Original article:

0.5% azithromycin gel as local drug delivery system in   management of chronic periodontitis

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ABSTRACT:

Introduction-Recent role of chemotherapeutic agents in form of local delivery system as an adjunct to mechanical therapy suggest improvement in periodontal health. The aim of present study was to evaluate clinical and microbiological effectiveness of locally delivered 0.5% azithromycin gel as an adjunct to scaling and root planing in managing chronic periodontitis patient.

Material and Methods-This was randomized control trial using split mouth study design. A total of 40 sites from 20 patients, who had pocket depth more than 5 mm and diagnosed as chronic periodontitis, were selected for the study. These were randomly categorized in two treatment groups: scaling and root planning only (control group) and scaling and root planning plus 0.5% azithromycin gel (test group). Clinical parameters such as gingival index, probing pocket depth and clinical attachment level were recorded at baseline, 6 weeks and 12 weeks at selected sites for both the groups.

Result & observations-Overall parameters improved from baseline in both test and control group. Mean pocket depth reduction from baseline to 12 weeks was 4.1500±0.6708 and 4.350±0.489 in test and control respectively, the difference being statistically significant at p<0.001.Mean clinical attachment level gain from baseline to 12 weeks, the difference being statistically significant at p<0.05. All microbiologic categories showed significant improvement in both groups, with greater improvement in the test group.

Conclusion Based on the results it was concluded that locally delivered Azithromycin might be a valuable adjunct to scaling and root planing in the treatment of chronic periodontitis.

Key words: 0.5% Azithromycin gel, local drug delivery system, periodontitis

INTRODUCTION

Periodontal disease is considered infection of periodontium as it involves the bacterial aetiology, inflammatory process and breakdown of periodontal structures[1]. The aetiology of periodontitis is multi-factorial, but it is an infection and caused by specific group of organisms that colonize tooth surface in form of biofilm called plaque which consist of microorganisms embedded in gelatinous matrix. This matrix protects microbes by inhibiting penetration of harmful chemicals and antibiotics in biofilm[2]. Greater emphasis has been placed on non-surgical approaches to periodontal therapy. Non-surgical therapy used to delay repopulation of pathogenic microorganism by controlling the bacterial plaque, by disrupting or removing the sub gingival gram-negative flora. But mechanical debridement may have certain limitations which include difficult to reach areas like root concavities, root furcations and extra

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dental sites which serve as reservoir of bacteria even after instrumentation. The failure of mechanical debridement to completely eradicate the putative pathogenic organism provides the need for an adjunct antimicrobial agent to suppress the bacterial load of inflammatory periodontal disease[3]. Putative pathogens associated with periodontal diseases are susceptible to variety of antiseptics and antibiotics[4, 5.] The variety of topical and systemic agent have been used which can block the pathway and progression of periodontal disease[4]. But systemic antibiotic therapy has certain disadvantages such as inability to achieve high GCF concentration, increased risk of adverse drug reaction, increased selection or multiple antibiotic resistant microorganisms, and uncertain patient compliance[6]. To overcome these shortcomings of antibiotic therapy, local drug delivery system was developed.

Goodson, et al[7] in 1979 first proposed the concept of controlled delivery in the treatment of periodontitis. Local drug delivery allows the use of concentration of approximately 100 times higher than does systemic administration. Site-specific, controlled release delivery systems have allowed us to administrate therapeutic levels of drugs on the site of infection for prolonged period of time[8].

