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Original Research Article

Azhitromycin and periodontal disease: Effect on clinical parameters, P.gingivalis and reactive oxygen species

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ABSTRACT

The current study was aimed at estimating the effect of azithromycin as an adjunct on clinical parameters, microbiological and biochemical parameters in chronic periodontitis subjects. Clinical parameters,namely gingival index, papillary bleeding index, probing depth, clinical attachment level, collection of subgingival plaque for P.gingivalis assessment and gingival crevicular fluid for estimation of reactive oxygen metabolites was done at baseline and 1st month in both the test subjects(30) receiving scaling and root planning along with azithromycin and the control subjects(30) who received scaling and root planning with placebo. The bleeding index and gingival index showed significant difference between the groups at the end of 1st month, whereas probing depth and clinical attachment level did not. Reactive oxygen metabolites levels reduced in both the groups and were significant on intergroup comparison at the end of one month. The mean percentage decrease in P.gingivalis levels was more in test group at the end of one month and was statistically significant. In addition to non surgical treatment, systemic administration of azithromycin seems to be beneficial in the management of chronic periodontitis.

1. Introduction

Periodontal disease, one of the most common and prevalent oral disease that results from the interplay between the periodontal pathogens and the host. Over the decades, the etiology and pathogenesis has evolved and now dysbiotic periodontal environment seems to set the stage towards periodontal disease with Porphyromonas.gingivalis emerging as the “key stone” pathogen.[1]

Once initiated, the host immune system modulates the disease progression, with the immune cells spewing a pleothora of inflammatory mediators and enzymes, leading to collateral damage, evident as attachment and bone loss. The periodontal milieu which was in a state of oxidative eustress is thrown in to distress with neutrophils and macrophages producing reactive oxygen species (ROS) via the respiratory burst mechanism as the part of the defense response to infection. ROS, basically beneficial have harmful effects when produced in excess and has been well substantiated in literature.[2] Measurement of free radicals and other ROS is are difficult owing to their shorter lifespan and biochemical instability, but byproducts like reactive oxygen metabolites (ROM) can be estimated.[3]

As the disease etiology and pathogenesis has changed over time, newer and adjunctive treatment modalities have also evolved, though nonsurgical and surgical periodontal therapy still remains the mainstay treatment.[4] However, the use of antimicrobials, either systemic antibiotics or local drug delivery agents as adjuncts to the nonsurgical/surgical periodontal therapy is justified but will not be effective if used as a monotherapy alone. Adjunctive antibiotics have been shown to aid treatment outcomes in patients with severe chronic periodontitis and aggressive periodontitis.[5]

In the recent times, Azithromycin (AZM), has gained popularity in the periodontal scenario because of its twin action, namely antimicrobial and anti-inflammatory. It

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affects the degranulation of neutrophils, and release of proinflammatory mediators.[6] it is patient friendly because of its one dose per day regimen, pharmacologic action and prolonged duration of action thus making it very suitable as an adjunctive antiobiotic in the management of periodontal disease. Literature on chronic periodontitis (CP) have shown it to be effective.[7] Buket Han et al 2012,[8] demonstrated that adjunctive azithromycin in combination with SRP therapy had no additional effect compared with non-surgical therapy alone on the periodontal pathogens other than F. nucleatum, and on levels of gingival crevicular fluid (GCF) matrix metalloproteinase 8(MMP-8) in patients with generalized severe chronic periodontitis.

Therefore, this study was undertaken to see the impact of azithromycin in combination with non-surgical periodontal therapy on clinical parameters like probing depth(PD), clinical attachment level(CAL), microbiologic parameter, namely P.gingivalis and oxidative stress biomarker, namely GCF ROM levels in subjects with CP over a 1-month period.

2. Materials and Methods

This clinical study was designed as a single centered, placebo-controlled, parallel group randomised control study of one month duration. The study population consisted of 60 subjects with generalized CP (35 to 65 years of age) belonging to both sexes and all subjects were randomly selected from the outpatient clinic of the department of periodontics. Subjects were divided into two groups of 30 subjects each as with Group I – control group (who received SRP plus a placebo) Group II – test group (who received SRP plus adjunctive azithromycin). Before participation, the study protocol was explained to all subjects and they gave written informed consent. Ethical committee approval was procured from the university.

