

The effects of aspirin gel and mouthwash on levels of salivary biomarkers PGE₂, TNF- α and nitric oxide in patients with periodontal diseases

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Abstract: Inflammation and its mediators have an important role in gingivitis and periodontitis. Prostaglandin is one of the eicosanoid involved in many chronic inflammatory diseases, including periodontal diseases. Aspirin irreversibly acetylates cyclooxygenase and inactivate this enzyme responsible for the production of PGE₂ that mediates pain and inflammation. The aim of the study was to prepare aspirin gel and mouthwash in 1% concentration and use it in patients with periodontal diseases during the non-surgical periodontal treatment and to assess its anti-inflammatory effects on salivary biomarkers PGE₂, TNF- α , and nitric oxide. Thirty patients were divided into three treatment groups, standard treatment group, second received scaling and root planning with gel application of 1% aspirin, third received scaling and root planning followed by rinsing with 1% aspirin mouthwash. Results indicated that the levels of PGE₂, TNF- α and nitric oxide in the groups of patients received gel treatment and mouthwash treatment was decreased to significant levels ($p \leq 0.001$) as compared to the group of standard treatment. Aspirin gel was found to be more effective in reducing inflammatory biomarkers in contrast to aspirin mouthwash ($p \leq 0.001$). We concluded from our study, that low concentration of aspirin oral preparations are highly active in reducing the inflammatory biomarkers associated with periodontal diseases.

Keywords: Aspirin, inflammatory biomarkers, PGE₂, TNF- α , nitric oxide, periodontal diseases

INTRODUCTION

Periodontal diseases are the most common cause of supporting tooth structure destruction. Inflammation and its mediators have an important role in gingivitis and periodontitis. This inflammatory process caused by the anaerobic bacteria leads to slow destruction of periodontal tissues. Macrophages and gingival fibroblasts released TNF- α and IL-1 β which are accountable to produce prostaglandin E₂, IL-6 and IL-8. Prostaglandin is one of the eicosanoid involved in many chronic inflammatory diseases, including periodontal diseases. The pro-inflammatory cytokines stimulate bone resorption by increasing osteoclastic activity which results in alveolar bone and attachment loss (Hasturk *et al.*, 2012).

The occurrence of nitric oxide in periodontal tissues reflects the contribution of another inflammatory mediator accountable for the progression of gingivitis and periodontitis. Pro-inflammatory enzymes, including cyclooxygenase and metalloproteinases generated by nitric oxide are responsible for these inflammatory conditions (Scarel-Caminaga *et al.*, 2017).

Saliva is a complex mixture of proteins, water, electrolytes, enzymes and antimicrobial substances, secreted by the salivary glands. Since last few years, saliva has been used as a non-invasive diagnostic tool for testing many inflammatory diseases, including periodontal diseases. The levels of PGE₂, TNF- α and nitric oxide are increased in saliva and gingival crevicular fluid in patients with periodontal diseases (Gupta *et al.*, 2015; Pirseanm *et al.*, 2019; Gomes *et al.*, 2019).

Aspirin irreversibly acetylates cyclooxygenase and inactivate this enzyme responsible for the production of PGE₂ that mediates pain and inflammation (Ornelas *et al.*, 2017). Researches were done on the anti-inflammatory effects of different NSAIDs in treating periodontal diseases and researchers found satisfactory results but the long term systemic use of NSAIDs may cause nausea, vomiting, epigastric distress, GI bleeding and ulceration (Faizuddin *et al.*, 2012; Cavagni *et al.*, 2016). Thus, the aim of the study was to prepare gel and mouthwash of aspirin in 1% concentration and assess its anti-inflammatory effects on salivary biomarkers of PGE₂, TNF- α and nitric oxide.

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MATERIAL AND METHODS

Acetylsalicylic acid was gifted from Kaizen Pharmaceutical (Pvt) Limited, Pakistan). Propylene glycol, sodium benzoate, triethanolamine, glycerin and flavoring agent were obtained from Nigheban pharmacy, Karachi.

Preparation of aspirin gel

For preparing 500 gm gel (Mitala *et al.*, 1984), 5 gm of acetylsalicylic acid was dissolved in tween 80. When acetylsalicylic acid was dissolved completely and a clear solution was attained, preservatives (Zabrzewska *et al.*, 2014) were added followed by triethanolamine (Abrar *et al.*, 2012) to adjust the pH of the solution. The final prepared solution was then added in 2% carboxymethylcellulose gel to get a lump free preparation.

