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KEYWORDS: Chronic Periodontitis, SRP, NBF gel, CHX gel, FISH technology

COMPARATIVE EVALUATION OF NANOBIOFUSION GEL AND CHLORHEXIDINE GEL AS AN ADJUNCT TO SCALING AND ROOT PLANING IN THE TREATMENT OF CHRONIC PERIODONTITIS: A CLINICO MICROBIOLOGICAL STUDY

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ABSTRACT

Periodontitis is a polymicrobial disease ,characterized by distinct pathogens which results in the destruction of periodontal tissues. Early identification of periodontal pathogens can aid in the rationale of conservative treatment approaches. Fluorescence in situ hybridization (FISH) is one of the rapid methods for easy identification of microbial pathogens. Nano biofusion (NBF)gel contains nanoparticles which are efficient in rapidly penetrating the cells possessing special property of substantivity, antimicrobial, and anti inflammatory activity.

Chlorhexidine gel(CHX gel) has long been used in dentistry and exhibits bacteriostatic, bactericidal and anti-inflammatory properties. Hence present study aimed to compare clinical and microbiological effectiveness of locally delivered NBF gel and chlorhexidine gel as an adjunctive therapy to scaling and root planning (SRP) in the treatment of chronic periodontitis. Microbiological assessment was done suing Fluorescent In situ Hybridisation (FISH) technology to visualize, identify and quantitate Porphyromonas gingivalis (known Periodontal pathogen), Filifactor Alocis, Synergistes species (Novel pathogens).

METHODOLOGY : The study sample consisted of 75 sites from Chronic periodontitis patients of both genders within the age group of 30-60 years. Study samples were divided into 3 treatment groups based on their inclusion and exclusion criteria as Group A ,Group B and Group C. GROUP A comprised of 25 sites which received Full mouth scaling and root planing alone. GROUP B comprised of 25 sites which received Full mouth scaling and root planing followed by NBF gel application .GROUP C comprised of 25 sites which received full mouth scaling and root planing followed by CHX gel application. Patients in all the three groups were evaluated at baseline, 6 weeks, and 12 weeks interval and clinical parameters such as plaque index, Modified sulcular bleeding index(MSBI),

Probing pocket depth(PPD) and clinical attachment level(CAL) were recorded. Subgingival samples were collected from 10 sites of each group(GROUP A, GROUP B, GROUP C) at baseline and after 6 weeks and the microbiological analysis were carried to visualize, identify and quantitate Porphyromonas gingivalis (known Periodontal pathogen), Filifactor Alocis, Synergistes species (Novel pathogens) from all the three groups using Fluorescent In situ Hybridisation (FISH) technology.

RESULTS : In the present study, group B(SRP + NBF gel) and group C (SRP+CHX gel) showed progressive improvements in all the clinical and microbiological parameters on evaluated time periods. However group C (SRP+CHX) has Shown significantly higher improvement in reducing the bacterial count as compared to Group B(SRP+NBF gel) and Group A(SRP alone). In NBF gel, the NBF of propolis along withVitamin C and Vitamin E has brought about the tremendous improvement in periodontal health of the patients. Chlorhexidine (CHX) gluconate present in hexigel has both bacteriostatic abactericidal activity and also exhibits anti-inflammatory properties.

CONCLUSION : From the above studies it can be noted that both NBF gel and CHX gel has improved the clinic-microbiological parameters when used as an adjunct to scaling and root planning.

INTRODUCTION

Periodontitis is a polymicrobial disease caused by complex interactions between distinct pathogens in a biofilm resulting in the destruction of periodontal tissues. Over a period of 12 years using molecular approaches and sequencing techniques, it has become feasible to reveal the existence of new periodontal pathogens.

Therefore, it is evident that in addition to conventional periodontal pathogens, other microbes might be involved in onset and progression of periodontitis. The novel pathogens enlisted under periodontal phylogeny include *Cryptobacterium curtum, Dialister pneumosintes, Filifactor alocis, Mitsuokella dentalis, Slackia exigua, Selenomonas sputigena,*

Firmicutes, Solobacterium moorei, Treponema lecithinolyticum, and Synergistes[1].

