Original Article

Metformin

Metformin- A Non-Conventional Drug for the Local Treatment of Periodontal Diseases: A Randomized Clinical Control Study

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ABSTRACT

Objective: The purpose of this study was to develop 1% gel and mouthwash of metformin in order to treat the periodontal diseases locally to avoid the systemic adverse effects associated with its oral use. Although metformin is not used in the treatment of periodontal disease but due to its unique anti-inflammatory effects an attempt was made to prepare the gel and mouthwash and applied it locally to treat gingivitis and periodontitis.

Study Design: Randomized control trial study

Place and Duration of Study: This study was conducted at Crown Dental Clinic, Karachi and HEJ Research Institute of Chemistry, University of Karachi conducted from March 2016 to September 2016.

Material and Methods: The local formulation of 1% metformin gel and mouthwash was developed. Patients were divided into four groups 1) Control group 2)Standard treatment group 3) Gel treated group4) Mouthwash treated group. The clinical and biochemical variables were measured at day 0 and after 30 days of treatment in the all groups.

Results: after 30 days highly significant reduction was observed in all clinical variables ($p \leq 0.001$) as compared to standard and control group by 1% metformin gel and mouthwash. After 30 days bleeding was also reduced ($p \leq 0.001$) by the standard treatment. Similar efficacy was observed in reducing the clinical variables by both preparations after 30 days of treatment ($p \leq 0.001$). the reduction in TNF-α,PGE_2 and nitric oxide levelafter 30 days ($p \leq 0.001$)by 1% metformin gel and mouthwash when compared to standard and control group.Significant decrease in TNF-α level after 30 days ($p \leq 0.001$) was also observed by the standard treatment group.1% metformin gel found to be more effective in reducing the TNF-α and PGE_2level ($p \leq 0.001$) when compared to 1% mouthwashwhereas metformin mouthwash found to be more effective in reducing the nitric oxide level ($p \leq 0.001$) as compared to gel.

Conclusion: This research recommends that metformin due to its unique anti-inflammatory effects can be used in treating gingivitis and periodontitis.

Key Words: gingivitis, periodontitis, periodontal pocket depth, attachment loss, scaling and root planning, metformin

Citation of article: Hasan F, Najam R, Simjee SU. Metformin- A Non-Conventional Drug for the Local Treatment of Periodontal Diseases: A Randomized Clinical Control Study. Med Forum 2017;28(6):162-166.

INTRODUCTION

In Asian population particularly in Pakistan, India and Bangladesh the two main inflammatory diseases of the peridontiumaregingivitis and periodontitis. These periodontal diseases occurs because of their traditional eating habits of pan, tobacco, and beetle nut [1]. Another reason of increasing prevalence of these diseases is the lack of awareness of maintaining oral hygiene and the lack of approach to the dentist because of poverty. In Pakistan the disease affects the people of low socioeconomic status more than people of high socioeconomic status [2].

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Received: April 11, 2017; Accepted: May 24, 2017

In gingivitis the disease is limited to the mucosal epithelial tissue without clinical attachment loss whereas in periodontitis clinical attachment loss is present resulting in "periodontal pocketing" and alveolar bone loss [3.]

Pro-inflammatory cytokines has a very important role in periodontal diseases. Increase level of TNF-α, PGE_2, and nitric oxide was found in the saliva of the patients with periodontitis which suggests their role in diagnosing and treatment of periodontal diseases. Increased level of PGE_2 is responsible for the bone resorption and clinical attachment loss. The release of cyclooxygenase and metalloproteinases are activated by nitric oxide which also damage the periodontal tissue [4,5,6].

Certain drugs are commonly prescribed by the dentist in the treatment of periodontal disease but nowadays researchers are investigating on new pharmacological agents to treat the periodontal disease by modifying the host response and reducing inflammatory biomarkers produced by bacteria [7].

Metformin is most commonly used drug to treat diabetes mellitus.

