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Research Article

COMPARISION OF LEVELS OF PERIODONTOPATHIC BACTERIA BY POLYMERASE CHAIN REACTION IN CHRONIC AND AGGRESSIVE PERIODONTITIS PATIENTS BEFORE AND AFTER NON-SURGICAL THERAPY WITH SUBGINGIVAL ANTIMICROBIAL (BIOTENE) IRRIGATION.

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ABSTRACT

INTRODUCTION: Periodontitis is an inflammatory disease of the supporting tissues of teeth caused by specific group of microorganisms resulting in destruction of periodontal ligament, alveolar bone with pocket formation, recession or both.

AIM : To evaluate the efficacy of the adjunctive use of Biotene as subgingival irrigation along with scaling and root planning in the treatment of chronic and aggressive periodontitis patients.

MATERIALS AND METHODS: A split mouth study was done in 40 patients having probing pocket depths of ≥4 mm and diagnosed with chronic, aggressive periodontitis. Patients were divided into placebo group and test group. Assessment of plaque index (PI), gingival index (GI), probing pocket depth (PPD), and clinical attachment levels (CAL) was done at baseline and 3 months. Microbiologic assessment with polymerase chain reaction was done for Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia, and Treponema denticola by collection of plaque samples.

RESULTS: Both the groups A and B have shown statistically significant results in terms of all clinical and microbial parameters pre operatively and post operatively with more significance observed.

CONCLUSION: The results revealed that there is reduction in all clinical parameters and microbial parameters in test groups following scaling and root planning and subgingival irrigation of Biotene when compared with SRP alone in placebo group.

Key words: Periodontitis, Biotene, Scaling and root planing, Microbial analysis.

Introduction

Chronic periodontitis is an infectious disease resulting in inflammation within the supporting tissues of the teeth, progressive attachment loss, and bone loss[1]. The most important periopathogens are anaerobic bacteria: Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia, Treponema denticola, Fusobacterium nucleatum and the relative anaerobe Aggregatibacter actinomycetemco-mitans.[2]

Aggressive periodontitis refers to the multifactorial severe, and rapidly progressive form of periodontitis, which primarily-but not exclusively affects younger patients. Non-constant charecteristics of the disease are: Amounts of microbial deposits are inconsistent with the severity of periodontal destruction, elevated proportions of Actinobacillus actinomycetemco-mitans, and in some populations Porphyromonas gingivalis may be elevated[3].

The successful treatment of Periodontitis requires suppression or elimination of the subgingival periodontopathogens and is generally

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accomplished by a combination of mechanical procedures performed by a dental professional and daily plaque control procedures performed by the patient. Anti-infective therapy includes both mechanical and chemotherapeutic approaches to minimize or eliminate microbial biofilm (bacterial plaque), the primary etiology of gingivitis and periodontitis[4].

Scaling and root planing (SRP) is one of the most commonly utilized procedures for the treatment of periodontal diseases and has been used as the 'gold' standard therapy against which other therapies have been compared[5]. Because the efficacy of non-surgical mechanical procedures and conventional home care in controlling the pathogenic flora decreases as probing depths increase, efforts are underway to develop therapeutic strategies using the adjunctive subgingival delivery of antimicrobial agents.

Biotene® is an antibacterial enzymatic mouthrinse which contain antibacterial enzymes Which are found naturally in human saliva. Biotene contains three primary enzymes –Glucose Oxidase, Lactoperoxidase, and Lysozyme[6]. These protiens are known to 1) limit bacterial or fungal growth, 2) interfere with bacterial glucose uptake or glucose metabolism, and 3) promote aggregation and, thus, the elimination of bacteria. Lysozyme provides its antimicrobial activity by a muramidase dependent mode and a cationic-dependent or structure-related bactericidal mechanisms. [7]

The study is designed to evaluate the efficacy of Biotene ® as subgingival irrigation to compare the levels of periodontopathogenic bacteria in chronic and aggressive periodontitis patients before and after non-surgical periodontal therapy, and also to evaluate its effect on clinical parameters like plaque, gingival scores and pocket probing depth and relative attachment level and its efficacy in suppressing the pathogenic anaerobic micro flora.