Macrolides antibiotics are a class that has been screened and found to be affective against periodontitis, it has been suggested that macrolides act as anti-inflammatory agents as well as antimicrobial agents[9]. Azithromycin is the first subclass of macrolides called azalides. It shows good bacteriostatic in vitro activity against a wide variety of organisms found in mouth[10]. Azithromycin has a wide antimicrobial spectrum of action toward anaerobic bacteria as well as Gram-negative bacilli.[11]. It is effective against periodontal pathogens like Aggregatibacter actinomyce-temcomitans (previously Actinobacillus actinomy-cetemcomitans) and Porphyromonas gingivalis[12-14] and this antimicrobial activity supports its use in the treatment of periodontal infections. Azithromycin has significantly less bacterial resistance to the subgingival microflora of chronic periodontitis compared to other commonly prescribed antibiotics[15].Azithromycin also has a long half-life and good tissue penetration[9] .It is characterized by its significantly higher uptake by fibroblasts and acute-phase reactant cells, such as polymorph-honeutrophils, monocytes, and lympho-cytes.[16,17]. The drug is subsequently delivered and released in higher concentrations to phagocytised bacteria at the site of infection.[18] A few studies have examined the adjunctive use of systemic Azithromycin the treatment of periodontitis and demonstrated improvement in clinical parameters. **Gomi et al**, 2007 [19] who used systemic administration of Azithromycin in conjunction with scaling and root planning demonstrate reduction in probing depth. **Smith et al.**[20] compared the clinical effects of Scaling and root planing plus placebo to Scaling and root planing plus systemic Azithromycin ;Scaling and root planing with the adjunctive use of Azithromycin was better at improving clinical parameters such as pocket depth and bleeding on probing (BOP). Due to these advantages of Azithromycin,, in the present study an attempt was made to evaluate its efficacy as an adjunct to scaling and root planning in treatment of patients with chronic periodontitis. The purpose of this study was to evaluate the efficacy of locally delivered 0.5% Azithromycin gel as an adjunct to scaling and root planing , with scaling and root planing alone in treatment of patients with chronic periodontitis.

MATERIALS AND METHOD

PARTICIPANTS

The patients for this study were selected from the outpatient section, Department of

Periodontia, Government Dental College and Hospital, Ahmedabad, India. Twenty patient, aged 25-55 yrs suffering from chronic periodontitis with probing pocket depth more than 5mm , radiographic evidence of bone loss and undergone no antibiotic therapy or periodontal therapy in the preceding 6 months were included .Patients with known or suspected allergy to the macrolide group, smokers, alcoholics, and pregnant or lactating females were excluded. A total of 40 sites from 20 patients were selected for the study. The duration of the study was for 3 months. Two sites were identified for the study in each patient. One site served as control site and the other site on the contralateral quadrant served as the test site.

The nature and design of the clinical trial was explained to the patients and consent was obtained for their participation in prescribed Performa. On screening day, patient evaluation was followed by impressions for fabrication of acrylic stents to standardize the point of entry to the periodontal pocket during scheduled appointments as seen in Figure I &II

Preparation of 0.5% Azithromycin gel:

The formulation was done in The National Institute of Pharmaceutical Education and Research (NIPER) Ahmadabad. The following gel is prepared under strictly aseptic conditions:(% w/w)

Azithromycin Dihydrate- 3.50

Chlorbutol BP- 0.50

Carbopol® 934P 2.50

NaOH (4% w/v solution) - 6.50

Water-S 87

Azithromycin dihydrate is dispersed in the sterile unneutralised Carbopolin water containing chlorbutol BP in solution. A sterile 4% w/v sodium hydroxide solution is then added with constant mixing to a final pH of 7.The prepared gels were packed in vials.

Estimation of Quantity of 0.5% AZM

The drug estimation was done by gradient high pressure liquid chromatography (HPLC), a variable wavelength programmable ultraviolet/visible spectroscopy (UV/VIS) detector, and a system controller; operating software was used for the analysis.

Intervention

Firstly, patients received full mouth scaling and root planing and were recalled after a week when baseline parameters were recorded. The test site received 0.2 ml of 0.5% azithromycin gel using a blunt cannula and no periodontal dressing was given after applying the drug as seen in figure V. Control sites received only scaling and root planing . After the insertion of local drug delivery system, patients were instructed not to floss or probe at the treated site. They were also advised to avoid the use of mouthwash throughout the study period.