Lookwood (2010) recommended the use of metformin as an anti-inflammatory agent as he found out that the normal inhibitory action of Zn^{+2} against cysteinyl proteases is raised by metformin[8]. Bak et al. (2010) also found that when metformin was given in rats with ligature induced periodontitis there was a decrease in bone loss and an increase in alveolar bone formation as a result of increase osteoblastic differentiation[9].

In periodontology, use of local drug delivery is getting very popular nowadays. The sub-gingival bacteria are not entirely removed even after scaling and root planning and they again form a biofilm. The reason behind this local drug delivery system is the removal of this biofilm which provides the complete eradication of the disease [10]. This method of treatment is much easier to execute, requires less instrumentation and provide good bioavailability of the drug. The drug adhered in the base of the periodontal pocket for a longer span with least adverse effects [11].

The objective of this research was to assess and compare the effectiveness of 1% metformin gel with mouthwash, to treat the periodontal diseases with minimum side effects which are frequently observed with the systemic use of the drugs used in treating periodontal diseases.

MATERIALS AND METHODS

This study was conducted at Crown Dental Clinic, Karachi and HEJ Research Institute of Chemistry, University of Karachi conducted from March 2016 to September 2016.

Metformin HCl tablets 500 mg (Merck), purchased from the market and the other chemicals were received from Nighebaan Pharmacy, Karachi.

Formulation of 1% metformin gel: Distilled water was used to dissolve 500 mg of 10 metformin tablets and mixed with 2% hydroxyethyl cellulose gel containing methyl paraben sodium, propyl paraben sodium and EDTA (preservatives). The pH of the preparation was adjusted by adding triethanolamine[12]. Before application into the periodontal pocket the gel was then finally evaluated for its physicochemical properties.

Formulation of 1% metformin mouthwash: Distilled water was used to dissolve 500 mg of 10 metformin tablets. Triethalnolamine was added to adjust the solution pH, glycerin and food dye was used for color and flavoring. To prevent the growth of the fungisodium benzoate was added as preservative. The mouthwash was then finally evaluated for its physicochemical properties.

Procedure involving pharmacological treatment:

Ethical approval, consent and permission: The study has been reviewed and was approved by the ethics review committee of University of Karachi, Pakistan.

Participants: 40 participants were included in this randomized clinical control study.

Inclusion criteria

- Patients above 20 years
- Patients without systemic disease

Exclusion criteria

- Patient received any medication from the past 6 months
- Patients having systemic disease
- Patient received periodontal treatment from the past 24 months
- Pregnant and lactating women

The participants were divided in to four categories

- Control group: no treatment
- Standard treated group: scaling and root planning only; each mouth quadrant per week for four consecutive weeks
- Gel treated group: scaling and root planning per quadrant per week for four weeks along with the intra-crevicular gel application by disposable syringe of 5 ml having 25 gauge blunted needle after 48 hours of every session
- Mouthwash treated group: mouthwash was used after the end of each treatment session for four consecutive weeks.

The clinical and biochemical variables were measured in each group at day 0 and after 30 days.

Sample collection and determination of biochemical variables: Saliva samples were obtained from each participant at day 0 and at 30 day. The samples were collected in a 5 ml sterilized coded vials, centrifuged at 12,000 g for ten minutes. The centrifuged fluids were shifted to -20°C refrigerator.

Analysis of TNF-α (Invitrogen, California), nitric oxide and PGE_2 (Glory science co., Ltd, USA) in salivary samples were done by using enzyme-linked immunosorbent assay (ELISA) kits [6, 13, 14.]

Determination of clinical variables: The clinical variables were measured by using CPITN probe.

Probing depth: Probing depth is measured from the gingival margin to the base of the sulcus or periodontal pocket. Six teeth were examined and in each tooth six sites were probed [15].

Attachment level: Teeth and sites used for probing were similar to the probing depth [15].

Tooth mobility: Horizontal and vertical tooth mobility was calculated by applying the moderate pressure in the in the facial-lingual direction and on incisal or occlusal surface of the tooth respectively[15, 16].