Periodontal pockets accommodate a multitude of bacterial phylotypes that make it difficult to differentiate between mere commensals and true pathogens. The profiles of these bacterial species differ on different oral surfaces, and this could be the reason why some Bacteria remain unidentified.[2,3]

Early identification of microbial pathogens is essential for rational and conservative therapy. Fluorescence in situ hybridization (FISH) is one of the rapid methods for easy identification of microbial pathogens. Binding of short fluorescence-labelled DNA or nucleic acid-mimicking PNA probes to ribosomes of infectious agents with consecutive analysis by fluorescence microscopy allows identification of bacterial and eukaryotic pathogens at genus or species level. FISH analysis leads to immediate differentiation of infectious agents without delay due to the need for microbial culture. As a microscopic technique, FISH has the unique potential to provide information about spatial resolution, morphology and identification of key pathogens in mixed species samples[4].

Scaling and root planning (SRP) remains the "gold standard" treatment for periodontal diseases against which other treatments are compared. However, comprehensive mechanical debridement of sites with deep periodontal pockets is difficult to accomplish[5]. This has led to the adjunctive use of antimicrobial agents delivered either systemically or locally. Local drug delivery systems allow the therapeutic agents to be targeted to the disease site. Thus, the dose can be minimized, reducing the systemic absorption and

subsequent risk of adverse side effects. Chlorhexidine is a highly effective antimicrobial agent that is extensively studied and shown to be effective as a mouth rinse[6] and also as a subgingival irrigant. It shows broad spectrum of topical antimicrobial activity, substantivity, effectiveness, safety, and lack of toxicity[7].

India has a rich history of using plants for medicinal purposes. Thus, an emphasis on usage of herbal agents such as propolis, *Aloe vera,* green tea extracts, neem, and curcumin have gained popularity in recent times. Propolis produced by honeybees is a resinous mixture collected from parts of plants, buds, and exudates. In 1908, the first scientific work with propolis, illustrating its composition and pleiotropic property, was published and was first patented in 1968. Propolis is a natural remedy in the different formulation in the field of medicine and dentistry.[8,9]

Nanotechnology deals with the physical, chemical, and biological properties of structures and their components at nanoscale dimensions. It is based on the concept of creating functional structures by controlling atoms and molecules on a one-by-one basis. With developments in materials science and biotechnology, nanotechnology is anticipated to provide advances in dentistry and innovations in oral health-related diagnostic and therapeutic methods[10].

Nano-Bio Fusion (NBF) Gingival Gel, which is a patented scientifically formulated, bio-adhesive antioxidant gel harvesting naturally occurring antioxidants for targeted action. The Nano Bio-Fusion technology amplifies the natural antioxidant power of Propolis, Vitamin C and Vitamin E. Once applied, NBF Gingival Gel creates nano-bioactive protective film which results in increased absorption, resulting in improved clinical effectiveness and visible results after application[11].

Thus, the present study aimed to compare clinical and microbiological effectiveness of locally delivered NBF gel and chlorhexidine gel as an adjunctive therapy to scaling and root planning (SRP) in the treatment of periodontitis.

MATERIAL AND METHODS:

Patients for the study were selected from the Outpatient Department of Periodontics, A.J. Institute of Dental Sciences, Mangalore.The study sample consisted of 75 sites from Chronic periodontitis patients of both genders within the age group of 30-60 years. Patients with good general health suffering from chronic Localised /generalized periodontitis were included in this study. Patients were explained about the procedure to be performed and an informed consent was taken. The following criteria was considered for the selection of patients.[11]

Inclusion criteria	Exclusion criteria
1. Patients willing to participate in the study. 2. Patients aged between 30-60 years. 3. Patients with good general health, suffering from chronic generalized/Localized periodontitis with a minimum of twenty teeth present. 4. Periodontal pocket of Probing depth of 4-6mm classified as localized/generalized chronic periodontitis. 5. Patients who had not received any periodontal treatment in the past 6 months	1. Patients on chemical/herbal drugs for the past 3 months 2. Periodontal therapy received in past 6 months 3. Use of Systemic or subgingival antimicrobial within the 6 months prior to the study 4. Use of Ant inflammatory therapy within the 6 months prior to the study 5. Allergy to CHX 6. Habit of Tobacco chewing and smoking 7. History of Systemic diseases that could influence the course of Periodontal disease or would require prophylactic antibiotics (not medically compromised) 8. Pregnancy and lactation 9. Aggressive periodontitis

Procedure

After obtaining the informed consent from the patients, all the parameters at the baseline were recorded. The initial examination recorded plaque index (PI) 1963,Modified Sulcus bleeding index(1987), Probing Pocket depth, and Clinical attachment level.

- GROUP A: (25 sites)- Full mouth scaling and root planning alone was performed.
- GROUP B: (25 sites)- Full mouth scaling and root planning was performed followed by NBF gel application in the existing periodontal pockets through a Syringe until it was detected in the gingival margin. Postoperative home care instructions including brushing two times daily with a soft brush was advised.
- GROUP C (25 sites) - full mouth scaling and root planing was performed followed by Chlorhexidine gel application through a syringe. Patients were advised to follow their regular oral hygiene methods.