Subjects diagnosed with severe generalized CP in the presence of the following criteria namely – minimum of 4 teeth with one or more sites exhibiting probing depth $\geq$ 4mm, clinical attachment level $\geq$2 mm, and radiographic evidence of bone loss were included in the study. The exclusion criteria were subjects with $\leq$15 teeth, current or previous smokers, pregnant or lactating women, systemic disorders like diabetes mellitus, immunologic disorders, history of any other systemic disease, hypersensitivity to any type of macrolide and patients on antibiotics or other medications and undergone periodontal treatment with in the past three months.

All data were recorded in a standard proforma. Oral examination was carried out with proper illumination using mouth mirror and graduated Williams's periodontal probe. The following indices and clinical parameters were evaluated for the subjects, namely gingival index (GI), papillary bleeding index (BI), probing depth (PD) and clinical attachment level (CAL)

At baseline, the indices and clinical parameters were recorded for all subjects. GCF and microbiologic samples were collected for assessment of ROM and microbiologic levels of P. gingivalis. The site with greatest probing depth was selected for GCF collection. After drying the area, supra-gingival plaque was removed and a standardized volume of 1μl was collected from each site with an extra-crevicular approach, using volumetric capillary pipettes. The collected GCF was transferred immediately to ependorff tubes and stored at -70°c until the time of assay. For collection of sub-gingival plaque, the site with greatest probing depth was selected and supragingival plaque was removed. Gracey curette Nr. 5/6 (Hu-Friedy, Chicago, USA) was inserted as deep as possible into the pocket till tissue resistance was felt and the plaque was removed with a single upward pull stroke. The samples were transferred in to a sterile container and 1 ml phosphate buffer saline was added into the container as transportation media and was transported to the lab for microbial assessment. For GCF collection, after drying the area with a blast of air, supra-gingival plaque was and the GCF was collected with an extra-crevicular approach, using volumetric capillary pipettes that were calibrated from 1-5μl. The collected GCF was transferred immediately to ependorff tubes and stored at -70°c until the time of assay.

In Group I &II subjects, non-surgical periodontal treatment (scaling and root planing) was completed in two visits within 24 hours using hand instruments (Gracey curettes).On completion of scaling and root planing, Group II subjects were administered azithromycin tablets (500mg), once daily for three consecutive days, whereas Group I received placebo for three days. Clinical data, GCF and microbiologic samples were re-evaluated at 1stmonth after therapy in Group I and Group II subjects to assess the changes. P. gingivalis was identified and assessed using PCR in both control and test groups at baseline and 1st month after therapy. Estimation of ROM was done according to methods described by other studies.[9]

2.1. Statistics

The data collected for various clinical parameters such as gingival index, bleeding index, probing depth and clinical attachment level were assessed at baseline and at 1st month in Group I and Group II patients. The data was analysed statistically to find the mean, standard deviation and test of significance of mean values for the various parameters between the groups. Independent sample t-test was used to compare mean values between test and control groups. Paired sample t-test used to compare mean values between baseline and one month post-operatively. Significance level was fixed at 0.05.

Table 1: Comparison of clinical parameters in group I and group II from baseline and one month

		Mean ±Std. dev	P-Value
GI	Baseline-1st month(Group –I)	2.60±0.507 1.13±0.834	<0.001
	Baseline-1st month(Group –II)	2.67±0.488 0.27±0.458	<0.001
BI	Baseline-1st month(Group –I)	2.80±0.414 0.87±0.640	<0.001
	Baseline-1st month(Group –II)	2.87±0.352 0.13±0.352	<0.001
PD	Baseline-1st month(Group –I)	4.80±0.676 3.27±0.799	0.001
	Baseline-1st month(Group –II)	5.00±0.655 3.13±0.743	0.001
CAL	Baseline-1st month(Group –I)	2.87±0.640 2.80±0.775	1.000
	Baseline-1st month(Group –II)	2.87±0.743 2.53±0.516	<0.05