MATERIALS AND METHODS:

This was a single blinded split mouth study undertaken to evaluate the effect of Biotene as an adjunct to SRP and SRP alone in treatment of chronic and aggressive periodontitis patients. In this clinical trial 20 patients with chronic periodontitis aged between 35-60 years and aggressive periodontitis between 16-30 years of age who reported to the Department of Periodontics, St. Joseph Dental College, Eluru between May 2013 to August 2014 were enrolled for the study. Approval of the study was obtained from the ethical committee of St. Joseph Dental College and an informed consent was taken from all participants before commencing of the study.

INCLUSION CRITERIA:

1) Patient having at least three teeth with probing pocket depth of 5‑8 mm that bleed on probing at the initial visit, Patients with good systemic health who have not received local and/or systemic antibiotic therapy within the last 6 months prior to the baseline examination of the study, Patients diagnosed with chronic and aggressive periodontitis.

EXCLUSION CRITERIA:

1) Pregnant women, Lactating mothers, Smokers, Patients who underwent previous periodontal surgery.

Before starting the trial, plaque samples were collected from all the sites and were stored in EDTA vial for microbial sampling, subsequently all the patients underwent full mouth scaling using an ultrasonic scalar and after 1 week SRP was done followed by sub gingival irrigation of Biotene and oral hygiene instructions.

The groups were treated as follows:

Chronic Group –**A**: Scaling and root planning was followed by irrigation of Biotene subgingivally at baseline visit. Biotene was irrigated subgingivally to base of pocket by means of cannula.

Chronic Group –**B**: Scaling and root planning was followed by irrigation of distilled water subgingivally at baseline visit.

Aggressive Group –**A**: Scaling and root planning was followed by irrigation of Biotene subgingivally at baseline visit. Biotene was irrigated subgingivally to base of pocket by means of cannula.

Aggressive Group –**B**: Scaling and root planning was followed by irrigation of distilled water subgingivally at baseline visit.

Four clinical variables including plaque index (PI) , gingival index , probing pocket depth, clinical attachment levels and microbiologic analysis of subgingival plaque samples for the "red complex"

periodontal pathogens(A. actinomycetemco-miotans, P.gingivalis, T. denticola, T. forsythia) using PCR was measured at baseline, 4 weeks interval. Subgingival plaque samples was collected by using a Gracey curette by inserting it subgingivally into the deepest portion of the periodontal pocket.

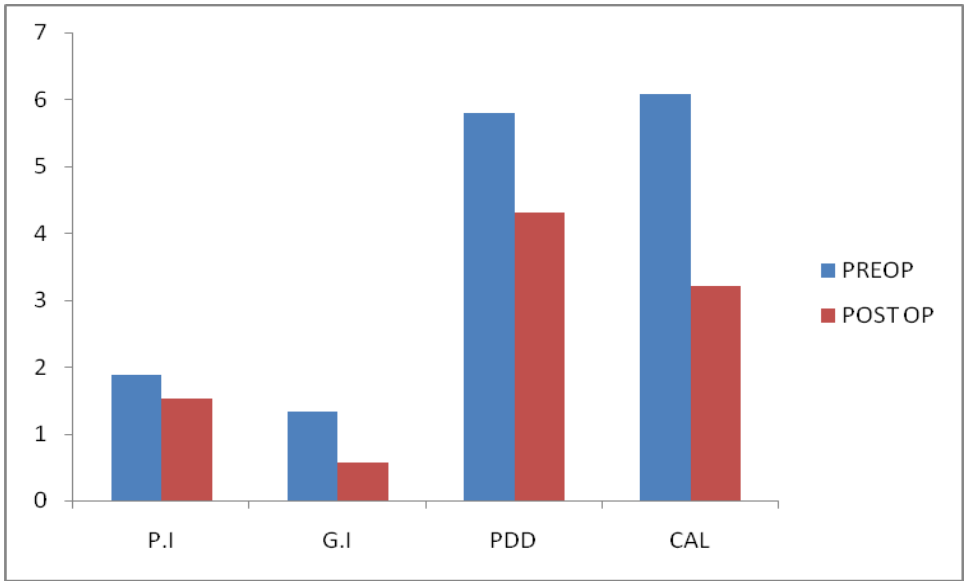
STASTICAL ANALYSIS: The data was analyzed sing statistical package for social sciences SPSS v 10.5, ibm, Chicgo, IL.

