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Effects of 1% amitriptyline gel and mouthwash in patients with periodontal diseases via local drug delivery system: A randomized control clinical trial

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Abstract: Amitriptyline, an agent universally used to treat depression, has an anti-inflammatory activity and a potential for lowering inflammatory mediators. Periodontal diseases like gingivitis and periodontitis if untreated contributes to gingival tissue destruction and bone resorption. These diseases are commonly treated with conventional non-steroidal anti-inflammatory agents and antibiotics along with standard periodontal treatment. The aim of this experimental, observational and randomized clinical control trial was to evaluate the anti-inflammatory effects of amitriptyline on clinical parameters and on inflammatory biomarkers in patients of periodontal diseases by developing 1% oral gel and mouthwash formulations. 30 patients participated in the study were grouped in three categories, patients received standard conventional treatment, patients received gel treatment for four weeks after standard treatment, patients received mouthwash for four weeks after standard periodontal treatment. Results showed that amitriptyline gel and mouthwash in 1% formulation showed promising results by significantly reducing periodontal parameters and inflammatory biomarkers ($p \leq 0.001$) as compared to standard treatment. Thus, we suggest that gel and mouthwash formulation of amitriptyline is highly efficacious in treating the periodontal diseases.

Keywords: Amitriptyline, periodontal diseases, local drug delivery, saliva, inflammatory biomarkers, clinical parameters

INTRODUCTION

Periodontal diseases are the most common inflammatory diseases of oral cavity worldwide, caused by the bacterial plaque (Kistler *et al.,* 2013). Gingivitis and periodontitis have the same clinical signs and symptoms of inflammation but as the gingivitis progresses in to periodontitis, there is pocket formation due to clinical attachment loss (Hasan and Palmer, 2014). There is also alveolar bone loss followed by tooth loss as a result of increased production of pro-inflammatory cytokines (Moradi *et al.,* 2014), matrix metalloproteinases (Popat *et al.,* 2014), neutrophilic enzymes, reactive oxygen species (Ramesh *et al.,* 2016; Indurkar and Verma, 2016) and nitrous oxide (Menaka *et al.,* 2009). Common drugs used to treat the pain and inflammation linked with these conditions is conventional non-steroidal anti-inflammatory agents such as NSAIDs (Salvi and Lang, 2005) and antibiotics (Azodo and Ojehanon, 2014). New researches are going on drugs having anti-inflammatory effects which are not commonly used to treat periodontal diseases, by giving them through local drug delivery system. This system provides best results without surgical interventions. The drug reaches in mechanically restricted areas and reassures the drug delivery to the base of dental pocket thus reducing infection and to avoid the adverse effects related with its systemic use (Malathi *et al.,* 2014).

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Amitriptyline is universally available old drug used to treat several disorders including depression (Shinohara *et al.,* 2019), obsessive compulsive disorder (Feighner, 1999), social anxiety disorder (Feighner, 1999), bulimia nervosa (Bacaltchuk and Hay, 2003) and many others (Kim *et al.,* 2013). It has anti-inflammatory activity as well as it has a potential of lowering tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and prostaglandin E_2 (PGE_2) levels (Gurgel *et al.,* 2013). The reduction of the inflammatory biomarkers is due to the analgesic action of amitriptyline at the periphery which inhibits the reuptake of serotonin and norepinephrine. It also binds to local anesthetic receptors thus, blocks sodium channels and producing the analgesic effects. Central analgesic action of the drug is due to the opening of many different K^+ channels. It also decreases pain intensity by blocking N-methyl-D-aspartate (NMDA) receptors. Over all it reduces the neuronal excitability which is responsible for its antihyperalgesic action (Lawson, 2017).

Thus, the aim of our study was to evaluate the anti-inflammatory effects of amitriptyline in patients with gingivitis and periodontitis, to treat periodontal diseases by developing amitriptyline oral gel and mouthwash in 1% formulation and to estimate the clinical and biochemical variables before and after scaling and root planning in selected patients of gingivitis and periodontitis.

MATERIALS AND METHODS

This experimental, observational and randomized clinical control trial study was done in the department of Pharmacology, University of Karachi and HEJ Research Institute of Chemistry, University of Karachi during December, 2017 to May 2018. 30 patients were selected for the study. A consent form was signed by each patient. After the consent, history of the patients was taken.

Ethical approval

All methods used in this study involving human subjects were according to Helsinki declaration (2000). The study was approved from the Board of Advanced Studies and Research committee of Karachi University and the Independent Ethics Committee of International Center for Chemical and Biological sciences ICCBS/IEC-029-HS-2017/Protocol/1.0.

