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COMPARATIVE EVALUATION OF ESSENTIAL OIL CONTAINING MOUTHWASH AND SYSTEMICALLY ADMINISTERED AZITHROMYCIN IN PROPHYLACTIC CONTROL OF BACTEREMIA SECONDARY TO SCALING AND ROOT PLANING

Dr Md. Aamir Ahsan[1], Dr Gaurav Malhotra[2], Dr Pradeep Shukla[3], Dr Prerna Kataria[4], Dr Varun Dahiya[5]

[1]Post Graduate Student, Department of Periodontology & Implantology, Divya Jyoti College of Dental Sciences & Research

[2]Professor, Department of Periodontology &Implantology, Divya Jyoti College of Dental Sciences& Research

[3]Professor & HOD, Department of Periodontology &Implantology, Divya Jyoti College of Dental Sciences & Research

[4]Professor, Department of Periodontology &Implantology, Divya Jyoti College of Dental Sciences & research

[5]Professor, Department of Periodontology &Implantology, Divya Jyoti College of Dental Sciences & Research

Conflicts of Interest: Nil

Corresponding author: Dr Md. Aamir Ahsan

Abstract:

Transient bacteremia frequently occur secondary to several periodontal procedures. The purpose of the present study is to Investigate the prevalence of bacteremia caused by scaling and root planning & comparative evaluation of essential oil containing mouthwash and systemically administered azithromycin in prophylactic control of bacteremia secondary to scaling and root planning.

Methods: 45 patients with chronic periodontitis were randomly assigned to three groups (control, EO, and AZM).The EO group received quadrant subgingival SRP & irrigation with EO containing mouthwash Oral administration of AZM was started 3 days before SRP in the AZM group. No adjunctive treatment was performed before SRP in the control group. Peripheral blood and GCF were collected at baseline and after 1 week. The second blood sample was taken 6 minutes after the initiation of quadrant SRP. The blood samples were cultured and analyzed for bacteremia. Quantitative analysis of periodontopathic bacteria in the sulcus was performed using the polymerase chain reaction Invader method.

Results: Bacteremia incidence rates were 90%, 70%, and 20% for the control, EO, and AZM groups, respectively. Significant reduction of the incidence of bacteremia was shown in the AZM group only ($P < 0.01$). Subgingival bacterial counts significantly decreased in both the EO and AZM groups ($P < 0.01$).

Conclusions: Quadrant SRP frequently induced bacteremia. Although AZM was effective in reducing bacteremia incidence, EO showed less effectiveness.

Keywords: Azithromycin, Essential oil

Introduction

The pathogenesis of periodontal disease (PD) is an inflammatory process, characterized by the host-mediated destruction of soft tissue caused by the induced production and activation of lytic enzymes and stimulated osteoclastogenesis.[1] PD is a continuous process or consists of episodes of exacerbation and remission.[2] The inflammatory process occurring in PD is characterized by the infiltration of leukocytes, which limit the level of bacterial invasion. Factors promoting leukocyte recruitment includes bacterial products, cytokines, cross-talk between innate and adaptive immune responses, chemokines, lipid mediators, and complement. Page and Schroeder[3] showed that bone resorption ceases when a 2.5-mm zone is created between the site of bacteria and bone. In PD there is uncoupling of bone resorption with subsequent bone formation so that a net loss of bone occurs.