Outcomes

Clinical parameters evaluated were gingival index, probing depth using UNC-15 graduated periodontal probe and clinical attachment level. Readings were taken at baseline.6 weeks and 12 weeks (Figure III & IV). Apart from clinical parameters, microbiological evaluation of subgingival plaque samples also done at baseline and 6 weeks. The supragingival plaque was removed to prevent contamination of the flora, then the area was isolated, and subgingival plaque was collected using sterilized Gracey curette by inserting it subgingivally into the deepest portion of the periodontal pocket parallel to the long axis of the tooth and moved coronally by scraping along the root surface as seen in Figure VI.The samples were then transferred to a bottle containing thioglycollate transport media. The procedure was again repeated after six week. The collected samples were taken to Microbiology laboratory for culturing. The samples were emulsified and inoculated into blood agar

plates. The plates were placed in the Dynax anaeroble jar. The anaerobic jar was kept in the incubator at 37 degree Centigrade for 48 hours. After 48 hours the anaerobic jar was opened and microbial colonies on the blood agar plates were identified. The colonies were identified based on colony morphology and Gram staining. Growth in each quadrant was given the scores in CFU/ml

➢ < 10,000 CFU/ml - three primary streaks in one quadrant

➢ 25 - 50,000 CFU/ml - growth in one complete quadrant

➢ 50 - 75,000 CFU/ml - growth in two complete quadrants

➢ 75 - 1, 00,000 CFU/ml - growth in three complete quadrants

➢ 1, 00,000 CPU/ml - growth in four complete quadrants.

TABLE I: CLINICAL PARAMETERS AT BASELINE (MEAN± SD) FOR ALL PATIENTS.

GROUP	GINGIVAL INDEX	POCKET PROBING DEPTH	CLINICAL ATTACHMENT LEVEL
CONTROL	2.517±0.18	6.350±0.745	5.650±0.587
TEST	2.500±0.1662	6.800±0.69	5.900±0.718
P-VALUE	0.76	0.56	0.23

TABLE II: CLINICAL PARAMETERS OF CONTROL AND TEST AT BASELINE,6 WEEKS AND 12 WEEKS.

	GROUP	MEAN±STD BASELINE	MEAN±STD (6 WEEK)	MEAN±STD (12 WEEK)	t – VALUE	P-VALUE
GINGIVAL INDEX	CONTROL	2.517±0.1829	1.485±1.880	1.1185±0.1008	0.308	<0.05
	TEST	2.500±0.1662	1.436±0.1993	1.064±0.7632	1.927	<0.001
PROBING POCKET DEPTH	CONTROL	6.3500±0.7451	5.000±0.7632	4.350±0.489	1.947	<0.05
	TEST	6.800±0.6958	4.400±0.7539	4.1500±0.6708	1.077	<0.001
CLINICAL ATTACHME-NT LEVEL	CONTROL	5.6550±0.5871	4.4500±0.6048	3.800±0.5231	1.205	<0.05
	TEST	5.9000±0.7181	4.3000±0.47016	3.750±0.6386	0.271	<0.05

TABLE III : COMPARISONS OF MEAN CHANGES FROM BASELINE TO 6 WEEKS IN COCCI, RODS, SPIROCHETES AND OTHERS (STRAIGHT RODS, FILAMENTS)

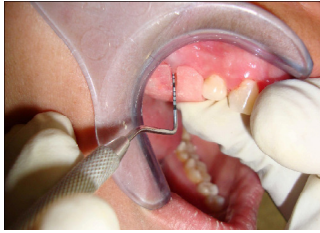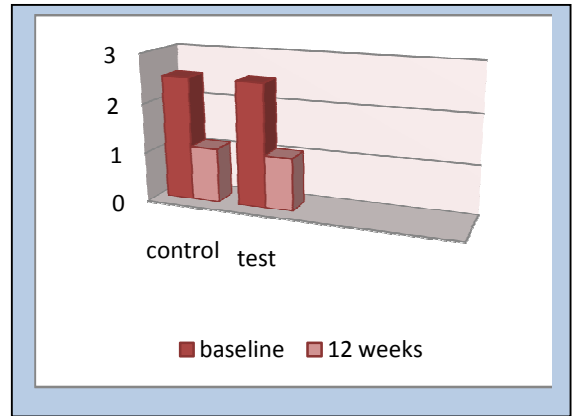|	CONTROL				TEST			
	MEAN± STD BASELINE	MEAN± STD (6 WEEK)	T-VALUE	P-VALUE	MEAN± STD BASELINE	MEAN± STD (6 WEEK)	T-VALUE	P-VALUE
COCCI	40100±17970.4	46350±21332.6	-3.674	<0.05	40400±17804	55200±20877	-8.799	<0.001
RODS	67350±12553.9	46900±17979	4.042	<0.001	74150±14327.9	43600±20877	4.092	<0.001
SPIROCHETES	1317±1362	441±481.5	3.893	<0.001	1380±1122	204±260	3.907	<0.001
OTHERS	731±1182.0	1053±1159	-1.149	<0.05	1038±1122.8	1771±1613	-5.321	<0.001