GI – Gingival Index
PD- Probing depth
CAL – Clinical attachment level
P – Significance

Table 2: Comparison of clinical parameters between group I and group II at baseline & 1 month

Group I/II	Mean ± Std. dev	P-Value
GI(Baseline)	2.60±0.507 2.67±0.488	0.710
GI(1st month)	1.13±0.834 0.27±0.458	0.004
BI(Base line)	2.80±0.414 2.87±0.352	0.630
BI(1st month)	0.87±0.640 0.13±0.352	0.001
PD(Base line)	4.80±0.676 5.00±0.655	0.402
PD(1st month)	3.27±0.799 3.13±0.743	0.593
CAL(Base line)	2.87±0.640 2.80±0.775	0.733
CAL(1st month)	2.87±0.743 2.53±0.516	0.214

GI – Gingival Index
BI – Papillary bleeding index
PD- Probing depth
CAL – Clinical attachment level
P – Significance

Table 3: Comparison of mean reactive oxygen metabolites (rom) levels between group I and group II

ROM level (CARRU)	Group	N	Mean	Std. Dev	t-Value	P-Value
Baseline	Group -I Group -II	30	457.13 456.83	66.75 46.59	0.014	0.989
1st month	Group -I Group -II	30	347.07 259.12	78.23 27.32	4.110	< 0.001

ROM - Reactive oxygen metabolites
t – t statistic
P – Significance

Table 4: Comparison of p. gingivalis levels between group I and group II

Group I/II	Mean ±Stddev	P-Value
Pg(Baseline)	13.24±0.126 13.25±0.093	1.000
Pg(1st month)	18.39±0.383 24.05±0.802	<0.001
Pg(% decrease)	38.85±0.764 57.61±0.318	<0.001

Pg– P. gingivalis
P – Significance

3. Results and Discussion

Results in the present study showed that all the clinical parameters showed improvement at the end of one month in both Group I and Group II, and was significant. However, for CAL, significance was seen only in test group at the end of one month whereas control group did not as seen in Table 1.

On comparing all the clinical parameters between test and control groups, the mean gingival index, papillary bleeding index, probing depth and CAL, significant difference was seen at end of one month only for GI & BI and it was nonsignificant for PD and CAL, as depicted in Table 2.

GCF ROM levels of control group at baseline were (457.13± 66.75CARRU) and at 1st month were (347.07±78.23CARRU) and of test group at baseline was (456.83±46.59CARRU) and at first month (259.12± 27.32CAARU). The intergroup comparison (Table 3) showed significance (<0.001) at end of one month. The mean CT (Threshold cycles) value of P. gingivalis of control group at baseline was (13.24±0.126) and of test group at baseline was (13.25±0.093) as seen in Table 4. There was decrease in P.gingivalis level in both the groups and was more in test compared to the control group (mean value inversely propotional to CT). The mean % decrease was also significant ((<0.001).