The quality control tests were performed before its application into the patient's periodontal pockets, which includes:

- Gel pH (Abrar *et al.*, 2012)
- Viscosity (Misal *et al.*, 2012)
- Syringeability (Aslani *et al.*, 2013)
- Spreadability (Misal *et al.*, 2012)
- Total microbial count in the preparation (Zamir *et al.*, 2015)
- Mucoadhesion test (Aslani *et al.*, 2018)

Preparation of aspirin mouthwash

10 gm of Acetylsalicylic acid (ASA) was added in propylene glycol gradually in little amounts until it was dissolved, with the aid of mechanical stirrer (IKA Works Inc.) at 500 RPM. Sodium benzoate, triethanolamine, flavor and water were then added in the cleared filtered mixture to make the final 1000 ml of 1% preparation. The mouthwash was finally evaluated for its quality control before its application into the oral cavity.

The quality control tests included:

- (a) Mouthwash pH (Zamir *et al.*, 2015)
- (b) Total microbial count in the preparation (Zamir *et al.*, 2015)

Study group

This was a randomized clinical control trial. Thirty patients were enrolled during January 2018 to April 2018. Detailed examination of patients was done for inclusion and exclusion criteria.

Thirty patients were randomly divided into three treatment groups equally (n=10). All groups received scaling and root planning in each mouth quadrant once a week for 4 consecutive weeks. First group (standard treatment group) received only scaling and root planning. The second group received scaling and root planning with gel application of 1% aspirin into the periodontal pocket

in treated quadrant after 48 hours of each session and the third group of patients received scaling and root planning followed by rinsing with 1% aspirin mouthwash after the completion of each session.

All the individuals who were engaged in the study were informed about the research protocol and the patients who signed the informed consent were included in the study. All the procedures were done according to Helsinki Declaration 1964 which were later amended in 2000. The study was done after got approved from board of advanced studies and research (BASR) KU and the Independent Ethics Committee of International Center for Chemical and Biological sciences ICCBS/IEC-029-HS2017/Protocol/1.0.

Inclusion criteria

Adult patients of twenty years or more
Patients with no former history of systemic disease

Exclusion criteria

Patients who were using any medications or antibiotics since the last six months
Pregnant or breast feeding women

Collection of salivary samples

All the samples were collected according to the standard protocol. Unstimulated salivary samples were collected. Salivary samples were collected before treatment and four weeks after completion of treatment. The collection was done in the early morning before 11 am. Before collection of saliva, patients were instructed to rinse the mouth with water to remove any food particles. For each patient two to three ml of whole saliva was collected in vials. The vials were then coded and immediately centrifuged. The supernatant clear fluids were then refrigerated at -20°C (Jeyasree *et al.*, 2018). Collected clear fluids were used to assess the levels of PGE₂ {Glory science company, Ltd, USA} (Gumus *et al.*, 2016), TNF- α {Invitrogen, California}} (Gumus *et al.*, 2016) and nitric oxide {Glory science company, Ltd, USA} Poorsattar *et al.*, 2014) by using ELISA kit.

STATISTICAL ANALYSIS

SPSS version 21 (IBM) was used for analysis of data. Values were plotted as mean $\pm$ SD. One way ANOVA was used for analysis and group comparison was done using Bonferroni's test, considering p-value of ≤ 0.05 as significant.

RESULTS

The levels of PGE₂, TNF- α and nitric oxide in the groups of patients received gel treatment and mouthwash treatment was reduced to significant levels ($p \leq 0.001$) as compared to the group of standard treatment. Levels of all

Table 1: Salivary concentration of PGE₂ (pg/ml) before and after treatment

Groups	Initial Examination (n=10)	Final Examination (n=10)
Standard treatment	95.60 ± 2.14	91.24 ± 2.56
Aspirin gel	95.51 ± 2.24	42.20 ± 2.42 ^{####}
Aspirin mouthwash	96.51 ± 1.04	50.35 ± 1.14 ^{###}

Table 2: Salivary concentration of TNF-α (pg/ml) before and after treatment

Groups	Initial Examination (n=10)	Final Examination (n=10)
Standard treatment	72.56 ± 1.78	63.59 ± 1.58
Aspirin gel	74.58 ± 1.62	17.52 ± 1.54 ^{####}
Aspirin mouthwash	73.86 ± 1.60	29.50 ± 1.58 ^{###}

Table 3: Salivary concentration of Nitric oxide (μmol/ml) before and after treatment

Groups	Initial Examination (n=10)	Final Examination (n=10)
Standard treatment	63.52 ± 0.82	62.81 ± 0.56
Aspirin gel	62.04 ± 0.91	28.86 ± 0.41 ^{####}
Aspirin mouthwash	63.60 ± 0.92	39.67 ± 0.68 ^{###}

Mean ± SD. ^{###} p ≤ 0.00, shows significance with standard treatment

^{!!!} p ≤ 0.001, shows significance between gel and mouthwash

inflammatory biomarkers were highly reduced in patients that received treatment with aspirin gel and mouthwash. High statistical significant difference was also observed between gel and mouthwash (p ≤ 0.001) preparations. The gel reduced the levels of all inflammatory biomarkers to a higher extent as compared to mouthwash.