FIGURE I: CONTROL GROUP AT BASELINE

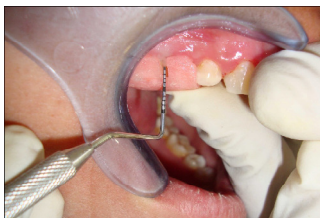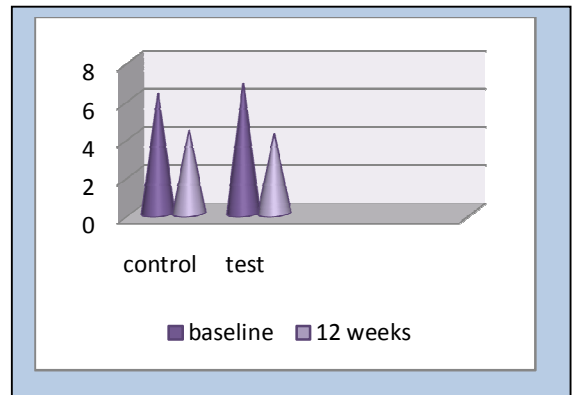FIGURE II: TEST GROUP AT BASELINE

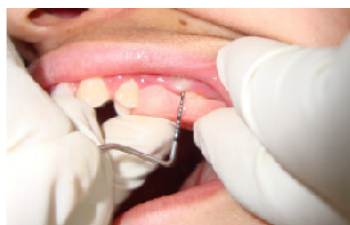FIGURE III: CONTROL GROUP AT 12 WEEKS

FIGURE IV: TEST GROUP AT 12 WEEKS

FIGURE V: PLACEMENT OF AZITHROMYCIN GEL INTO THE POCKET

STATISTICAL ANALYSIS- Mean and standard deviations were estimated from the samples of each group. Student-t-test was performed in test and control sites at each time interval.

RESULTS

This study was designed to evaluate and compare the effects of SRP (control) and SRP plus 0.5% AZM via local drug delivery (test) on the clinical and microbiologic parameters (GI, PD, and CAL) in patients with chronic periodontitis. Twenty patients were enrolled in the study and were distributed equally in two groups..Forty treatment sites were evaluated for clinical parameters at baseline 6 and 12 weeks; microbiologic parameters were recorded at baseline and at 6 weeks No adverse reaction was observed in any subject from the test group, and no patient reported any discomfort. No statistically significant difference in baseline values was observed between the groups (P >0.05) as in table I. Intragroup analysis showed that both groups presented significant changes in GI, PD, and CAL at all time periods compared to baseline (P<0.05). Significant improvements in microbiologic criteria were also observed in both groups at 6 week interval compared to baseline.

GINGIVAL INDEX(GI)

The mean GI reduction for both groups is presented in table II. A intra group analysis showed significant differences at all time intervals compared to baseline. After 12 weeks, the reduction in GI was greater in test group compared to control (1.064±0.7632 and1.1185±0.1008 respectively) with difference being significant at p<0.001.

POCKET DEPTH (PD)

Pocket depth reduction was statistically significant within both groups compared to baseline at all time intervals (table II).At 6 weeks,the reduction in pocket depth in test and control were 4.400±0.7439 and 5.000±0.7632 respectively.After 12 weeks

there was significant reduction in pocket depth in test and control(4.1500±0.6708 and4.350±0.489 respectively)The difference being significant at p<0.001.

CLINICAL ATTACHMENT LEVEL (CAL)

CAL gain was greater in test compared to control at all periods. The difference from baseline was also statistically significant in both groups (table II).