Patients in all the three groups were evaluated at baseline, 6 weeks, and 12 weeks interval and the clinical parameters were assessed

Microbiological Analysis

Subgingival samples were collected from 10 sites of each group (GROUP A, GROUP B, GROUP C) using a curette from the deep portion of the pockets, at baseline and after 6 weeks. The collected plaque sample was transported in a suitable transport media(Tris-EDTA buffer) to Central Research Laboratory, Maratha Mandal Dental College, Belgavi. Microbial analysis was done to visualize, identify and quantitate Porphyromonas gingivalis (known Periodontal pathogen), Filifactor Alocis, Synergistes species (Novel pathogens) from all the three groups using Fluorescent In situ Hybridisation (FISH) technology.

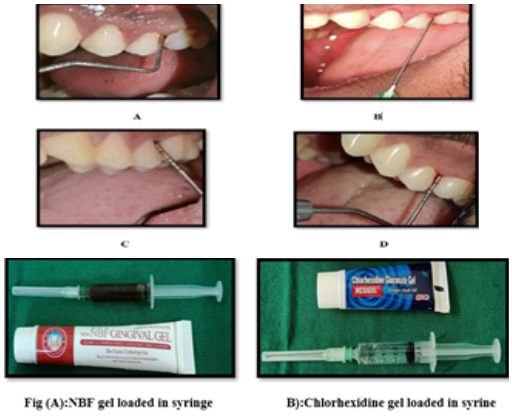
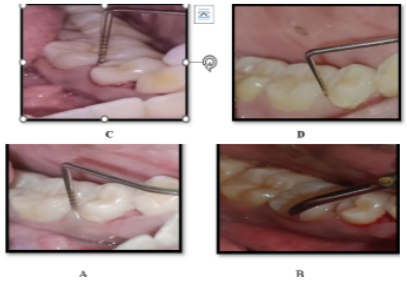


Fig (A):NBF gel loaded in syringe B):Chlorhexidine gel loaded in syrine

GROUP 1 :SCALING AND ROOT PLANING ALONE

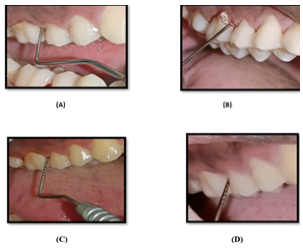
A)Probing pocket depth at baselibe (PPD=6mm irt mesiolingual site of 36) (B)SRP performed using ultrasonic instruments(C)Probing pocket depth after 6 weeks(PPD=3mm irt mesiolingual site of 36)(D)Probing pocket depth after 12 weeks (PPD=5mm irt mesiolingual site of 36)



GROUP 2 : SCALING AND ROOT PLANING+NBF GEL APPLICATION

A)Probing pocket depth at baselibe (PPD=5mm irt mesiobuccal site of 26)(B)NBF gel application through syringe(C)Probing pocket depth ater 6 week s(PPD=3mm irt mesiobuccal site of 26)(D)Probing pocket depth after 12 weeks (PPD=3mm irt mesiobuccal site of 26)

Group 3:Scaling and root planing+Chlorhexidine gel application



A)Probing pocket depth at baselibe (PPD=5mm irt mesiobuccal site of 16)(B)CHX gel application using syringe(C)Probing pocket depth after 6 weeks(PPD=3mm irt mesiobuccal site of 16)(D)Probing pocket depth after 12 weeks (PPD=4mm irt mesiobuccal site of 16)

Microbiological analysis using FISH

GROUP A

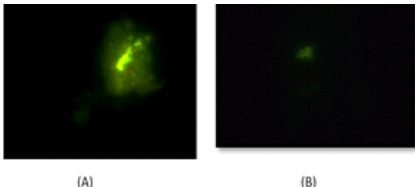


FIG (A)depicts the detection of P.gingivalis,F.Alocis and Synergistes at baseline and after (B) 6 weeks, after FISH and fluorescence microscopy in subgingival plaque sample

GROUP B

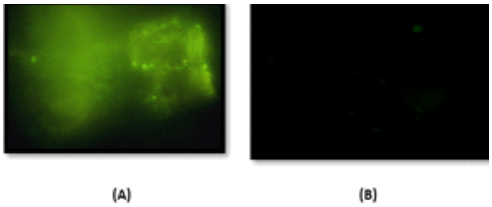


FIG (A)depicts the Detection of P.gingivalis , F.Alocis, Synergistes at baseline and (B) after 6 weeks(after FISH and fluorescence microscopy in subgingival plaque sample.