Bacteremia occurs when bacteria enters the blood stream transiently and can be detected by laboratory blood culture techniques. Though bacteremia are transient, it has long been recognized that oral bacteria may cause distant infections There is evidence that components of these causative bacteria of oral infections, particularly lipopolysaccharide, may promote atherosclerosis, affect blood coagulation, the function of platelets and prostaglandin synthesis. The micro-organisms more frequently associated with periodontitis are *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans*, *Tanerella*

forysthus, *Echinella corrodens*, *Campylobacter rectus*, *Micromonas micros*, *Treponema denticola*, *Fusobacterium Nucleatum*, and *Prevotella intermedia* Bacteremia frequently occurs after scaling and root planing, periodontal probing, periodontal surgery, suture removal, sometimes chewing, subgingival irrigation,and oral hygiene procedures such as tooth brushing and flossing

Azithromycin (AZM) is a systemic antibiotic, belongs to a class of macrolide antibiotics called azalides. It has better oral absorption & work in a bacteriostatic fashion by interfering with the 50s component of the bacterial ribosome, thus inhibiting translation of mRNA and preventing proper protein synthesis. It is broad spectrum & more effective against certain Gram-negative bacteria, especially *Actinobacillus actinomycetemcomitans*.[4] AZM improved clinical parameters when used as an adjunct to SRP.

Essential oil-containing antiseptic (EO) is an over-the-counter mouth wash containing 2 phenol-related essential oils; EO is associated with only minimal side effects and kills a wide range of microorganisms by disrupting their cell walls and inhibiting their enzyme activity.[5] EO is capable of extracting bacterial endotoxins, Moreover, EO penetrates the plaque biofilm and is active against biofilm-embedded bacteria.

Multiple PCR is a variant of PCR enabling simultaneous amplification of many targets of interest in one reaction by using more than one pair. It consists of multiple prime sets within a single PCR mixture to produce amplicons of varying sizes that are specific to different DNA sequences. By targeting multiple genes at once, additional information may be gained from a single test-run. Annealing temperatures for each of the primer sets must be optimized to work correctly within a single reaction, and amplicon sizes. That is, their base pair length should be different enough to form distinct nabds when visualized by gel electrophoresis.

Present study is done to investigate the prevalence of bacteremia caused by SRP, and to evaluat the efficacies of two prophylactic methods for bacteremia secondary to SRP: subgingival irrigation and rinsing with EO, and systemic administration of AZM.

Matrials & Method

Patients were selected from the department of Periodontology and Implantology , D.J. College of Dental Sciences and Research, Modinagar in collaboration with immunology laboratory of Maratha Mandal Dental College, Belgaum (Karnataka). The whole study protocol was explained to them and patient's consent was taken for the same.

Systemically healthy 45 patients of Age group of 25 – 55 years who possessed a minimum of 20 teeth with generalized moderate- to-severe chronic periodontitis defined as having >3 teeth with probing depth (PD) >5 mm in each quadrant, with no history of antibiotic or periodontal therapy in the preceding 6 months were included. Subjects excluded were: Patients with congenital valve defects or at risk situation for infectious endocarditis, cardiovascular disease and diabetes; low levels of hematocrit or hemoglobin or allergy to macrolides also Patients who had taken systemic antibiotics, anti-inflammatory drugs, or immunosuppressive drugs within 3 months before the experiment and Patients who had received periodontal treatment within the previous 6 months, regularly used an oral irrigation device or mouthrinse, and had an incompatible dentition (e.g., orthodontic bands, partial dentures,Teeth unsuitable for extensive ultrasonic scaling).

Clinical examination

A thorough medical and drug history of each patient was obtained. Smoking habits were registered (number of cigarettes per day and years of smoking). For over a month before the study, all subjects received a few visits of standard oral hygiene instructions. One week before starting the study, full-mouth periodontal examination was performed including :Pocket Depth , clinical attachment level (CAL),and bleeding on probing (BOP). PD and CAL were recorded at six sites per tooth (mesio-buccal, buccal disto-buccal, mesio-lingual, lingual, disto-lingual) with a periodontal probe. The quadrant exhibiting the most severe periodontal condition was selected as the site for SRP on the basis of clinical findings