In our study, the severity of the periodontitis was similar in both control and test groups at the beginning and was nonsignificant (Table 1). The significant improvements in clinical parameters at the end of one month in gingival index and bleeding index could be attributable to the SRP. SRP therapy induces the resolution of the inflammatory response and cessation of the progression of periodontal disease, and thereby results in a reduction of PD. Our results are in accordance with Buket Han et al 2012, who reported a reduction in probing pocket depth and bleeding on probing in both the test and control groups attributed it to the fact that CP patients had markedly inflamed gingiva in addition to periodontal breakdown. Therefore, clinical improvement occurring because of gingival shrinkage tended to be greater after SRP, which resulted in decrease in gingival and bleeding indices 1 month later. Latif SA et al 2016 [10] showed a statistically significant reduction in gingival inflammation and gingival bleeding scores in AZM+ SRP group. They also supported the adjunctive use of AZM tablet (500mg). Significant reduction of gingival index in azhithromycin (Group II) compared to Group I is similar to the results obtained by VidyaDodwad et al 2012, [11] where a reduction in gingival index was seen following subgingivally delivered 0.5% controlled release azithromycin gel. Our results are also at par with Smith SR et al 2002 [12], in which the bleeding index of both the groups were significantly decreased when compared with baseline values and additionally the BI of AZM group was significantly lower than the control group , which is observed in our study. As it is well known, the application of AZM can be effective since high concentrations of this drug have been identified in the inflamed tissue, [13] another reason for the better gingival and bleeding indices score recorded in the AZM group. Though mean reduction in the probing depth from baseline to end of one month was observed in both the groups, it was more in AZM group than in Group I and inter group significance not evident in both the groups between baseline and one month. Mascarenhas P et al 2005 [14] has also reported mean per patient average reduction in PPD from baseline and has stated few reasons which might have led to this namely, good compliance with AZM therapy, faster wound healing due to quickened decrease in bacteria at the area of infection, reduction in cross infection potential, high concentration of the drug in the inflammatory cells and lastly the low bacterial resistance to AZM. When analysing CAL, nonsignificance was seen between Group I and II at one month duration and both Group I and II did not show significance between baseline and one month interval. Our results are in par with that of Latif SA et al 2016 [10] who did not observe any significant improvement in CAL in the AZM+SRP group. Literature shows that gain in attachment levels were seen as a result of SRP therapy in sites with initial PD >6 mm. [15] Similarly Mascarenhas P et al 2005 [14] has shown the same CAL gain trend for deeper PD sites, where CAL changes were statistically significant for both groups when compared to baseline. In the present study subjects were of moderate periodontitis cases with shallow to moderate pockets, probably no significant gain in the CAL was observed.

Significant mean reduction was seen in P.gingivalis levels at end of one month, and was more in azithromycin group and was statistically significant between the groups. Oteo A. et al 2010,[16] reported that the prevalence of P.gingivalis decreased significantly after one and six months and non-significant reduction was seen in the placebo group. Buket Han et al 2012[8] has shown that P.gingivalis levels significantly decreased at 2 weeks, 1 month, and 6 months compared with the baseline levels in both groups.

GCF ROM levels decreased significantly in both the groups at the end of one month and alsoon intergroup comparison, significance (<0.001) was noted. ROM is a good indicator of ROS. SohiniChaudhary et al 2014[17] have shown decrease in plasma ROM levels at 1^{st}, 2^{nd} months after nonsurgical treatment. Therefore, it is reasonable to extrapolate the same findings to GCF ROM, which has shown a reduction in both the groups following SRP. However, the significant reduction in Group II compared to Group I may be assigned to the effect of AZM on respiratory bursts of neutrophils, by being highly concentrated at the inflamed site. It has been demonstrated that 0.5μg/ml of AZM reduced active oxygen generation by neutrophils.[18]

It has been recommended that systemic antibiotics have to be given immediately after completion of SRP for it to be effective once the subgingival biofilm is cleared.[19] Till date, there is no clearcut guideline about the correct time for prescribing antibiotic as an adjunct. Recently, it has been recommended for nonsurgical therapy to be complete within a short time-period and for the antibiotic intake to be started on the day of treatment completion[5] and a similar protocol was followed in this study.

It is clear that azithromycin has attributes that makes it ideal for the treatment of periodontitis. The MIC (Minimal inhibitory concentration) of azithromycin for these periodontopathogens is maintained in the gingiva for a period of 7–14 days.[20] Patient compliance is a major plus point – it is administered in one dose of 500 mg every 24 h for only 3 consecutive days, compared to tetracycline that has to be administered for a period of 14–21 days, and other agents that are prescribed for 7–10 days. One limitation of the study that has to be acknowledged is the sample size and also the changes in the GCF ROM level associated with generic factors such as oral hygiene. The present study indicates that using azithromycin along with SRP shows better clinical results than non-surgical therapy alone as evident on bleeding index, gingival index and probing depth, levels of P. gingivalis and on GCF ROM levels in patients with periodontitis. Therefore, within reason, prescription of AZM in the management of periodontal disease is beneficial. However further long term studies with a larger sample size are required to shed more light on this observed benefit.

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5. Source of Funding

None.

6. Conflicts of interest

The authors declare no conflicts of interest in the present study

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