Bleeding on probing: To evaluate bleeding, the probe was gently insertedalong the gingival tissue wall of the sulcus [15, 16.]

Plaque index: In each arch 3 teeth were examined followed by examining four surfaces of each tooth[15, 16].
Gingival index: In each arch 3 teeth were examined followed by examining four surfaces of each tooth [15, 16].
Statistical analysis: Statistical analysis was done by one way ANOVA and post hoc analysis by Bonferroni test.

RESULTS

Table 1a and 1b shows that after 30 days highly significant reduction was observed in all clinical variables ($p \leq 0.001$) as compared to standard and control group by 1% metformin gel and mouthwash.

Table No.1a: Measurement of clinical parameters

Groups	Periodontal Pocket Depth		Attachment Level		Bleeding on Probing	
	Day 0	Day 30	Day 0	Day 30	Day 0	Day 30
Control Group	3.69 ± 1.08	3.91 ± 0.83	3.96 ± 0.81	3.97 ± 0.81	1.00 ± 0.25	0.90 ± 0.32
Standard Group	3.90 ± 0.83	3.77 ± 0.95	3.79 ± 0.55	3.57 ± 1.00	0.90 ± 0.32	0.54 ± 0.25***
1% Metformin Gel	4.28 ± 1.04	2.57 ± 1.07***###	4.43 ± 1.00	2.74 ± 1.01***###	0.95 ± 0.11	0.00 ± 0.00***###
1% Metformin Mouthwash	4.01 ± 1.00	1.99 ± 0.84***###	4.20 ± 0.99	2.13 ± 0.86***###	0.95 ± 0.11	0.00 ± 0.00***###

n=10, mean ± SD. *** $p \leq 0.001$= highly significant with control group.
$p \leq 0.001$= highly significant with standard group.

Table No.1b: Measurement of clinical parameters

Groups	Tooth Mobility		Plaque Index		GingivalIndex	
	Day 0	Day 30	Day 0	Day 30	Day 0	Day 30
Control Group	0.60 ± 0.70	0.80 ± 0.53	2.66 ± 0.21	2.67 ± 0.76	2.45 ± 0.36	2.47 ± 0.35
Standard Group	0.70 ± 0.67	0.70 ± 0.67	2.64 ± 0.18	2.31 ± 0.15	2.63 ± 0.37	1.89 ± 0.77
1% Metformin Gel	1.20 ± 0.79	0.40 ± 0.52***###	2.47 ± 0.48	0.47 ± 0.50***###	2.43 ± 0.38	0.40 ± 0.41***###
1% Metformin Mouthwash	1.20 ± 0.63	0.50 ± 0.53***###	2.28 ± 0.55	0.52 ± 0.45***###	2.37 ± 0.40	0.46 ± 0.42***###

n=10, mean ± SD. *** $p \leq 0.001$= highly significant with control group.
$p \leq 0.001$= highly significant with standard group.

Table No. 2: Measurement of biochemical variables

Groups	TNF-α		PGE_2		Nitric Oxide	
	Day 0	Day 30	Day 0	Day 30	Day 0	Day 30
Control Group	75.88 ± 2.51	75.96 ± 2.57	90.83 ± 1.93	91.54 ± 1.76	60.76 ± 1.62	61.26 ± 1.75
Standard Group	74.26 ± 1.78	41.85 ± 1.58***	94.90 ± 2.26	92.9 ± 2.47	62.47 ± 0.75	61.72 ± 0.67
1% Metformin Gel	78.03 ± 1.23	3.15 ± 1.37***###+++	85.90 ± 1.55	29.72 ± 1.85***###+++	69.35 ± 2.29	37.6 ± 2.59***###
1% Metformin Mouthwash	73.66 ± 1.75	21.83 ± 1.62***###	80.53 ± 1.63	40.31 ± 1.28***###	62.41 ± 2.38	24.94 ± 2.46***###+++

n=10, mean ± SD. *** $p \leq 0.001$= highly significant with control group. ### $p \leq 0.001$= highly significant with standard group. +++ $p \leq 0.001$= highly significant in comparison of metformin gel v/s metformin mouthwash.