Clinical protocol

A total of 45 subjects were randomly assigned to three groups (control, n = 15; EO, n = 15; and AZM, n = 15) based on the treatment protocol. At baseline, peripheral blood and GCF were collected. After 1 week, GCF was taken once again. Subsequently, the quadrant SRP was performed by hand instruments

under local anesthesia with 2% lidocaine (plus 1:80,000 epinephrine). The second sample of peripheral blood was taken 6 minutes after the initiation of SRP. The quadrant SRP was completed within 40 minutes. Fig:1

Essential oil group

Quadrant subgingival irrigation with 100 ml of EO was performed gently for 10 minutes at baseline and 10minutes (for clearance of any bacteria introduced by irrigation) before SRP. A professional, pulsating, low-pressure irrigation device was used at a setting of mild degree, with an irrigation tip. After initial sampling, mouth rinsing at home four times daily for 20 seconds with 20ml of EO was continued for 1week.

Oral administration of azithromycin

The subjects started taking azithromycin once a day for 3 days before quadrant SRP was performed. Stimulated GCF of 45 chronic periodontitis patients was taken at baseline & after treatment with #40 paper points preoperatively in vial called eppendorf tube containing 0.5 ml of tris- EDTS. These samples were send to laboratory of Maratha Mandal dental college, department of Microbiology of Belgaum for RT-PCR test.

Blood was obtained by venipuncture in the antecubital fossa. Before each sampling, the skin overlying the vein was swabbed with ethyl alcohol and then chlorhexidine tominimize the number of potential skin contaminants. Each sample comprised 10 ml of blood, which was obtained using a 22-gauge butterfly and safety lock blood collection set and 30-ml syringe

Result

The three bacterias (P.gingivalis, T.forsythia and P.Intermedia.) were analysed in GCF sample both before and after treatment by PCR. The presence of particular bacteria was evaluated as positive or negative. Also the blood sample is collected for culturing and is evaluated for bacteremia. And number of copies were calculated preoperatively and postoperatively.

Wilcoxon Signed Rank test was used to compare bacteria present preoperatively with postoperatively and also used to compare the median value of bacteria in males and females present pre treatment and post treatment.

McNemar test was used to compare the number of patients showing reduction in number of bacteria after treatment.

Spearman's rho test was used to find the correlation of bacteria with depth separately in each sex.

Comparision of bacteria present preoperatively with postoperatively

With application of Wilcoxon signed Ranks Test, at 5% level of significance it was found bacteria named P.gingivalis were found to show significant difference wherasTf bacteria does not show significant difference. It means there is a significant decrease in number of bacteria count after treatment w.r.t two bacteria i.e P.Intermedia, P.gingivalis, wheras bacteria T.forsythia does not decrease after treatment (**Table1)**

COMPARISION OF PREVALENCE OF BACTERIA PRESENT PREOPERATIVELY WITH POSTOPERATIVELY

Preoperatively prevalence rate of three bacteria (P.Intermedia,P.gingivalis and T.forsythia) are 56.6%, 83.3%and 46.6% respectively. After treatment their prevalence rates were reduced to 10%, 40%,and 16.6% respectively. **(Table 2)**

COMPARISION OF NUMBER OF PATIENTS SHOWING REDUCTION IN NUMBER OF BACTERIA AFTER TREATMENT.

McNemer test was applied for evalution of number of patients which shows reduction in number of bacteria and chi square test (2 sided) was used to find correlation between number of positive patients before and after treatment.

Out of 45 patients P.Intermedia. was positive in 25 patients, P.gingivalis was positive in 37 patients and T.forsythia was positive in 21 patients. On post treatment evalution there was reduction in number of initially positive cases in all 3 bacteria. In case of P.Intermedia, number of patients were reduced from 25 to 3 (88.2%) , in case of P.gingivals positive patients were reduced from 37 to 8 (78.5%), in case of P.gingivals positive patients were reduced from 21 to 4(21.4%),.**(Table 3).**