MICROBIAL PARAMETERS

In the present study, microbial parameters were assessed at baseline and at the end of 6 weeks. Mean±Std for cocci at baseline and at 6 week in control group respectively, 40100±17970.4 and 46350±21332.6 (P<0.05) and for test group 40400±1780 and 55200±20877.0 (P<0.001), show significant improvement in no. of coccoid cell at 6 weeks.Mean ±Std for Rods at baseline and at 6 week in control group respectively, 67350±12553.9 and 46900± 17979 and for test group 74150±14327.9 and 43600±20877 (P<0.001). Mean±Std for spirochetes at baseline and at 6 week in control group respectively, 1317±1362 and 441± 481.5 and for test group 1380 ± 1122 and 204±260, (P<0.001).Mean±Std for others (straight rods and filaments) at baseline and at 6 week in control group respectively, 731±1182.0 and 1053± 1159,P<0.05 and for test group 1038 ± 1122.8 and 1771±1631 (P<0.001)Microbiological results were statically significant for both test and control group as in table III

DISCUSSION

There is increase evidence that organisms present in microbial plaque constitute the primary and possibly the only extrinsic etiologic agent participating in the cause of inflammatory periodontal disease[21].Mechanical debridement reduces microbial load however, sometimes mechanical therapy not able to remove tissue invasive pathogens hence adjunctive chemotherapeutic agent provides additional benefit

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in controlling the disease. Systemic use of antibiotic dramatically improves clinical outcome although it is associated with inherent adverse effects. Hence to overcome these drawbacks, local drug delivery systems has been developed.

Azithromycin is a semisynthetic, acid stable antibiotic, which represents the prototype of a novel class of macrolides named azalides. Furthermore, GCF concentration achieved by locally delivered Azithromycin gel is 2041μg/ml retained in site for up to 28 days .[22,23]Azithromycin has been found useful in the treatment of periodontal infections, because of its increased acid stability, increased tissue distribution and decreased binding to plasma proteins and rapid absorption. Other important characteristics of this drug are the increased concentration found in cells such as neutrophils, macrophages, fibroblasts, monocytes and epithelial cells, which may explain the high level of Azithromycin found in the infected tissue[24]. Furthermore, among all macrolides Azithromycin is most effective against gram-negative anaerobes i.e Fusobacterium species, Bacteroid species, Actinobacillus actinomy-cetemcomitans, Selemonas species[25] . Gomi et al [26] compared the effects of full-mouth SRP combined with systemic AZM to conventional SRP; they showed that full-mouth SRP with systemically administered AZM was a clinically and bacteriologically useful basic periodontal treatment for patients with severe chronic periodontitis. In a study by Sayyed et al 2012 [27] , adjunctive use of systemic azithromycin show significant clinical benefit in the treatment of chronic periodontitis. In a study by Mascarenhas[28] et al., AZM combined with SRP for the treatment of moderate to severe periodontitis in smokers provided significantly greater PD reduction and CAL gain at moderate and deep pockets 6 months post-therapy than SRP alone.

The result of this investigation demon-strated an overall improvement in all parameters at various time interval in both test and control groups (table II). Severity and quantity of gingival inflammation assess by gingival index. A significant change in gingival index found within both control and test group. Gingival index was 2.517±0.1829 mm and 2.500±0.1662 mm at baseline for control and test sites, and at end of our study (12 weeks) mean score was 1.118±0.1008 mm and 1.064±0.7632 mm respectively. *Eickholz P[29] et al, 2002* observed significant reduction of gingival index for both control and test sites which were 1.81± 0.55 and 1.1±1.0 respectively. These observations are consistent with the observation of present study. Reduction in probing depth is the major clinical outcomes measured to determine the success of a treatment. A significant reduction in probing depth found within both groups compared to baseline at all time intervals. Probing pocket depth for control and test sites were reduced to 4.350±0.489 mm and4.1500±0.6708 mm respectively at 12 weeks (p<0.001)**.** *Pradeep et al, 2008*[23] evaluated the effect of subgingivally delivered 0.5% Azithromycin in the treatment of chronic periodontitis as an adjunct to scaling and root planning observed significant reduction in probing pocket depth 4.40 ± 1.12 mm and 4.00 ± 0.76 mm for control and test sites respectively with p value (<0.05). These observations are consistent with results of present study, which shows greater pocket depth reduction in test as compared to control sites. *Gomi et al, 2007*[26] who used systemic administration of Azithromycin in conjunction with scaling and root planning demonstrate reduction in probing depth 1.77 mm at the end of 13 weeks. A significant gain in clinical attachment level also found within both groups compared to baseline at all time intervals. But between groups , the value did not reach statistical significance at end of our

study. This may be because chronic periodontitis is a chronic disease which progresses in episodic manner and rate of progression of disease is slow, so a 12 week period may be not sufficient to record noticeable differences in attachment loss. Our results are in accordance of study by Han et al,2012[30] which reported no significant difference in clinical attachment level of sites with 4 to 6 mm pocket by adjunctive use of azithromycin with non surgical periodontal therapy in chronic periodontitis patients..