RESULTS:

TABLE 1: MEAN, STANDARD DEVIATION AND TEST OF SIGNIFICANCE FOR PLAQUE INDEX, GINGIVAL INDEX, PROBING DEPTH, CLINICAL ATTACHMENT LEVEL OF AGGRESSIVE PLACEBO GROUP PRE AND POST OPERATIVELY.

VARIABLES	PRE OPERTIVE		POST OPERTIVE		P Value
	MEAN	SD	MEAN	SD	
Plaque index	1.87	0.49	1.52	0.41	0.0001S
Gingival index	1.33	0.32	0.56	0.26	0.000S
Probing depth	5.80	0.79	4.30	0.67	0.000S
Clinical attachment level	6.07	1.01	3.20	0.92	0.000S

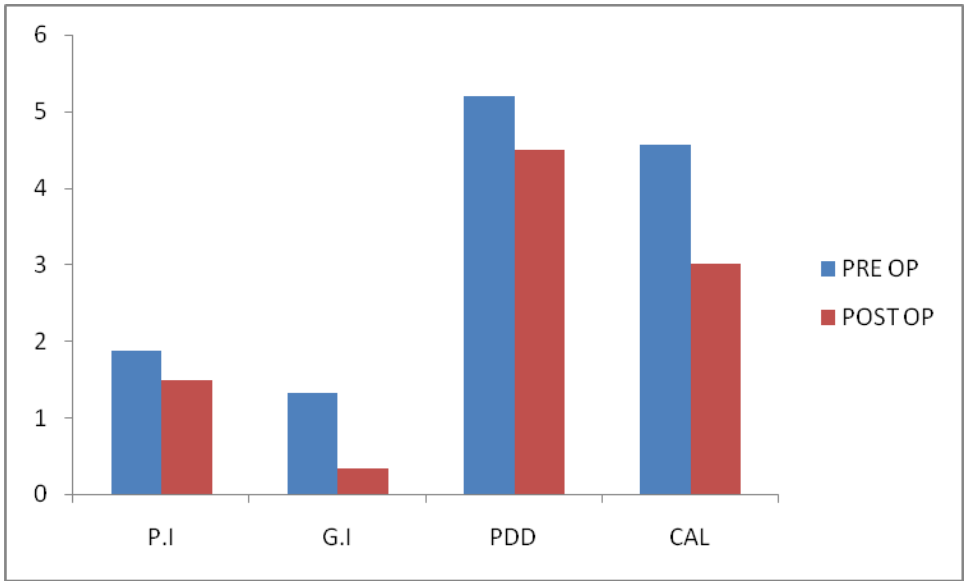
Statistical Analysis: Paired t test. Statistically significant P<0.05.



GRAPH 1: MEAN, VALUES FOR PLAQUE INDEX, GINGIVAL INDEX, PROBING DEPTH CLINICAL ATTACHMENT LEVEL OF AGGRESSIVE PLACEBO GROUP PRE AND POST OPERATIVELY.

TABLE 2: MEAN, STANDARD DEVIATION AND TEST OF SIGNIFICANCE FOR PLAQUE INDEX, GINGIVAL INDEX, PROBING DEPTH, CLINICAL ATTACHMENT LEVEL OF CHRONIC PLACEBO GROUP PRE AND POST OPERATIVELY.

VARIABLES	PRE OPERTIVE		POST OPERTIVE		P Value
	MEAN	SD	MEAN	SD	
Plaque index	1.87	0.58	1.48	0.47	0.000S
Gingival index	1.32	0.39	0.33	0.19	0.000S
Probing depth	5.20	0.63	4.50	0.53	0.0013S
Clinical attachment level	4.57	0.94	3.00	0.95	0.000NS