Incidence of bacteremia

The incidence of bacteremia at baseline and after SRP in the groups is shown in Table. No bacterial growth was detected at baseline in any of the groups. A total incidence of bacteremia after SRP was 60% (27/45); the frequency was 90% (13/15) in the control

group,70% (11/15) in the EO group, and 20% (3/15) in the AZM group. The incidence rate of bacteremia among the groups was different (P = 0.008).Another statistical analysis was also performed tocompare each combination (control group versus EO group, control group versus AZM group, and EO group versus AZM group). Rate of incidence in the AZM group was significantly lower than that of the control group (P = 0.003). However, the incidence rate between the EO group and control group (P = 0.291) or AZM group (P = 0.035) was not significant. Table:4

Identification of microorganisms isolated from blood

The bacterial species detected in the positive blood samples are shown in Table. All isolates were facultative or obligate anaerobes. There were significant differences in a-Streptococcus between the control group and other groups (P = 0.014). Three subjects of the EO group exhibited polymicrobial bacteremia (growth of >1 microorganism from the blood sample). No polymicrobial growth was observed in the other groups.

Bacteria in GCF

The mean SD for intra group comparisons of subgingival bacterial counts. Numbers of total bacteria and P. gingivalis in the control group were significantly increased after 1 week compared to the baseline (P <0.05). Meanwhile, counts of total bacteria, P. gingivalis, and T. forsythia in the EO group were significantly decreased after 1 week compared to the baseline (P <0.01). The AZM group after 1 week had significantly greater reductions in counts of all species compared to the baseline (P <0.0001).

The numbers of all kinds of species at baseline were comparable among three groups, except for T. for sythia between the EO group and the AZM group (P <0.01). The counts of all kinds of species in the AZM group after 1 week were significantly lower compared to the other groups(P <0.01). Also, numbers of the total bacteria and T. forsythia in the EO group were lower than that of the control group (P <0.01).

Table 1: Comparison of bacteria present preoperatively with postoperatively

Bacteria	**Pre treatment(n=45) Median (min-max)**	**Post treatment (n=45) Median(min-max)**	**P value**	**Significance (s)**
PI	1000(0-26000)	0(0-18000)	0.001	S
Pg	4000(0-38000)	0(0-61000)	0	S
Tf	0(0-31000)	0(0-26000)	0.088	NS

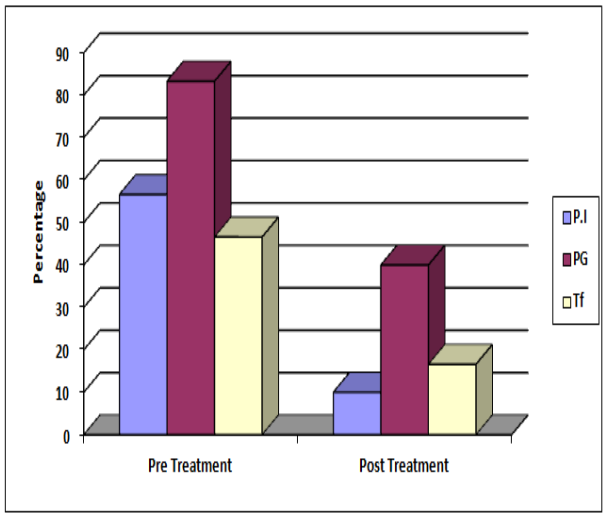
Table 2: Comparison of prevalence of bacteria present preoperatively with postoperatively