In the present study, microbial parameters were assessed at baseline and at the end of 6 weeks for test control and test group 46350±21332.6 and 55200±20877.0 (P<0.001), show significant improvement in no. of coccoid cell at 6 weeks. Our results showed marked decrease in no. of spirochetes and rods at the end of 6 weeks. Microbiological results were statically significant for both test and control group. These results are in accordance with study conducted by *Pradeep et al, 2008*[23] found coccoid cell ,rods, spirochetes and others(straight rods and filaments)

in test group show highly significant after gel application are highly significant (P< 0.001) at the end of 6 week..*Gomi et al., 2007*[19] found a reduction in the total number of bacteria and black pigmented rods after the adjunctive use of systemic Azithromycin with full-mouth Scaling and root planning. With regard to microbial parameters, all bacteriologic categories in both groups showed a shift toward health.

CONCLUSION

Within the limit of this study, it was found that the adjunctive use of 0.5% Azithromycin gel with scaling and root planing enhances the outcome of non-surgical treatment of chronic periodontitis.. However, limitations of this study was that it was a short term study, but as the findings were encouraging it justifies the need for long term study to evaluate the true value of the 0.5% Azithromycin gel. Further ,long term trials using different vehicles and concentrations of azithromycin, are desired to comment on its application in periodntal disease as adjunct to scaling and root planning to support the observations of our study.

REFERENCES

1. Haffajee AD, Socransky SS. Microbial and biological agents destructive periodontal diseases. Periodontol 2000 1994; 5: 78-11

2. Darveau P.R. Tanner,Page R.C.The microbial challenge in periodontitis.Periodontaol 2000;1997;14.12-32

3. Slots J, Rosling B. Suppression of the periodontopathic microflora in localized juvenile periodontitis by systemic tetracycline. J Clin Periodontol 1983; 10: 465-86.

4. Van Winkelhoff AJ, Rams TE, Slots J. Systemic antibiotic therapy in periodontics. Periodontol 2000 1996; 10: 47-78.

5. Drisko CH. Non-surgical therapy: Pharmacotherapeutics. Ann Periodontol 1996; 1:491-518.

6. Position Paper. Systemic Antibiotics in Periodontics. J Periodontol
2004; 75: 1553-1565.

7. Goodson JM, Haffajee A, Socransky SS. Periodontal therapy by local
delivery of tetracycline; J Clin Periodontol 1979: 6 : 83 – 92

8. Rams T, Slots J: Local delivery of antimicrobial agents in the periodontal pocket. Periodontology 2000 1996; 10: 139-159.

9. Maizumi N, Tamura Y, Kanai H, Tsutsui T. Quantitative comparison of the cytocidal effect of seven macrolide antibiotics on human periodontal ligament fibroblasts. J Periodont Res 2002; 37: 250-254.

10. Sefton AM, Maskell JP. Beighton D, Whiley A, Shain H, Foyle D, Smith SR. Smales FC, Williams JD. Azithromycin in the treatment of periodontal disease. Effect on microbial flora. J Clin Periodontol 1996; 23: 998-1003

11. Peters DH, Friedel HA, McTavish D. Azithromycin: A review of its antimicrobial activity, pharmacokinetic properties and clinical efficacy. Drugs 1992;44:750-799.

12. Pajukanta R, Asikainen S, Saarela M, Alaluusua S, Jousimies-Somer H. In vitro activity of azithromycin compared with that of erythromycin against Actinobacillus actinomycetemcomitans. Antimicrob Agents Chemother 1992;36:1241-1243.

13. Pajukanta R. In vitro antimicrobial susceptibility of Porphyromonas gingivalis to azithromycin, a novel macrolide. Oral Microbiol Immunol 1993;8:325-326.