After 30 days bleeding was also reduced ($p \leq 0.001$) by the standard treatment. Similar efficacy was observed in reducing the clinical variables by both preparations after 30 days of treatment ($p \leq 0.001$).

Table 2 indicates the reduction in TNF-α,PGE_2 and nitric oxide levelafter 30 days ($p \leq 0.001$)by 1% metformin gel and mouthwash when compared to standard and control group.Significant decrease in TNF-α level after 30 days ($p \leq 0.001$) was also observed by the standard treatment group.1% metformin gel found to be more effective in reducing the TNF-α and PGE_2level ($p \leq 0.001$) when compared to 1% mouthwashwhereas metformin mouthwash found to be more effective in reducing the nitric oxide level ($p \leq 0.001$) as compared to the gel.

DISCUSSION

Periodontal problems are very common worldwide nowadays. To maintain the periodontal health adequate oral hygiene is required because if the oral hygiene is not maintained there is a building of oral plaque which is responsible for the growth of anaerobic bacteria. There is a destruction of collagen supporting the periodontium and alveolar bone resorption in the late stage of periodontitis. The increased production of pro-inflammatory cytokines, neutrophilic enzymes and reactive oxygen species as a result of activation of neutrophils is due to proliferation and entrance of anaerobic pathogens in the periodontium [17, 18].

Periodontal diseases are treated mainlyby scaling and root planning as well as by oral drugs such as non-steroidal anti-inflammatory agents and different types of antibiotics [19, 20]. Nowadays, researchers are interested in different drugs that are not used in treating periodontal diseases but have significant effects on inflammation. These drugs also acts as an anti-oxidants, works as an anti-septic and also have anti-collagenase activity [7].

Treatment of periodontal diseases by the local drug delivery system is provided by the dentist but regular visits are required to treat the diseases which not only reduces systemic administration of drugs but also minimizing the adverse effects associated with its use.

In this study we observed the anti-inflammatory effect of metformin in patients with periodontal disease by developing 1% metformin gel and 1% metformin mouthwash. These oral preparations not only reduced the clinical parameters but also have a beneficial effect on biochemical variables. The anti-inflammatory effects of both the preparations was probably due to the mechanism depends on AMP activated protein kinase. This anti-inflammatory effects of the drug is non-hepatic and independent from the other metabolic effects of metformin [21]. Thus, it produces a completely different anti-inflammatory mechanism in treating periodontal disease as compared to commonly used NSAIDs. Both the preparations reduced the tooth mobility by increasing the osteoblastic activity results in alveolar bone formation [9]. The reduction in tooth mobility may also be due tothe reduced formation of reactive oxygen species and through apoptosis within the cell [22].

Both the gel and mouthwash preparation highly reduced the level of TNF-α, PGE_2 and nitric oxide. The mRNA expression of SREBP-1c, PPARγ and FASN genes and the other pro-inflammatory cytokines are under control by metformin in the hepatocytes [21]. Metformin decreases the level of these genes and the pro-inflammatory cytokines. Reduction in the inflammatory biomarkers decreased bleeding and also decrease the attachment level and pocket depth.

CONCLUSION

We concluded from our study that the both preparations are highly effective in reducing the periodontal disease in its local form without producing any adverse effects. Although both the gel and mouthwash is highly effective in reducing the clinical and biochemical variables but the gel produced better results when compared to mouthwash possibly because of its muco-adhesive property as the drug is available for extended period of time in the periodontal pocket. Thus, the gel form is effective in chronic forms of periodontal diseases and the mouthwash plays a role in those conditions where there is an acute form of the disease.

Recommendations: This research recommends that metformin due to its unique anti-inflammatory effects can be used in treating gingivitis and periodontitis. Further researches are also required on anti-inflammatory effects of new pharmacological agents in treating periodontal diseases.

Conflict of Interest: The study has no conflict of interest to declare by any author.

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