Bacteria	Preoperatively	Postoperatively
PI	56.6%	10%
Pg	83.3%	40%
Tf	46.6%	16.6%

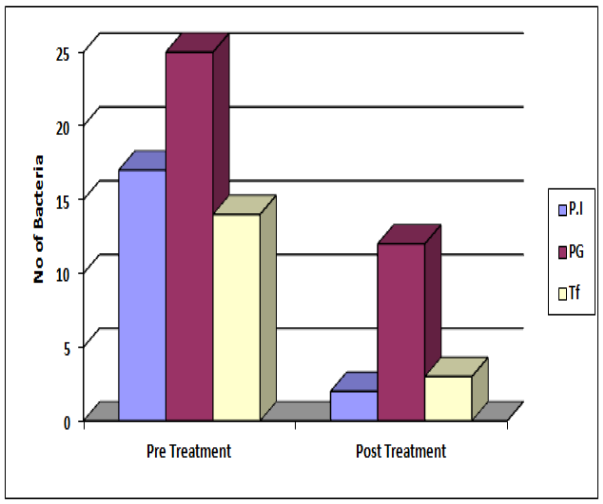
Table 3: Comparison of number of patients showing reduction in number of bacteria after treatment

Bacteria	no. of patients positive for respective bacteria(pretreatment)	No. of patients positive for respective bacteria (post-treatment)	total no. of patients which show reduction in no. of bacteria (positive value)	P value	Significance (S)
PI	**25**	**3**	**22**	**0.01**	**S**
Pg	**37**	**8**	**29**	**0.01**	**S**
Tf	**21**	**4**	**17**	**0.022**	**S**

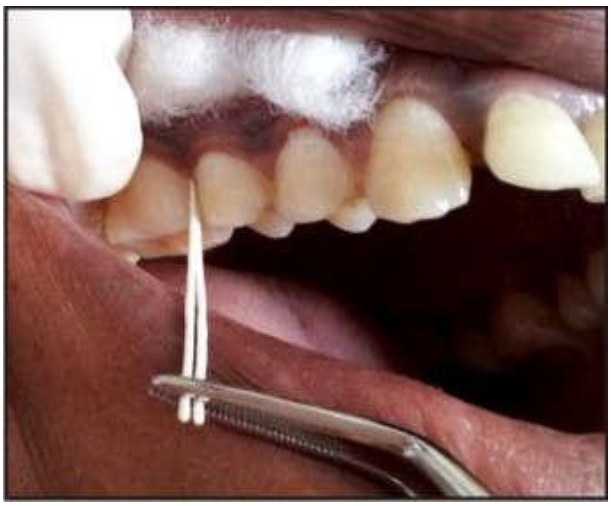
*****SIGNIFICANT AT 5% LEVEL OF SIGNIFICANCE



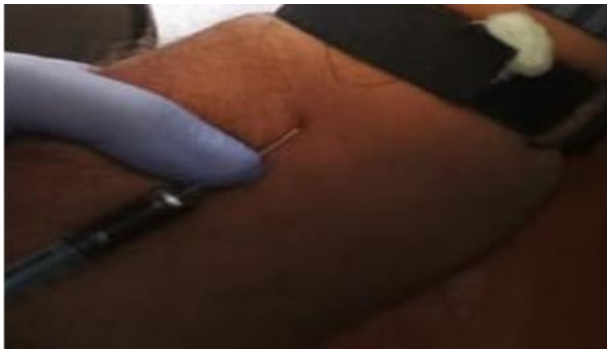
Graph 1: Showing comparison of prevalence of bacteria Pretreatment and Post treatment



Graph 2: Showing comparison of number of patients showing positivity for bacteria in pretreatment and Post treatment.