14. Filatova NA, Kuznetsov EA, Dmitrieva LA, Tsarev VN, Kalinin AI. The prospects for using the new macrolide antibiotic azithromycin (Sumamed) in combined treatment of periodontitis (in Russian). Stomatologiia (Mosk)1995;74:12-

15. Van Winkelhoff AJ, Herrera D, Winkel EG, Dellemijn- Kippuw N, Vandenbroucke-Grauls CM, Sanz M. Antibiotic resistance in subgingival microflora in patients with adult periodontitis. A comparative survey between Spain and the Netherlands (in Dutch). Ned Tijdschr Tandheelkd 1999;106:290-294

16. Culic O, Erakovic V, Cepelak I, et al. Azithromycin modulates neutrophil function and circulating inflammatory mediators in healthy human subjects. Eur J Pharmacol 2002;450:277-289.

17. Pajukanta R, Asikainen S, Saarela M, Alaluusua S, Jousimies-Somer H. In vitro activity of azithromycin compared with that of erythromycin against Actinobacillus actinomycetemcomitans. Antimicrob Agents Chemother 1992;36:1241-1243

18. Blandizzi C, Malizia T, Lupetti A, et al. Periodontal tissue disposition of azithromycin in patients affected by chronic inflammatory periodontal diseases. J Periodontol 1999;70:960-966

19. Gomi K, Yashima A, Iino F, et al. Drug concentration in inflamed periodontal tissues after systemically administered azithromycin. J Periodontol 2007;78:918-923.

20. Smith SR, Foyle DM, Daniels J, et al. A double-blind placebo-controlled trial of azithromycin as an adjunctto non-surgical treatment of periodontitis in adults:Clinical results. J Clin Periodontol 2002;29:54-61.

21. Goodson JM, Offenbacher S, Fair DH and Hogan PE. Periodontal Disease Treatment by Local Drug Delivery; J Periodonlogy 1985: 265 – 272

22. JW Krayer,RS Leite,KL Kirkwood; Dent Clin N Am 2010;54:13-33.

23. A.R. Pradeep, S.Vidya Sagar, and Happy Daisy,J Periodontol 2008;79:2125-35

24. Argani H, Pourabbas R, Hassanzadeh D, Masri M, Rahravi H.treatment of cyclosporine induced gingival overgrowth with Azithromycin containing toothpaste. Exp Clin Transplant 2006; 4:420-424

25. Williams JD, Meskell JP, Shain H, et al. Comparative in vitro activity ofAzithromycin macrolides (erythromycin, clarithromycin and spiramycin) andstreptogramin RP 59500 against oral organism. JAntimicrob Chemother1992;30:27-37

26. Gomi K, Yashima A, Nagano T, Kanazashi M, Maeda N, Arai T. Effects of full mouth scaling and root planing in conjunction with systemically administered azithromycin. J   Periodontol 2007;78:422-429.

27. Seyyed Ali Banihashemrad, Majid Reza Mokhtari, Mohammad Hassan Najafi Neshli1, Golsa Akbarian, Fatemeh Farazi. Clinical Effects of Systemic Azithromycin as an Adjunct to Scaling and Root Planing in the Treatment of Chronic Periodontitis: International Journal of Stomatological Research 2012, 1(3): 24-30

28. Mascarenhas P, Gapski R, Al-Shammari K, Hill R, Soehren S, Fenno CJ et al. Clinical Response of Azithromycin as an Adjunct to Non-Surgical Periodontal Therapy in Smokers J Periodontol 2005;76:426-436

29. Eickholz P, Kim TS, Bürklin T, Schacher B, Renggli HH, Schaecken MT, Holle R, Kübler A, Ratka-Krüger P: Non-surgical periodontal therapy with adjunctive topical doxycycline: a double blind randomized controlled multicenter study. J Clin Periodontol 2002; 29: 108–117

30. Buket Han, Gulnur Emingil, Guven O zdemir,Taina Tervahartiala,Caner Vural, Gul et al.Azithromycin as an adjunctive treatment of chronic periodontitis;clinical microbiological and biochemical parameters.J Periodontol 2012;83:1480-1491.