GROUP C

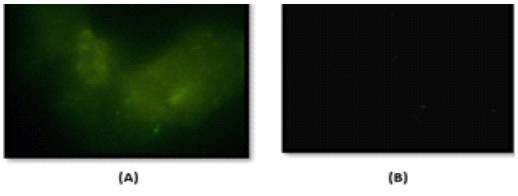


FIG (A)depicts the Detection of P.gingivalis , F.Alocis, Synergistes at (A)baseline and (B)after 6 weeks after FISH and fluorescence microscopy in subgingival plaque sample.

STATISTICAL ANALYSIS

Statistical analysis of the data was performed using SPSS 20.0. The Categorical variables were presented as frequency and percentage. The continuous variables were presented as mean±SD . Comparison categorical variables were performed using chi square test . comparison of plaque index(Sillnes and Loe 1964), modified sulcus bleeding index(A Mombelli et al,1987), pocket probing depth and clinical attachment level between group A , group B and C was done using Bonferroni test .pre post comparison was done using paired t test . A p value<0.05 was considered statistically significant.GroupTimeMeanStd. Deviationp valueGroup ABase line2.1000.306p<0.001 Week61.2520.380Week121.2040.337 Group BBase line2.0400.344p<0.001 Week61.0680.287 Week120.8120.213 Group CBase line2.2040.284p<0.001 Week61.1840.248Week120.8680.189

Table1: Showing Mean and standard deviation of plaque index in group A, group B and group C .

Group	Time	Mean	Std. Deviation	p value
Group A	Base line	2.100	0.306	p<0.001
	Week6	1.252	0.380	
	Week12	1.204	0.337	
Group B	Base line	2.040	0.344	p<0.001
	Week6	1.068	0.287	
	Week12	0.812	0.213	
Group C	Base line	2.204	0.284	p<0.001
	Week6	1.184	0.248	
	Week12	0.868	0.189	

In group A, B, and C, the analysis showed statistically significant effect of time on plaque index score with p<0.001..

Graph no.1 : Pre and post plaque index in group A ,group B and group C

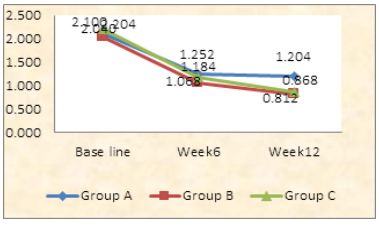


Table no. 2: Showing Mean and standard deviation of modified sulcus bleeding index in group A ,group B and group C .

Group	Time	Mean	Std. Deviation	p value
Group A	Base line	2.100	0.306	p<0.001
	Week6	1.252	0.380	
	Week12	1.204	0.337	
Group B	Base line	2.040	0.344	p<0.001
	Week6	1.068	0.287	
	Week12	0.812	0.213	
Group C	Base line	2.204	0.284	p<0.001
	Week6	1.184	0.248	
	Week12	0.868	0.189	

In Group A, B, and C, the analysis showed statistically significant effect of time on modified sulcus bleeding index with p<0.001.

Graph no.2 : Pre and post modified sulcus bleeding index in group A ,group B and group C

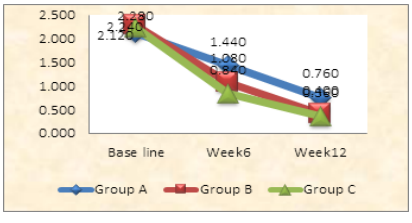


Table no.3: Showing Mean and standard deviation of pocket probing depth in group A ,group B and group C .

Group	Time	Mean	Std. Deviation	p value
Group A	Base line	5.240	0.723	p<0.001
	Week6	4.280	0.542	
	Week12	3.760	0.523	

Group B	Base line	5.160	0.746	p<0.001
	Week6	3.600	0.645	
	Week12	2.560	0.507	
Group C	Base line	5.440	0.651	p<0.001
	Week6	3.440	0.507	
	Week12	2.320	0.476	

In group A,B, and C , the analysis showed statistically significant effect of time on pocket probing depth with p<0.001

Graph no.3 : Pre and post probing pocket depth in group A ,group B and group C

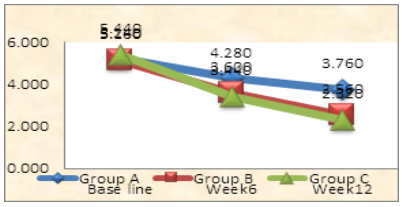


Table no.4: Showing Mean and standard deviation of clinical attachment level in group A ,group B and group C .