Collection of GCF



COLLECTION OF BLOOD

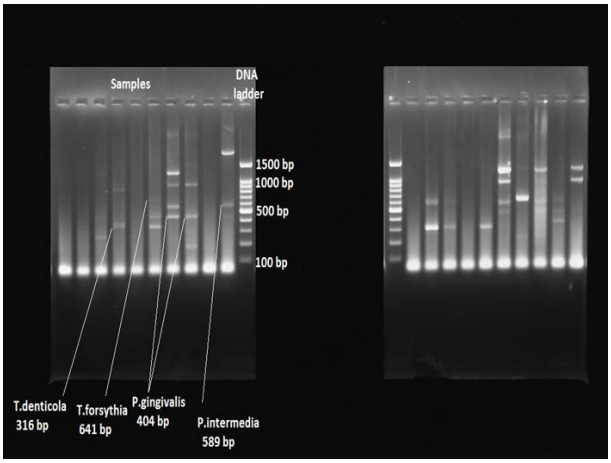
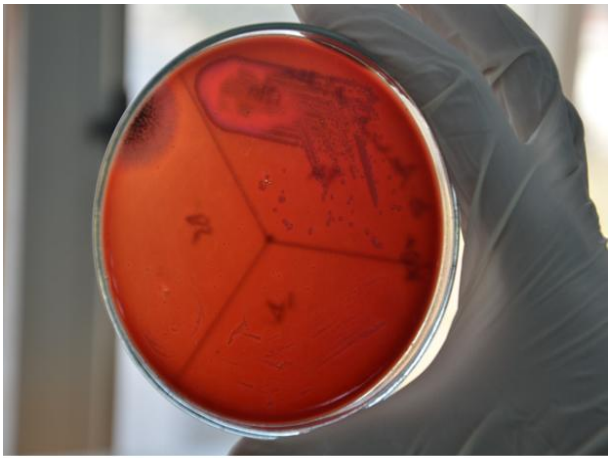


Image for gel casting

Growth of bacteria in culture media



Growth of bacteria is decreased after treatment

DISCUSSION

The initiation and progression of periodontal disease is attributed to presence of elevated levels of pathogenic bacteria within gingival crevice30. Socransky et al and Mullally BH et al demonstrated that species such as Porphyromonasgingivalis, Tannerela forsythia and Prevotellaintermedia routinely occur together in the subgingival biofilm[6] Microorganisms associated with periodontitis can be detected by various techniques like cultural techniques, immunofluorescence and ELISA. Bacterial culturing is the classic diagnostic method.[7]

PCR is extremely sensitive, being able to detect even one copy of the searched DNA target and does not require rigorous conditions for transport of samples from the clinical department to the laboratory.

It is rapid with results being available within hours of sample acquisition, cheaper and less labour intensive.For PCR, sample material like GCF, saliva and subgingival plaque have been used for detection of periodontopathogenic bacteria.

The present study We demonstrated that SRP induced a high level of detectable bacteremia. Furthermore, we observed that AZM prophylaxis significantly reduced the incidence of bacteremia.

Our data in this study indicate the incidence of bacteremia in 90% of subjects after SRP in the control group. There is a wide variation in the frequency of bacteremia induced by SRP in the literature reviewed, which ranged from 18.5% to 80.9%40. This variability could be attributed to a number of factors that play a role in the detection of a transient bacteremia: the techniques used, application of a tourniquet, component of anesthetic, the timing of blood sample collection, the degree of periodontal inflammation, quantity and composition of the gingival flora, and identification methods for the isolation of microorganisms.[8]

In fact, the subjects used in studies by Wakiet al.[9]in 1990 showed 18.5% prevalence, whereas Lofthusmet al.[10]in 1991 reported 50%prevalence, were all in the periodontal maintenance phase. Even Messini et al. (1999) found 83% aerobic and anaerobic bacteria in blood after SRP and Lafaurie GI,[11] in 2007 showed 80.9% prevalence. Meanwhile, in the present study, patients had not received any periodontal treatments in the sulcus within the previous 6 months. Also, Wakiet al.[9] collected blood 2 minutes after the initiation of SRP, whereas we collected blood 6 minutes after the start in the present study.

It is well known that the number and proportion of Gram-negative bacteria in periodontal pocketsincrease with the progression of periodontal disease;[12] moreover, aggressive periodontitis involves specific microorganisms, such as A. actinomycetemcomitans,[13] P. gingivalis, and T.forsythia.[14] Hence, it should be considered that this result was attributed to the difference of the subgingival microflora and the level of gingival inflammation.