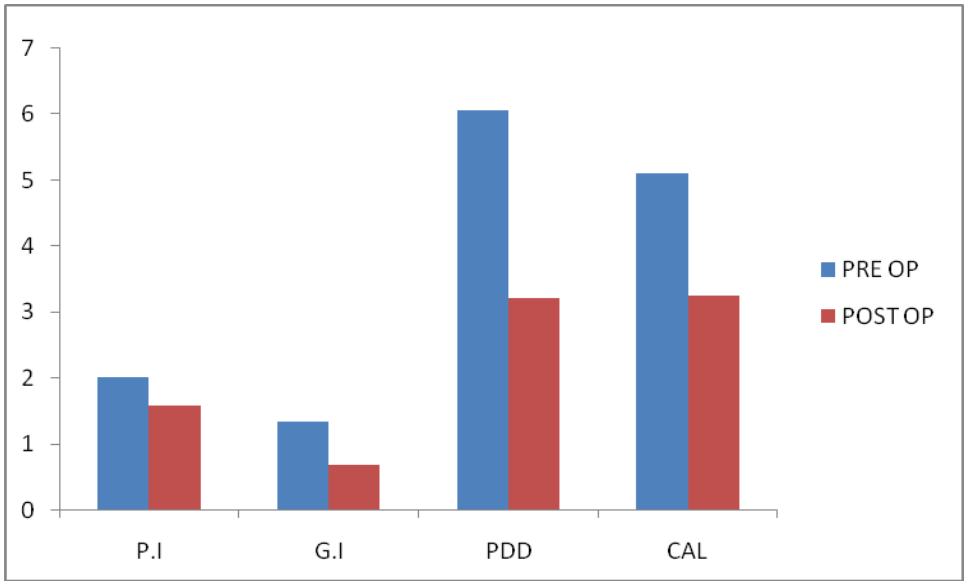


GRAPH 2: MEAN VALUES FOR PLAQUE INDEX, GINGIVAL INDEX, PROBING DEPTH CLINICAL ATTACHMENT LEVEL OF CHRONIC PLACEBO GROUP PRE AND POST OPERATIVELY.

TABLE 3: MEAN, STANDARD DEVIATION AND TEST OF SIGNIFICANCE FOR PLAQUE INDEX, GINGIVAL INDEX, PROBING DEPTH, CLINICAL ATTACHMENT LEVEL OF AGGRESSIVE TEST GROUP PRE AND POST OPERATIVELY.

VARIABLES	PRE OPERTIVE		POST OPERTIVE		P Value
	MEAN	SD	MEAN	SD	
Plaque index	2.01	0.43	1.57	0.34	0.0002S
Gingival index	1.33	0.39	0.67	0.36	0.000S
Probing depth	6.07	1.01	3.20	0.92	0.000S
Clinical attachment level	5.10	0.84	3.25	0.63	0.000S

Statistical Analysis: Paired t test. Statistically significant P<0.05.

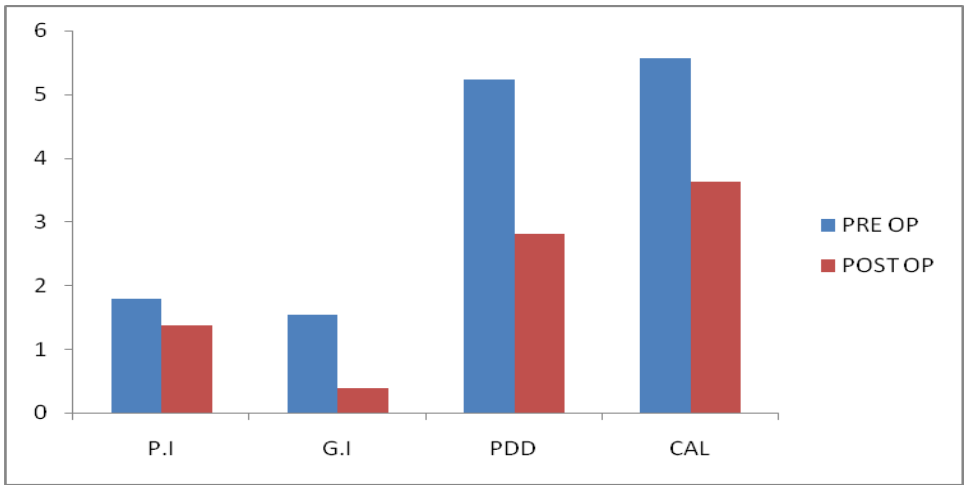


GRAPH 3: MEAN VALUES FOR PLAQUE INDEX, GINGIVAL INDEX, PROBING DEPTH CLINICAL ATTACHMENT LEVEL OF AGGRESSIVE TEST GROUP PRE AND POST OPERATIVELY.