Group	Time	Mean	Std. Deviation	p value
Group A	Base line	4.120	0.526	p<0.001
	Week6	3.640	0.490	
	Week12	3.040	0.676	
Group B	Base line	4.400	0.500	p<0.001
	Week6	3.240	0.436	
	Week12	2.080	0.702	
Group C	Base line	4.320	0.476	p<0.001
	Week6	2.720	0.614	
	Week12	1.600	0.645	

In group A, B and C, the analysis showed statistically significant effect of time on clinical attachment level with p<0.001.

Graph no.4 : Pre and post clinical attachment level in group A ,group B and group C

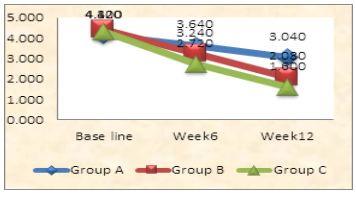


Table no.5 shows Showing comparison in Plaque index, modified sulcus bleeding index,Probing pocket depth and clinical attachment Level between the three groups

Microbes		Group			Total	P value
		Group A	Group B	Group C		
Filifactor Alocis	0	4	8	7	19	p>0.05
		40.0%	80.0%	70.0%	63.3%	
	1+	6	2	3	11	
		60.0%	20.0%	30.0%	36.7%	
Porphyromonas Gingivalis	0	0	4	6	10	P<0.05
		0.0%	40.0%	60.0%	33.3%	
	1+	3	6	4	13	
		30.0%	60.0%	40.0%	43.3%	
	2+	7	0	0	7	
		70.0%	0.0%	0.0%	23.3%	P<0.05
Synergistes	0	0	5	8	13	
		0.0%	50.0%	80.0%	43.3%	
	1+	6	5	2	13	
		60.0%	50.0%	20.0%	43.3%	
	2+	4	0	0	4	
		40.0%	0.0%	0.0%	13.3%	

In plaque index, base line to week 12 Difference is significantly high in group B as compared to A.Group C showed significantly high difference than group A.The comparison of group B and C showed no significant difference between them.

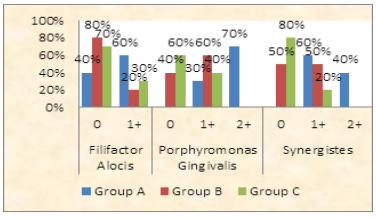
In modified sulcus bleeding index(MSBI),baseline to week 12 difference is significantly high in group B as compared to A. Group C showed significantly high difference than group A. The comparison of group B and C showed no significant difference between them In probing pocket depth(PPD) , base line to week 12 difference is significantly high in group B as difference than group A. The comparison of group B and C showed no difference them.

Table no.6 :showing comparison of week 6 grading of Filifactor Alocis, Porphyromonas Gingivalis and Synergistes between group A ,group B and group C.

Microbes		Group			Total	P value
		Group A	Group B	Group C		
Filifactor Alocis	0	4	8	7	19	p>0.05
		40.0%	80.0%	70.0%	63.3%	
	1+	6	2	3	11	
		60.0%	20.0%	30.0%	36.7%	
Porphyromonas Gingivalis	0	0	4	6	10	P<0.05
		0.0%	40.0%	60.0%	33.3%	
	1+	3	6	4	13	
		30.0%	60.0%	40.0%	43.3%	
	2+	7	0	0	7	
		70.0%	0.0%	0.0%	23.3%	P<0.05
Synergistes	0	0	5	8	13	
		0.0%	50.0%	80.0%	43.3%	
	1+	6	5	2	13	
		60.0%	50.0%	20.0%	43.3%	
	2+	4	0	0	4	
		40.0%	0.0%	0.0%	13.3%	

In clinical attachment level(CAL) , base line to week 12 difference is significantly high in group B as compared to A. Group C showed significantly high difference than group A. The comparison of group B and C showed no difference between them.

Graph no.12 :showing comparison of week 6 grading of Filifactor Alocis, Porphyromonas Gingivalis And Synergistes between group A ,group B and group C



Improvement in Filifactor Alocis does not vary significantly between the groups(p>0.05). Porphyromonas Gingivalis showed significant association with p<0.05.The data shows 60% with no bacterial count in group C followed by 40% in group B and 0% in group A. Synergistes showed significant association between the groups. The data shows 80% in group C with no bacterial count followed by 50% in group B and 0% in group A.