Formulation of 1% amitriptyline intra-crevicular gel and mouthwash

Amitriptyline hydrochloride tablets 25mg (Obs Pharma) were bought from the pharmacy and the other chemicals were gifted from Nigheeban Pharmacy, Karachi. The gel was prepared from 25 mg amitriptyline tablets dissolved in distilled water. The dissolved preparation was mixed in 2% hydroxyethylcellulose gel (Cizmarik *et al.,* 2014) having propyl paraben sodium, methyl paraben sodium, and ethylenediaminetetraacetic acid (Zabrzewska *et al.,* 2014). The preparation was stirred properly to avoid lump formation. Final pH was adjusted by adding triethanolamine in the preparation (Abrar *et al.,* 2012).

1% mouthwash was formulated by using 25 mg amitriptyline tablets dissolved in distilled water. Sodium benzoate (preservative), glycerin (sweetener), food dye color, flavor and triethanolamine (pH adjustment) were added in the formulation (Mariappa *et al.,* 2015).

The characteristics of the gel and mouthwash were assessed before its application into the periodontal pocket. Total microbial count in the preparation was also evaluated to make sure that no contaminating microorganism was found in the gel and mouthwash.

Inclusion criteria

- Patients with no past history of any major disease

Exclusion criteria

- Patients received any periodontal treatment in the last two years
- Patients taking any drug treatment since last six months
- Pregnant and lactating females

The patients were grouped in three categories

1. Patients received only scaling and root planning (standard periodontal treatment)
2. Patients received standard periodontal treatment followed by the application of gel in periodontal pockets. The gel was applied in the periodontal pockets by using 25 gauge needle one day after the treatment for four weeks
3. Patients received standard periodontal treatment followed by the application of mouthwash for four weeks

   The following periodontal parameters were measured and recorded on dental chair by using CPITN probe.

Probing depth

Six teeth were assessed and probed for six sites. The probe was inserted in the pocket following tooth length till resistance was felt (Funosas *et al.,* 2009; Varghese *et al.,* 2014).

Attachment level

Six teeth were assessed and probed for six sites. The clinical attachment level was then calculated according to the standard criteria (Funosas *et al.,* 2009; Varghese *et al.,* 2014).

Tooth mobility

Tooth mobility was assessed, which occurs due to loss of bone that supports the teeth. When periodontal tissues are inflamed the traumatic occlusion of teeth leads to severe bone loss (Bisson *et al.,* 2018).

Plaque index

Plaque index was assessed according to Silness and Loe criteria (Obulareddy *et al.,* 2018).

Gingival index

Gingival index was assessed according to Loe and Silness criteria (Morgan *et al.,* 2018).

Bleeding on probing

It was assessed by gently moving a blunt probe in the periodontal pocket (Obulareddy *et al.,* 2018).

Saliva sample collection and estimation of TNF-α, PGE_2 and NO

The sample collection in vials was done around 11-12 am according to the standard protocol (Henson and Wong 2010). Unstimulated saliva samples were collected, centrifuged and refrigerated. The samples were assessed for TNF-α (Invitrogen, California), prostaglandin E_2 and nitric oxide (Glory science company, Ltd, USA) using commercially available ELISA kits. The levels of TNF-α, of PGE_2 and NO were measured using the sandwich technique as per manufacturer instructions against their specific antibodies. The sample absorbance concentration curve was observed by plotting it on the standard curve of TNF-α (pg/ml), PGE_2 (pg/ml) and NO (μmol/L) against their respective absorbance (Eivazi *et al*., 2017; Gumus *et al.,* 2017; Poorsattar *et al.,* 2014).

Table 1: Measurement of PPD, AL and BOP

Groups	PPD		AL		BOP	
	Before treatment (n=10)	After 30 days of treatment (n=10)	Before treatment (n=10)	After 30 days of treatment (n=10)	Before treatment (n=10)	After 30 days of treatment (n=10)
Conventional Periodontal treatment	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% Amitriptyline gel	3.31±0.20	1.27±0.19***	3.54±0.20	1.40±0.19***	0.88±0.25	0.00±0.00***
1% Amitriptyline mouthwash	3.37±0.16	1.33±0.20***	3.44±0.24	1.44±0.22***	0.92±0.18	0.00±0.00***

Mean ± SD. *** ($p \leq 0.001$) = significant with standard treatment.
PPD= Periodontal Pocket Depth; AL= Attachment level BOP= Bleeding on Probing