TABLE 4: MEAN, STANDARD DEVIATION AND TEST OF SIGNIFICANCE FOR PLAQUE INDEX, GINGIVAL INDEX, PROBING DEPTH, CLINICAL ATTACHMENT LEVEL OF CHRONIC TEST GROUP PRE AND POST OPERATIVELY.

VARIABLES IN GROUP A	PRE OPERTIVE		POST OPERTIVE		P Value
	MEAN	SD	MEAN	SD	
Plaque index	1.78	0.70	1.37	0.57	0.0007S
Gingival index	1.54	0.36	0.39	0.13	0.000S
Probing depth	5.23	1.14	2.80	1.19	0.000S
Clinical attachment level	5.57	0.82	3.63	0.67	0.000S

Statistical Analysis: Paired t test. Statistically significant P<0.05.

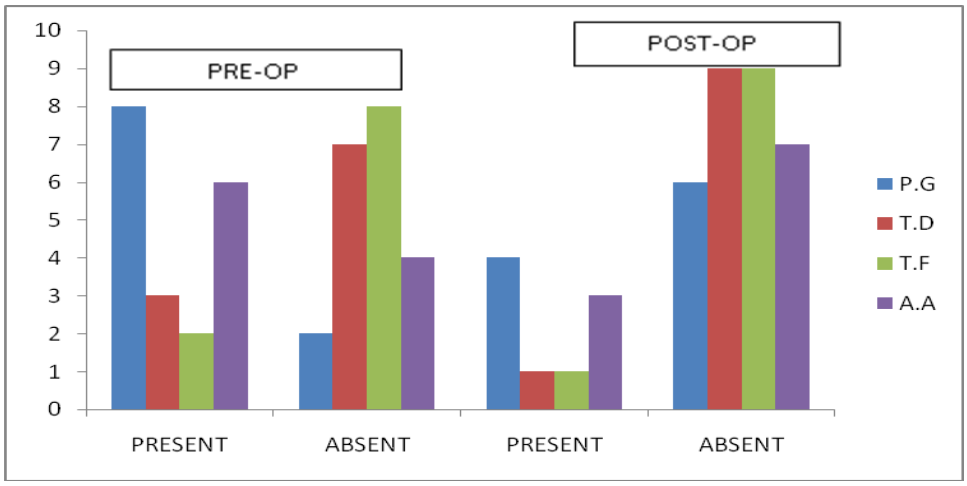


GRAPH 4: MEAN VALUES FOR PLAQUE INDEX, GINGIVAL INDEX, PROBING DEPTH CLINICAL ATTACHMENT LEVEL OF CHRONIC TEST GROUP PRE AND POST OPERATIVELY

TABLE 5: COMPRISION OF PCR VALUES OF AGGRESSIVE PLACEBO GROUP PRE AND POST OPERATIVELY.

PCR ORGANISM	PRE OP		POST OP		P value
	PRESENT	ABSENT	PRESENT	ABSENT	
P.g	8	2	4	6	0.068NS
T.d	3	7	1	9	0.264NS
T.f	2	8	1	9	0.531NS
A.a	6	4	3	7	0.178NS

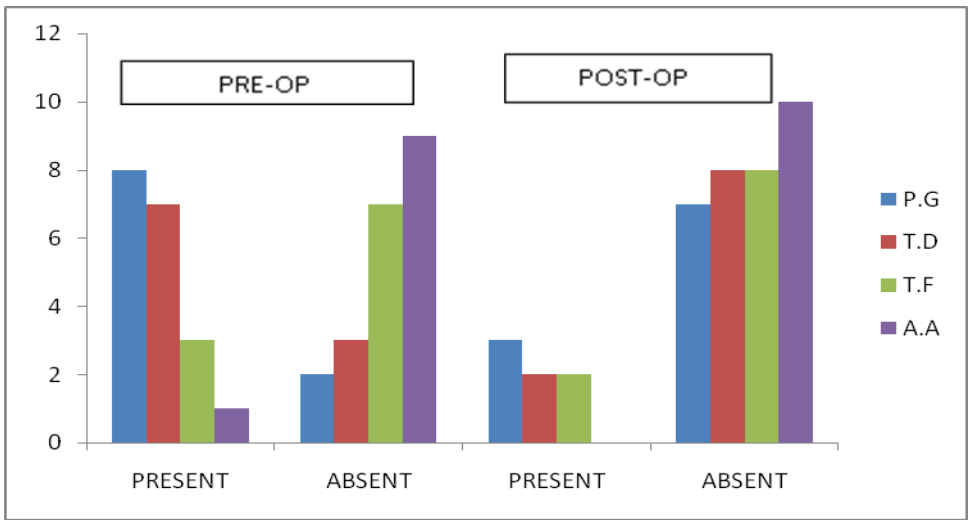
Statistical Analysis: Chi- square test.