Dependent Variable	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.
PI	Group A	Group B	-.33200*	.09176	.002
		Group C	-.44000*	.09176	.000
	Group B	Group A	.33200*	.09176	.002
		Group C	-.10800	.09176	.729

MSBI	Group A	Group B	-.52000*	.20827	.044
		Group C	-.52000*	.20827	.044
	Group B	Group A	.52000*	.20827	.044
		Group C	.00000	.20827	1.000
PPD	Group A	Group B	-1.12000*	.22331	.000
		Group C	-1.64000*	.22331	.000
	Group B	Group A	1.12000*	.22331	.000
		Group C	-.52000	.22331	.068
CAL	Group A	Group B	-1.24000*	.23646	.000
		Group C	-1.64000*	.23646	.000
	Group B	Group A	1.24000*	.23646	.000
		Group C	-.40000	.23646	.285

Discussion

Periodontal pockets accommodate a multitude of bacterial phylotypes that make it difficult to differentiate between mere commensals and true pathogens. The profiles of these bacterial species differed on different oral surfaces, and this could be the reason why some Bacteria remain unidentified. In addition to conventional periodontal pathogens, other microbes might be involved in onset and progression of periodontitis. A few of these include, Filifactor alocis, Selenomona, Synergistes, and Dialister pneumosintes that have been identified in a number of independent studies. Hence, the role of these novel pathogens in periodontal pathogenesis needs attention2,3. For many decades, research in the field oforal microbiology failed to identify certain subgingival microbiota due to technical limitations but, over a period of 12 years using molecular approaches and sequencing techniques, it has become feasible to reveal the existence of novel periodontal pathogens. The development of culture-independent methods has allowed the identification of periodontitis-associated uncultured and fastidious species, providing a more detailed look at the bacterial communities in periodontal tissues1. FISH can be applied for the detection of pathogenic microorganisms in primary material, which further shortens the time-to-result by saving the time for culturing and allows identification at genus and species level. Therefore, in mixed-species infections, FISH can help to identify the key pathogens and thus underpin the initiation of an effective pathogen specific antibiotic Therapy[12].

In dentistry, FISH has been mainly used as a research tool to study the interrelationship between different oral bacteria in in vitro created biofilm (13-17) and to study the invasive capability of these bacteria on buccal epithelial cells. Bhat et al 12 conducted a study to evaluate the applicability of FISH technique for the detection of Aa in 77 healthy and 77 patients suffering with chronic periodontitis. The data obtained after the procedure revealed that plaques from 84.5% of healthy individuals and 98.7% of chronic periodontitis showed the presence of Aa. The author proposed that this method can be directly applied to the clinical samples and can be used as a rapid diagnostic tool in periodontics.

Thus In this study ,the microbial analysis was carried out using (FISH) technology so as to visualize, identify and quantitate 3 microrganisms- Porphyromonas gingivalis (known Periodontal pathogen), Filifactor Alocis(Novel pathogens), Synergistes species (Novel pathogens) from subgingival plaque samples of patients suffering from chronic periodontitis.

Local drug delivery systems allow the therapeutic agents to be targeted to the disease site and with the local drug delivery system, we can adjust the dose which helps in reduction of systemic absorption and subsequent risk of adverse side effects . Higher concentration of a therapeutic agent can be attained in subgingival sites by local drug delivery compared with a systemic drug regimen[18].

Among the various local drug delivery agents used Chlorhexidine(CHX) has long been the gold standard for subgingival chemical plaque control regimens 19. It shows a broad spectrum of topical antimicrobial activity, safety, effectiveness, substantivity and lack of toxicity . CHX is a well established effective agent in plaque inhibition, and has added advantage of substantivity, safety, ease of use and economical15.Goswami et al20 conducted a study to compare and evaluate the clinical and microbiological effects of subgingival administration of CHX gel when used as an adjunct to SRP(experiment group) and SRP alone(control group) in patients with chronic periodontitis. CHX gel was shown to improve the clinical and microbiological parameters compared to SRP alone proving that it is an efficacious adjunct to SRP in the treatment of chronic periodontitis. This was in accordance with results observed from the study conducted by Jain et al21 to evaluate the clinical effectiveness of Xantham based CHX gel as an adjunct to SRP(experiment group) and SRP alone(control group) in the treatment of chronic periodontitis. In this study CHX gel(Hexigel) was used as one of the local drug delivery agent adjunct to SRP in the treatment of chronic periodontitis. Group C showed statistical significant results in all the clinical parameters assessed compared to Group A at the end of 12 weeks.