DISCUSSION

Aspirin is commonly used to treat pain and inflammation (Eccleston *et al.*, 2017) and is the drug to which all the other anti-inflammatory agents are compared. Since many years the mode of treatment for periodontal diseases is the removal of dental biofilm with scaling and root planning (Graetz *et al.*, 2017). As scaling instruments are unable to reach completely into periodontal pockets, the incomplete mechanical removal of plaque and calculus and due to the complex nature of biofilm, the bacteria again recolonize which produces the new biofilm and thus increases inflammation (Kalsi *et al.*, 2011). The role of aspirin is very important in reducing the inflammation of periodontal tissues damaged by dental biofilm (Sehran, 2017).

Several studies were conducted on the anti-inflammatory effect of different NSAIDs and the researchers found that there is decreased activity of osteoclasts and the smaller rate of alveolar bone loss. But the dose which produces these beneficial effects is higher and long period of treatment is required to produce these results (Cavagni *et al.*, 2016). In our study, we prepared the low concentration of aspirin gel and mouthwash which not only reduce the inflammation but also prevent the side effects related to its long term systemic use.

Periodontal tissues are affected by infection with gram negative anaerobic bacteria. These bacteria produce multiple inflammatory mediators which are responsible for injured periodontium (Dahlen and Preus, 2017). Salivary concentration of PGE₂, TNF-α and nitric oxide are higher in patients with periodontal diseases (Menaka *et al.*, 2009; Gumus *et al.*, 2017; Goms *et al.*, 2019) as compared to healthy individuals. Several studies showed that gingivitis and periodontitis are linked with increased levels of different inflammatory mediators (Yucel-Lindberg *et al.*, 2013; Petit *et al.*, 2019). Increased expression of TNF-α stimulates the inflammatory cells to release metalloproteinases at the site of infection which affect the periodontal fibers, ultimately leading to alveolar bone loss (Yucel-Lindberg *et al.*, 2013). Liao *et al.* (2014) worked on a rat periodontitis model and found increased expression of TNF-α in the diseased group as compared to control group. Our study also observed the same findings in which there is increased concentrations of TNF-α in patients with periodontal diseases, and following the treatment with anti-inflammatory preparation containing aspirin, this inflammatory biomarker in the saliva was reduced to a very low level which strongly suggests that this inflammatory mediator has a role in inflammatory diseases, including gingivitis and periodontitis and by decreasing its concentration recovery could be early initiated.

Another important mediator of inflammation is PGE₂. During inflammation the increased release of PGE₂ causes alveolar bone loss. Increase production of PGE₂ also stimulates the release of TNF-α so the activity of both these mediators is interlinked (Yucel-Lindberg *et al.*, 2013). It is a known fact that aspirin is an inhibitor of prostaglandin synthesis (Ratchford *et al.*, 2017) and our

study also proved the anti-inflammatory effect of low concentration of aspirin gel and mouthwash in reducing the level of PGE₂ significantly.

An additional mediator role was also found by many researchers in periodontal diseases and that is nitric oxide (Wattamwar *et al.*, 2016; Cintra *et al.*, 2016). The inflammatory cells express nitric oxide at the site of infection. It has a role in protection of periodontium by destroying the bacteria that may cause infection, however, too much production of nitric oxide results in destruction of host periodontal tissue by oxidation and nitration reactions and injury to DNA (Menaka *et al.*, 2009). In this study, level of nitric oxide was highly increased in patients before treatment but, after treatment of 4 weeks the level was also decreased to a significant level which also proves the effective role of aspirin in reducing the inflammation.

CONCLUSION

We concluded from our study that low concentration of aspirin oral preparations are highly effective in reducing the inflammatory biomarkers connected with periodontal diseases. So it is safe to use these low concentration preparations as an adjuvant to root planning and scaling rather than prescribing these drugs orally, as to avert the adverse reactions linked with oral use of aspirin. Gel form has more retainability hence shows more pronounced effects as compared to mouth wash.

However, this is a preliminary research with a small number of patients included and future studies are also required to study the effects of aspirin gel and mouth wash in different concentrations to treat periodontal diseases.

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