GRAPH 5: COMPRISION OF PCR VALUES OF AGGRESSIVE PLACEBO GROUP PRE AND POST OPERATIVELY.

TABLE 6: COMPRISION OF PCR VALUES OF AGGRESSIVE TEST GROUP PRE AND POST OPERATIVELY.

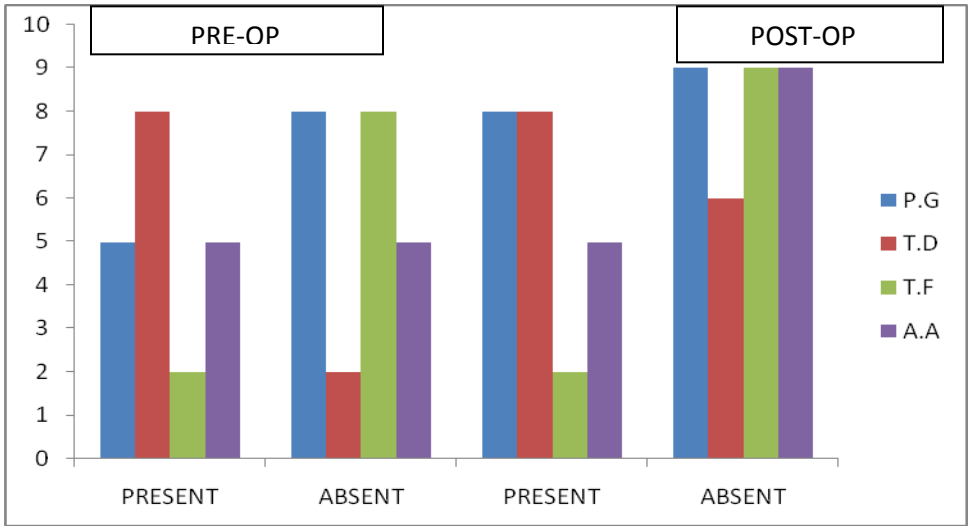
PCR ORGANISM	PRE OP		POST OP		P value
	PRESENT	ABSENT	PRESENT	ABSENT	
P.g	8	2	3	7	0.025S
T.d	7	3	2	8	0.025S
T.f	3	7	2	8	0.606NS
A.a	1	9	0	10	0.305NS



GRAPH 6: COMPRISION OF PCR VALUES OF AGGRESSIVE TEST GROUP PRE AND POST OPERATIVELY.

TABLE 7: COMPRISION OF PCR VALUES OF CHRONIC PLACEBO GROUP PRE AND POST OPERATIVELY.