Emphasis on usage of herbal agent such as propolis, have gained popularity in recent times.Propolis, nature's powerful antibiotic and healing substantial, is natural resinous product extracted by honeybees (Apis mellifera) from botanical provenance to protect the hive from invasion and infection. Propolis is rich in a wide range of bioflavonoids and has been used since ancient times for its pleiotropic properties. Test tube and in vivo studies have shown various antioxidant, anti-inflammatory, and anticancer properties of propolis22-24.Antibiotic properties of propolis are retained in commercial formulations, as 1 mg/ml has exhibited the minimum inhibitory concentration of propolis extract across various periodontopathogens25. Nano-Bio Fusion (NBF) gingival gel used in this study is a patented scientifically formulated, bioadhesive antioxidant gel harvesting naturally occurring antioxidants for targeted action. It mainly acts based on the NBF technology which allows the ultrafine antioxidants to surpass the moist intraoral environment, enter the cells and rejuvenate, revitalize, support, protect, and optimize gum and soft oral tissue. Debnath et al10 conducted a study to evaluate clinically and microbiologically the effectiveness of locally delivered NBF technology gel as an adjunctive therapy to scaling and root planing (SRP) in the treatment of chronic periodontitis. Result showed that from baseline to a period of 3 months, all the clinical parameters showed statistically significant difference between both groups along with the significant reduction of colony-forming units of aerobic periodontopathogens. it was concluded that Locally delivered NBF gel exhibited a significant improvement compared with SRP alone in treatment of chronic periodontitis. This was in accordance with studies of Srivastava et al26. The present study was conducted to evaluate the clinico- microbiological effectiveness of locally delivered nanobiofusion gel(Group B) and chlorhexidine gel(Group C) as an adjunct to SRP(Group A) in the treatment of chronic periodontitis. Results showed significant reduction in plaque index (PI) in all the three groups(Group A, Group B, Group C) from baseline and at 12 weeks. Group B and Group C showed significantly higher difference than Group A at the end of 12 weeks. Group B and Group C showed no significant difference at the end of 12 weeks. Changes in PI scores were dependent on patients' compliance. Group B(SRP+NBF gel) showed statistical significant results compared to Group A(SRP alone). Results from the current study was in accordance with the study conducted by Ashok et al where there was significant reduction in PI score after the subgingival application of propolis extract .These observations were in agreement with a study conducted by Koo et al in which propolis solution rinse significantly decreased the plaque score as compared to placebo and, also stated that the mechanism of plaque reduction of propolis is by the inhibition of glucosyltransferases enzyme which is required for plaque formation. Group C(SRP+CHX) showed statistically significant Results compared to Group A(SRP alone).Results from the current study was in accordance to the study

conducted by Goswami et al.,2021 wherein CHX adjunct to SRP showed statistically significant results at the end of 3 months compared to SRP alone. This could be due to the antiplaque and antimicrobial activity of CHX

There was statistically significant reduction in gingival bleeding(Modified sulcus bleeding Index(MSBI) observed in all the three groups(Group A ,Group B, Group C)from baseline and at 12 weeks.Group B and Group C showed statistically significant difference compared to A at the end of 12 weeks. However, Group B and Group C the results were not statistically significant at the end of 12 weeks.Group B(SRP+NBF gel) showed statistical significant results compared to Group A(SRP alone).

Results from the current study was in accordance to study conducted by Debnath et al.10 where Group B(SRP+NBF gel) showed statistically significant difference at 6 weeks interval and clinically significant difference at 3 months period compared to Group A (SRP alone).This can be attributed to the diverse characteristic property of propolis present in the NBF gel.Propolis as an anti-inflammatory agent has shown to inhibit synthesis of prostaglandins, activate the immune system by promoting phagocytic activity, stimulate cellular immunity, and facilitate healing effects on epithelial tissues. Group C(SRP+CHX) showed statistically significant results compared to Group A(SRP alone).Results from the current study was in accordance with the study conducted by goswami et al.20 where statistically significant results were observed in the experimental sites (SRP+CHX) compared to control sites(SRP alone).