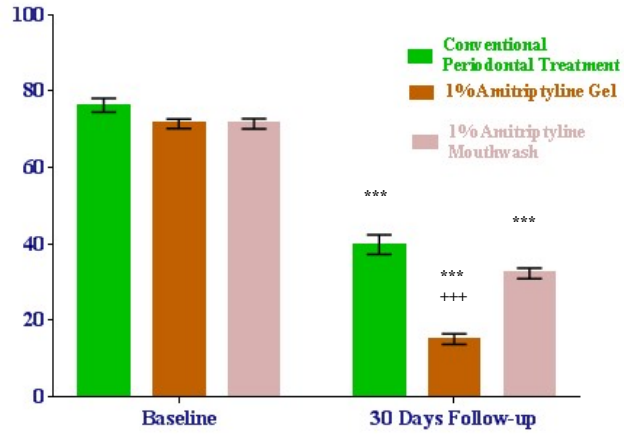
Table 2: Measurement of TM, PI and GI

Groups	T M		PI		GI	
	Before treatment (n=10)	After 30 days of treatment (n=10)	Before treatment (n=10)	After 30 days of treatment (n=10)	Before treatment (n=10)	After 30 days of treatment (n=10)
Conventional Periodontal treatment	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% Amitriptyline gel	0.80±0.42	0.40±0.52	2.55±0.35	0.40±0.52***	2.32±0.51	0.36±0.33***
1% Amitriptyline mouthwash	0.70±0.48	0.50±0.53	2.48±0.41	0.59±0.49***	2.45±0.32	0.42±0.28***

Mean ± SD. *** ($p \leq 0.001$) = significant with standard treatment.
PI= Plaque Index; GI= Gingival Index; TM= Tooth mobility

STATISTICAL ANALYSIS

Data analysis was done by SPSS version 21 (IBM). Comparative analysis of clinical parameters and biochemical variables was done by one way analysis of variance (ANOVA) among treatment groups and standard conventional treatment group. Comparison among both groups was done by Bonferroni test considering p-value ≤ 0.05 significant.

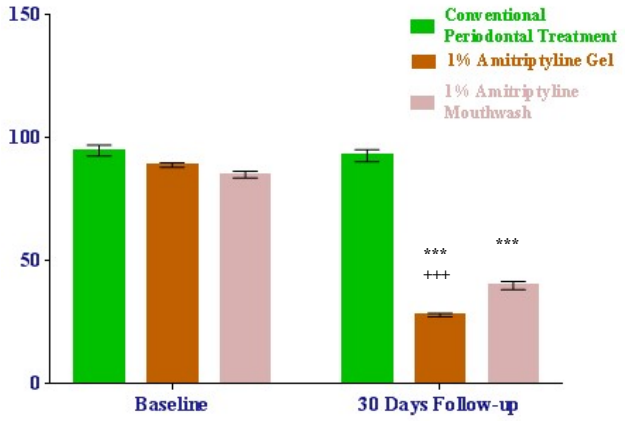


Mean ± SD. *** ($p \leq 0.001$) = significant with standard treatment.
+++$p \leq 0.001$= significant in comparison of amitriptyline gel versus amitriptyline mouthwash.
TNF-α= Tumor necrosis factor alpha

Fig. 1: Measurement of TNF-α (pg/ml)

RESULTS

Significant reduction was observed in pocket depth, attachment level, bleeding, plaque and gingival index ($p \leq 0.001$) by gel and mouthwash preparation as compared to standard periodontal treatment. (table 1 and table 2).

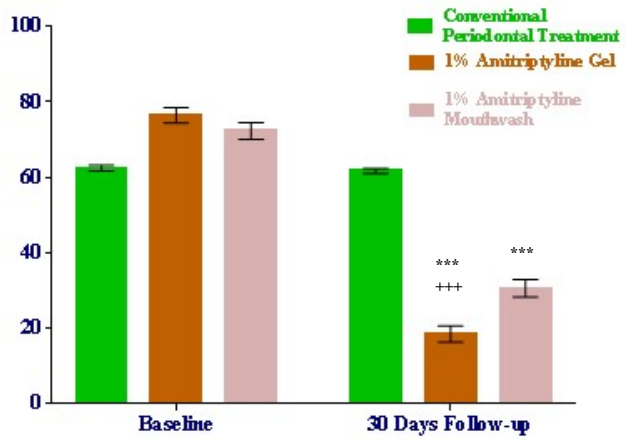


Mean ± SD. *** ($p \leq 0.001$) = significant with standard treatment.
+++$p \leq 0.001$= significant in comparison of amitriptyline gel versus amitriptyline mouthwash.
PGE_2= Prostaglandin E_2

Fig. 2: Measurement of PGE_2 (pg/ml)

The levels of TNF-α, PGE_2 and nitric oxide ($p \leq 0.001$) were highly reduced with the gel and mouthwash as compared to standard periodontal treatment. Gel is found to be more efficacious ($p \leq 0.001$) in reducing the

inflammatory mediators level as compared to mouthwash (figs. 1, 2 & 3).