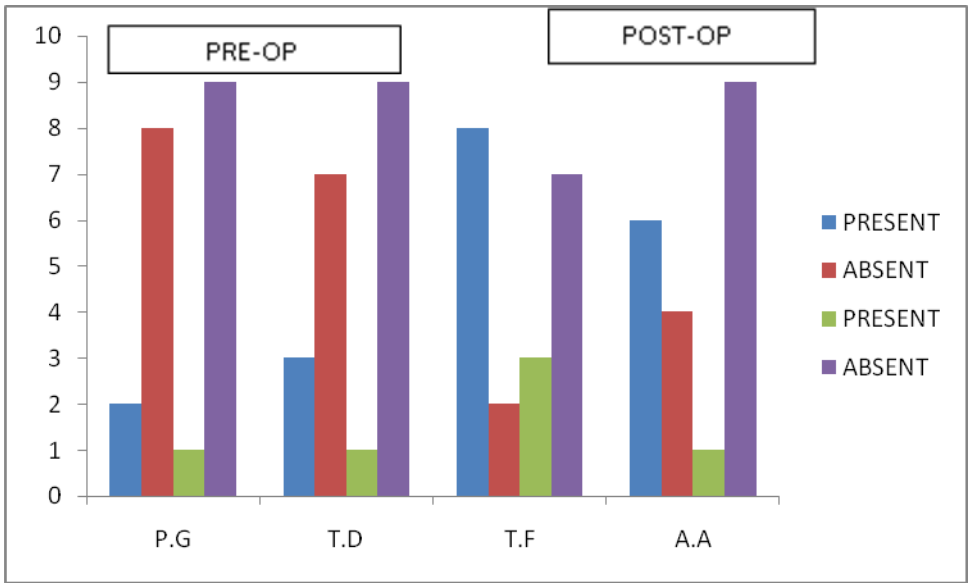
PCR ORGANISM	PRE OP		POST OP		P value
	PRESENT	ABSENT	PRESENT	ABSENT	
P.g	5	8	8	9	0.051NS
T.d	8	2	8	6	0.068NS
T.f	2	8	2	9	0.531NS
A.a	5	5	5	9	0.264NS



GRAPH 7: COMPRISION OF PCR VALUES OF CHRONIC PLACEBO GROUP PRE AND POST OPERATIVELY.

TABLE 8: COMPRISION OF PCR VALUES OF CHRONIC TEST GROUP PRE AND POST OPERATIVELY.

PCR ORGANISM	PRE OP		POST OP		P value
	PRESENT	ABSENT	PRESENT	ABSENT	
P.g	2	8	1	9	0.531NS
T.d	3	7	1	9	0.264NS
T.f	8	2	3	7	0.025S
A.a	6	4	1	9	0.019S



GRAPH 8: COMPRISION OF PCR VALUES OF CHRONIC TEST GROUP PRE AND POST OPERATIVELY.

DISCUSSION:

The study is designed to evaluate the efficacy of Biotene ® as subgingival irrigation to compare the levels of periodontopathogenic bacteria in chronic and aggressive periodontitis patients before and after non-surgical periodontal therapy, and also to evaluate its effect on clinical parameters like plaque, gingival scores and pocket probing depth and relative attachment level and its efficacy in suppressing the pathogenic anaerobic micro flora.

The use of antimicrobial mouthrinses is an approach to limiting the accumulation of dental plaque, with a primary objective of controlling the development and progression of periodontal diseases. Antimicrobial mouthrinses allow patients to deliver active agents in a cost-effective manner, while controlling dosage and timing. Depending on the chemistry of the rinse preparation, active agents can have sustained therapeutic benefits on the hard- and soft-tissue surfaces of the oral cavity, thereby extending their therapeutic benefits[8]. In the treatment of periodontal diseases, irrigation is used as a lavage to flush away the bacteria that are in contact with the periodontal tissues[9].

Biotene® is an antibacterial enzymatic mouthrinse which contain antibacterial enzymes Which are found naturally in human saliva. Biotene contains three primary enzymes –Glucose Oxidase, Lactoperoxidase, and Lysozyme[6]. These protiens are known to 1) limit bacterial or fungal growth, 2) interfere with bacterial glucose uptake or glucose metabolism, and 3) promote aggregation and, thus, the elimination of bacteria. Lysozyme provides its antimicrobial activity by a muramidase dependent mode and a cationic-dependent or structure-related bactericidal mechanisms. These antimicrobials, either alone or in combination with other antimicrobial molecules, have been incorporated in oral health care products to restore the antimicrobial capacity of saliva[7].

The present study was an interventional split mouth study conducted in 20 chronic and 20 aggressive periodontitis patients who were randomly divided into test groups and placebo groups over a period of 4 weeks.

There is a significant reduction in PI values in all the groups from baseline to follow-up-visits. P-values are as follows: Aggressive placebo group (p=0.0001), chronic placebo group (p=0.000), Aggressive test group (p=0.0002), Chronic test group (p=0.0007). This can be attributed to the fact that there was reduction in supragingival plaque after SRP and oral hygiene instructions received during preliminary visits. The results of this study were consistent with Sato K. Yoneyama et.al (1993)[10], Cugini MA et al (2000)[11].