In the present study, Probing Pocket depth(PPD) and CAL showed statistically significant reduction in all the three groups from baseline and at 12 weeks. Group B and Group C showed significantly higher difference compared to A at the end of 12 weeks. Group B and Group C showed no statistical significant difference at the end of 12 weeks. Group B(SRP+NBF gel) showed statistically significant results compared to Group A(SRP alone). Results from the current study was in accordance with study conducted by Debnath et al10., where NBF gel with an appropriate drug carrier agent at baseline has shown a statistically significant reduction in PD,CAL . According to the results of the clinical studies by Gebara et al., Coutinho, Sanghani et al , Propolis had anti-inflammatory substances which causes tissue suppression of leukotrienes and prostaglandins synthesis by macrophages and have inhibitory effects on myeloperoxidase activity. The radical scavenging effect of ethanolic extract of propolis is equal in effectiveness when compared to that of Vitamin C, removal of radicals by flavonoids in propolis along with its anti-inflammatory response aids in tissue healing and regeneration. Group C showed statistically significant results compared to Group A. Results from the current study was in accordance with study conducted by Goswami et al.,20 where experiment group(SRP+CHX) showed statistically significant differences in CAL and PPD compared to control group(SRP alone).Vaish et al30 (2016) conducted a study on 1.5% CHX gel. They found that the use of the CHX gel and chip, when used as an intra-sulcular antimicrobial agent, significantly reduced the PPD and Relative Attachment Level(RAL) from the baseline to 3 months.

In the present study, microbial analysis using FISH , identified, visualized and quantified microrganisms present in the plaque sample. Results showed significant reduction in the microbial count of F.alocis, P.gingivalis, Synergistes in all the 3 groups from baseline and at 6 weeks. Intergroup assessment of F.alocis ,in Group A , 20% had no bacterial count at base line and in the sixth week it has increased to 40%.In group B, 40% had no bacterial count at the base line and at sixth week, increased to 80% .In group C, 30% had no bacterial count at the base line and in the sixth week it has increased to 70%.On inter group assessment of Filifactor Alocis there was no significant differences between the groups(Group A, Group B, Group C(p>0.05).

On intergroup assessment of P.gingivalis ,in group A ,10% had no bacterial count at baseline and in the sixth week it has increased to 30%. In group B, 0% had no bacterial count at the base line and at the sixth week it has increased to 40%.In group C, 0% had no bacterial count at the base line and in the sixth week it has increased to 60%. On intergroup assessment at 6th week for P.gingivalis, in Group C 60% had no bacterial count followed by 40% in Group B, and 0% in Group A. It was concluded that Group C(SRP+CHX) has shown to be more efficient in reducing the bacterial count(P.gingivalis) when compared to other groups.

On inter group assessment of Synergistes ,in group A there was no statistically significant difference between the groups(Group A ,Group B, Group C).In group B, 30% had no bacterial count at the base line . At sixth week it has increased to 50%. In group C, 30% had no bacterial count at base line and at sixth week ,increased to 80%.On inter group assessment at 6th week for Synergistes in Group C 80% had no bacterial followed by 50% in Group B, and 0% in Group A. It was concluded that group C,(SRP+CHX) has shown to be more efficient in reducing the bacterial count(Synergistes) when compared to other groups.

In the present study, both group B and group C showed progressive improvements in all the clinical and microbiological parameters on evaluated time periods.The present study influenced the beneficial outcome of propolis along with Vitamin C and E and the nanotechnology amplified this effect in preventing disease progression. CHX gel had significantly reduced probing depth and microbial count at the end of the study period, suggesting the effectiveness of the CHX gel as an adjunct to SRP. From the above studies it can be noted that both NBF gel and CHX gel has improved the clinic-microbiological parameters when used as an adjunct to scaling and root planning.Further research with a larger sample size is warranted to have a better understanding of the effectiveness of NBF gel in the protection of periodontium

Limitations of the study

1.Small sample size
2.The study was site specific ,so there could be bias
3.Microbiological assessment of only 2 novel periodontopathogen were analysed
4.Patients were followed up only for 12 weeks.

CONCLUSIONS

Within the limitations of study NBF gel and CHX gel has shown to improve the clinico-microbiological parameters when used as an adjunct to scaling and root planning. The study proved that CHX gel as a chemotherapeutic agent showed both bacteriostatic and bactericidal activity substantially decreasing the bacterial load in subgingival plaque when placed locally in the periodontal pocket. The study influenced the beneficial outcome of propolis along with Vitamin C and E and the nanotechnology amplified this effect in preventing disease progression. The results indicate anti-inflammatory, antibacterial, and antioxidant property of the NBF gel. Therefore CHX and NBF gel can be used as an adjunct to SRP in improving the periodontal status of an individual. FISH technique was simple, rapid and can be easily adaptable with high sensitivity and has the ability to detect a single bacterial cell. This technique can be directly applied to the clinical samples and can be used as a rapid diagnostic tool in periodontics.

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