Mean ± SD. *** ($p \leq 0.001$) = significant with standard treatment.
+++ $p \leq 0.001$= significant in comparison of amitriptyline gel versus amitriptyline mouthwash
NO=Nitric oxide

Fig. 3: Measurement of NO (µmol/ml)

DISCUSSION

Maintenance of oral hygiene is important for dental and periodontal health (Cascaes *et al.,* 2014). Poor oral hygiene is accountable for plaque formation and rapid growth of anaerobic bacteria which is responsible for the development of the two most important inflammatory conditions, gingivitis and periodontitis in oral cavity (Hasan and Palmer, 2014). The collagen embracing the periodontium is destroyed which leads to the migration and resorption of alveolar bone. The gingival epithelium is migrated from the side of tooth surface and forms a "pocket" which provides an excellent location for the replication of gram negative and facultative anaerobes. Invasion and activation of neutrophils by the anaerobes cause increased production of IL-1β, IL-6, TNF-α and reactive oxygen species superoxide. Increased production of these mediators is responsible for the damage of periodontal ligaments, loss of alveolar bone with loss of tooth (Kaur *et al.,* 2012; Escobar *et al.,* 2018).

There are different treatment protocols through which these conditions are treated. Dentists usually prescribe antibiotics and anti-inflammatory agents to treat gingivitis and periodontitis (Azodo and Ojehanon, 2014; Nagi *et al.,* 2015). Surgical treatments are also used to manage these conditions including pocket reduction surgery, soft tissue grafts, bone grafts and bone surgery (Ogihara and Tarnow, 2014) but it requires time, compliance of patient and expertise of periodontologist. Cost is also another important factor associated with people of under-developed countries.

A good option to treat gingivitis and periodontitis without complicated surgeries is supra and sub gingival scaling and root planning with the application of gel in the inflamed pockets or the use of anti-inflammatory mouthwashes during these procedures (Rao *et al.,* 2013).

Different studies on animals found out that amitriptyline decreases inflammation by lowering the level of the IL-6 and TNF-α in forelimb flexor of rats (Manning *et al.,* 2014). The role of heterocyclic antidepressants as an anti-inflammatory agent was also observed. It was found out that these effects were due to neutrophil migration and mast cell stabilization (Gurgel *et al.,* 2013). Reduced bone loss and destruction of collagen in periodontal disease was also observed by using fluoxetine during the treatment of chronic stress in rats (Aguiar *et al.,* 2013).

In this study, our prepared gel and mouthwash of amitriptyline reduced both the clinical parameters and inflammatory biomarkers in the human saliva of the patients received the treatment. These effects were due to the drop in the level of PGE_2 and TNF-α at the periphery. This peripheral drop resulted by the decrease in norepinephrine and serotonin which are hyperalgesic (Verdu *et al.,* 2008). Nitric oxide is an important inflammatory biomarker in periodontal diseases and there is an imbalance in its level during periodontal inflammation (Gupta *et al.,* 2015). Our studies showed that both the gel and mouthwash significantly reduced the nitric oxide level which amplifies the use of amitriptyline in reducing periodontal inflammation.

In this study both gel and mouthwash of amitriptyline significantly reduced the clinical and biochemical variables but the gel is found to be more effective than the mouthwash. In gingivitis with severe bleeding where immediate reduction in bleeding is required, mouthwash is highly effective and its use would be preferred by the dentist. In chronic periodontitis, there in increase pocket depth and mobility, the gel would be preferred because of its mucoadhesive nature and viscosity and its ability to retain in the gingival pockets for long period of time without affecting its efficacy (Tiwari *et al.,* 2010).

Periodontal diseases are hard to treat and to achieve adequate results systemic drugs have to be given for longer periods (Shiloah *et al.,* 2014). This may results in adverse effects. The outcome of our study is that the prepared 1% formulations of amitriptyline showed full efficacy when applied locally to the diseased area which proves that the desire results can be achieved in short time period with no side effects.

CONCLUSION

The use of amitriptyline in the treatment of depression, severe pain associated with chronic migraine, tension headache, fibromyalgia, neuropathic pain and cancer pain

is well established but this study suggests that amitriptyline in local formulations is safe to treat gingivitis and periodontitis without systemic adverse effects. The reduction in the clinical parameters and biochemical variables showed that these formulations retained their efficacy during the treatment. Our study is pilot with limited resources and time. Therefore, in accordance to our results we strongly recommend that further studies are also required to observe the effects of amitriptyline in patients with periodontal diseases in large groups for longer period of time by preparing its gel and mouthwash in different concentrations.

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