This explanation may be supported by the consequence that there is a positive but weak correlation between the level of gingival inflammation and the presence of microorganisms in blood after scaling.[15]

In addition, we have performed PCR analysis of P. intermedia, P. gingivalis, and T. forsythia using the blood samples as described by Kinane et al.[8] Although PCR is acknowledged as an accurate and sensitive method for identification of bacterial isolates.

Culture method used in the study, none of the target bacteria was amplified in samples (data not shown). This may indicate that those bacteria are below the detectable level in the blood samples.

As one of the two preventive measures, subgingival irrigation and rinsing with EO was attempted. Before starting the experiment, we hypothesized that both irrigation and mouth rinsing with EO and AZM administration could drastically reduce the incidence of bacteremia.

However, pretreatment with EO hardly had an effect on the incidence rate. No bacteremia was detected in any blood after irrigation in the preliminary experiment; therefore, irrigation was carried out gently to preclude an induction of bacteremia.

This study observed that microorganisms isolated in the blood of the EO group were characterized by the growing frequency of obligate anaerobes, such as P. micra and F. nucleatum. Considering the data showing obvious reduction of microbiota in the sulcus, a possible cause would be that the composition of subgingival microflora was altered by irrigation and mouth rinse: aerobe and facultative anaerobes existing in the shallow zone of the sulcus were antisepticized, resulting in increase of the proportion of the obligate anaerobes.

In the present study, we used AZM as a prophylactic antibioticon SRP-triggered bacteremia. This antibiotic is known to have a long half-life, good tissue penetration, and higher tissue concentration.[16]

In addition, AZM is preferentially taken up by phagocytes, and therefore its levels in infected tissues are much higher than noninfectedsites.[17]

In this trial, the setting is aimed at the reduction of the intraoral bacterial counts before SRP by oral administration of antibiotics, rather than bactericidal action in the bloodstream after mechanical debridement.

We found that the bacteremia occurred in two subjects.

This suggests that the prophylactic effects of AZM for bacteremia secondary to quadrant SRP was by no means perfect. Similar results have been obtained on examination of bacteremia in patients subjected to dental treatments including dental restoration and tooth extraction, under general anesthesia with different antibiotic prophylaxis.

Our data in this study elucidate the substantial effectiveness of AZM for bacteremia. Studies have exhibited the favorable pharmacokinetics of AZM in gingival tissue, such as long duration and high concentration.[18,19]

Furthermore, adjunctive application of AZM for periodontal treatment has recently attracted attention: it was demonstrated that systemic administration of AZM in combination with SRP contributes to statistically significant improvement in PD, CAL, or BOP. Therefore, we consider that the use of AZM is beneficial in both the prevention of bacteremia and improvement of the effect of periodontal therapy.

Thus this study proposes that with help of non surgical therapy like scaling and root planning count of three bacteria (PI,PG,TF) are reduced if we compare preoperatively with postoperatively, So scaling and root planning plays a very important part in treating the patients of chronic periodontitis.

Limitation of the present study is that we are not able to explain the different strains of same periodontal pathogens present in Gcf & blood culture, Various studies are going on to find the different strain of periodontal pathogens present in GCF and blood and also to find different periodontal pathogens in patients of choronic periodontitis in different conditions like diabetes mellitus, hypertension and in smokers.

CONCLUSION

In conclusion this study revealed that; Quantity of three bacteria (P.Intermedia, P. gingivalis, T. forsythia) were reduced significantly after scaling and root planning. Out of 45 patients PI was positive in 17 patients and after treatment it was positive in 2 patients.In case of T.forsythia patient number reduced from 25 to 0 showing 100% reduction, in case P. gingivalis, patients showing positive for pg reduced from 25 to 12 showing 52% reduction Above mentioned conclusion simply that mechanical therapy like scaling and root planning is effective in treating the patients of choronic periodontitis. Also that blood sample is very useful diagnostic tool for detection of bacteria by using RTPCR test Moreover GCF can be used as a diagnostic tool for detection of bacteria by using RTPCR test

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