest.

References

1. Ferreira MC, Dias-Pereira AC, Branco-de-Almeida LS, Martins CC, Paiva SM. Impact of periodontal disease on quality of life: a systematic review. J Periodontal Res. 2017;52(4):651-665. doi:10.1111/jre.12436
2. Cardoso EM, Reis C, Manzanares-Céspedes MC. Chronic periodontitis, inflammatory cytokines, and interrelationship with other chronic diseases. Postgrad Med. 2018;130(1):98-104. doi:10.1080/00325481.2018.1396876
3. Fujita T, Yoshimoto T, Kajiya M, et al. Regulation of defensive function on gingival epithelial cells can prevent periodontal disease. Japanese Dental Science Review. 2018;54(2):66-75. doi:10.1016/j.jdsr.2017.11.003
4. Abe-Yutori M, Chikazawa T, Shibasaki K, Murakami S. Decreased expression of E-cadherin by Porphyromonas gingivalis -lipopolysaccharide attenuates epithelial barrier function. J Periodontal Res. 2017;52(1):42-50. doi:10.1111/jre.12367
5. Tâlvan ET, Mohor CI, Chisnoiu D, et al. Correlation of chronic periodontitis progression with sTREM-1 and E-Cadherin salivary levels. Rev Rom Med Lab. 2018;26(3):373-376. doi:10.1515/rrlm-2017-0038
6. Kadeh H, Arbabi Kalati F, Ramezaninejad M. Expression Patterns of E-Cadherin and N-Cadherin Proteins in the Periodontal Pocket Epithelium of Chronic Periodontitis. J Dent (Shiraz). 2023;24(1 Suppl):125-131. doi:10.30476/dentjods.2022.92474.1652
7. Ramesh PK, Devi RR, Nalini HE, Prasad PAK. Immunolocalization of gingival E-cadherin expression in smokers and non-smokers with chronic periodontitis. Journal of Indian Dental Association. 2020;14(9). doi:10.33882/jida.14.27157
8. wadie karim, Bashir M, Abbass M. Epithelial–mesenchymal transition in gingival tissues from chronic periodontitis patients: A case–control study. Dent Med Probl. 2021;58(3):311-319. doi:10.17219/dmp/133514
9. Takahashi N, Sulijaya B, Yamada-Hara M, Tsuzuno T, Tabeta K, Yamazaki K. Gingival epithelial barrier: regulation by beneficial and harmful microbes. Tissue Barriers. 2019;7(3):e1651158. doi:10.1080/21688370.2019.1651158
10. Li Y, Li B, Liu Y, et al. Porphyromonas gingivalis lipopolysaccharide affects oral epithelial connections via pyroptosis. J Dent Sci. 2021;16(4):1255-1263. doi:10.1016/j.jds.2021.01.003
11. Liu Y, Yang J, Sun W. Upregulation of IL-10 expression inhibits the proliferation of human periodontal ligament stem cells [published correction appears in Braz Oral Res. 2020;34:e030err]. Braz Oral Res. 2020;34:e030. Published 2020 Mar 27. doi:10.1590/1807-3107bor-2020.vol34.0030
12. Oh C, Kim HJ, Kim HM. Vitamin D maintains E-cadherin intercellular junctions by downregulating MMP-9 production in human gingival keratinocytes treated by TNF-α. J Periodontal Implant Sci. 2019;49(5):270. doi:10.5051/jpis.2019.49.5.270
13. Salam Ali S, Ali MS, AL-Gebori AM, Ahmed Hadi H. The Influence and Assessment of E-Cadherin Sera Levels with Periodontitis and Gingivitis. 2023; 10 No. 3S: Special Issue 3: 3151-3161. doi:https://doi.org/10.17762/sfs.v10i3S.1128
14. Yang F, Lu J, Yu Y, Gong Y. Epithelial to mesenchymal transition in Cyclosporine A-induced rat gingival overgrowth. Arch Oral Biol. 2017;81:48-55. doi:10.1016/j.archoralbio.2017.04.024
15. Eka Fitria Augustina, I. Ketut Sudiana, Soetjipto, Muhammad Rubianto. Expression of NF-κB and MMP-7 on defenses of the gingival epithelium injected lps porphyromonas gingivalis with the administration of curcumin. Journal of International Dental and Medical Research. 2019;12(6): 941-946.