There is a significant reduction in GI scores in all the groups (p=0.0000). This may be due to elimination of local etiological factors which harbor numerous pathogenic strains. This is in accordance with Becker et al (1988)[12], Boretti.G et al (1995)[13], Cugini M.A et al (2000)[11].

The difference in mean probing depth reduction is significant in all the groups i.e. Aggressive placebo group (p=0.0004), Chronic placebo group (p=0.0013), Aggressive test group (p=0.000), Chronic test group (p=0.000). This is in accordance with the study conducted by Haffajee.AD et al (1997)[14], Jingbo Liu et al (2013)[15], and Mari Raquel et al (2013)[16]. The difference in mean probing pocket depth reduction is significant in both the test groups when compared to placebo groups i.e. Aggressive test group (p=0.0068), Chronic test group (p=0.0006). This can be attributed to the anti-inflammatory mechanism of Lactoferrin present in Biotene®, which shows its action through the inhibition of pro-inflammatory cytokines such as interferon-γ, TNF-α, and IL-1α, IL-2, IL-6. This is in accordance with Yesim Er Oztas et al (2005)[17], SA.Gonzalez-chavez et al (2009)[18].

There is a significant clinical attachment gain in all the groups (p=0.000). This cal gain is may be due to the effectiveness of SRP. This is in accordance with the studies conducted by Hammerle CHF et al (1991)[19], Cugini MA et al (2000)[11], Mestinik M J et al (2012)[20], and Jinbo Liu et al (2013). There is a slight but not statistically significant gain in mean CAL levels in test group when compared to the placebo groups i.e. (p=0.8888), (p=0.1037) in Aggressive and Chronic test groups respectively. This is attributed to the presence of Lysozyme in Biotene® which has an anti-elastase activity by which Lysozyme binds to the elastin component of elastic fibers and prevents degradation of elastic fibers an exerts a protective effect on elastic fibers at sites of tissue injury. This is in accordance with Pyong Woo Park et al (1996)[21], Younes R et al (2009)[22].

The microbiologic essay (PCR) has shown a proportionate reduction in A.actinomycetemco-mitans, P.gingivalis, T.forsythia, and T.denticola in placebo groups which was not statistically significant. The reduction in microbial flora in placebo groups is due to SRP. This is in accordance with the study conducted by Haffajee at al (1997), M.A Cugini et al (2000)[14], Jingbo Liu et al (2013).

There is a significant reduction in A.a and P.g levels in both the test groups i.e. Aggressive test group (p=0.025; p=0.025), in chronic test group (p=0.019; p=0.025) respectively for A.a and P.g. this is may be due to the presence of Lactoferrin in Biotene® which contributes on killing of A.a by neutrophils and that it may play a significant role in innate secretory defense against this potential periodontopathogen. This is in accordance with the study conducted by J.R.Kalmar et al (1988)[23].

The decrease in A.a levels was also may be due to the presence of Lysozyme in Biotene® which has a major function of hydrolyzing the peptidoglycan of the bacterial cell wall resulting in cell lysis. This is in accordance with V.J.Lacno et al (1983)[24].

The decrease in levels of P.g was may be due to the bacteriostatic action of Lactoferrin on P.gingivalis by removal of hemoglobin receptor from the bacterial cell surface. Lactoferrin may have an additional antibacterial mechanism by which growth of P.gingivalis is suppressed. This is in accordance with the tudy conducted by O.Aguilera et al (1998)[25].

CONCLUSION:

In conclusion, within this 4 week clinical trial our data suggests that scaling and root planning combined with subgingival antimicrobial irrigation (Biotene®) have a significantly better and prolonged effect compared to scaling and root planning alone. The subgingival antimicrobial irrigation can be effective as an adjunct to mechanical therapy in treatment of chronic and aggressive periodontitis. To further elucidate the use of subgingival antimicrobial (Biotene®) irrigation adjunct to nonsurgical periodontal therapy in the treatment of Chronic and aggressive

periodontitis, a long term study with large sample of subjects should be carried out.

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