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A Comparative Clinical, Microbiological and Biochemical Evaluation of Guided Periodontal Pocket Recolonisation (GPR) using Synbiotics as an Adjunct to Scaling and Root Planing in Patients with Chronic Periodontitis: A Pilot Project

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ABSTRACT

Background: *With increase in the resistance to antibiotics, the paradigm of periodontal treatment in recent years is slowly shifting from specific bacteria elimination to altering bacterial ecology by probiotics. With this objective in mind, the present study was carried out to assess the use of a symbiotic preparation as a guided pocket recolonisation (GPR) procedure in patients with chronic periodontal disease.* ***Materials and methods:*** *Total 180 patients with chronic periodontitis with presence of true periodontal pockets; 4 mm-6 mm in depth involving minimum 3 or more quadrants were selected and divided into 3 groups wherein the first group only scaling and root planing was done without GPR application; in the 2nd group, GPR was carried out as a single application following SRP and in the 3rd group, multiple applications of GPR with SRP was done. Clinical measurements; microbiological analysis of periodontal pathogens by anaerobic culture and biochemical assessment of alkaline phosphatase and interleukin-6 using ELISA and spectrophotometry was carried out at baseline, 3 and 6 months respectively.* ***Results:*** *All the 3 groups showed significant changes in clinical ($p<0.05$) as well as in microbiological and biochemical parameters ($p<0.05$) within the groups. Intergroup comparisons revealed significant changes in group 2 and 3 with biochemical parameters. Positive correlation was observed with clinical parameters and alkaline phosphatase levels and P. intermedia counts in group 1 and 3.* ***Conclusion:*** *Within the limitations of the present study, it could be concluded that symbiotic therapy may have some additional benefit to Scaling and root planing.*

Keywords: Synbiotics, Guided pocket recolonisation, Chronic periodontitis, Scaling, Root planing

INTRODUCTION

The current concept concerning the etiology of periodontitis considers 3 groups of factors that determine whether active periodontitis will occur in a subject: a susceptible host, the presence of pathogenic species and the absence of so called "beneficial bacteria" [1]. Beneficial species of the indigenous oral microbiota and their role in epithelial colonization of oral pathogens are largely unexplored. The worldwide treatment strategy applied for periodontal disease is based on mechanical subgingival debridement eventually including periodontal surgery to reduce the depth of the periodontal pocket improving the oral hygiene. This shifts the sub gingival flora to a less pathogenic composition, characterized by high proportions of gram-positive aerobic species.

Although reductions in the total sub gingival microbiota of up to two-log values can easily be achieved, a recolonization, primarily by less pathogenic bacteria, towards baseline numbers occurs within 1-2 weeks [2]. The shift towards a less pathogenic microbiota is only temporary, with the re-establishment of a more aggressive microbiota within weeks to months. The dynamics of this recolonization depends on the level of oral hygiene, the efficacy of the sub gingival debridement and the residual probing depth. The use of antibiotics or antiseptics, either locally or systemically, does not really improve the long-term effect of periodontal therapy. Therefore, some authors have started to focus on the third etiological factor for plaque-related periodontal inflammation, namely the reduction or absence of so-called

beneficial bacteria [3]. The term "probiotic" literally means "for life" and is used when referring to bacteria associated with beneficial effects on humans and animals.

The use of microorganisms to promote health is very ancient and can even be traced back to the classical Roman literature where food fermented with microorganisms was used as a therapeutic agent. Research in the probiotic area has progressed considerably in the last 20 years, and significant advances have been made in the selection and characterization of specific probiotic cultures and substantiation of health claims relating to their consumption [4].

The term 'probiotics', the antonym of the term 'antibiotics', was introduced by Lilly and Stillwell as 'Substances produced by microorganisms which promote the growth of other microorganisms' [5]. The currently used consensus definition of probiotic was put forward by the World Health Organization and by the Food and Agriculture Organization of the United States in 2001. They defined probiotics as 'Live microorganisms which when administered in adequate amounts confer a health benefit on the host' [6]. It is clear that this definition restricts the use of the word probiotic to products that contain live microorganisms and points out the need for providing an adequate dose of probiotic bacteria in order to exert the desirable effects.

Prebiotics

'Prebiotics' on the other hand, are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and /or activity of one or a limited number of bacterial species already established in the colon, and thus in effect improve host health. These include inuline, fructo-oligosaccharides, galactooligosaccharides and lactulose. Combinations of prebiotics and probiotics have been suggested to have additive and synergistic effects in providing better oral health [7].

Synbiotics are defined as 'mixtures of probiotics and prebiotics that beneficially affect the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract of the host'. According to WHO synbiotics are live microorganisms present in digestible food ingredients, which, when administered in adequate amounts, beneficially affects the host by selectively stimulating the growth or activity of one or a limited number of bacterial species already established in the colon, thus in effect improve host health and confer a health benefit on the host [8].

Synbiotics

A combination of prebiotics and probiotics as mentioned earlier; can help prevent and treat disease through several mechanisms.

1. Direct interaction
2. Competitive exclusion
3. Modulation of host immune response

The most commonly-used synbiotic strains belong to the genera, *Lactobacillus*, *Bifidobacterium* [9-11] and *Streptococcus* [4]

Proposed Mechanism

Synbiotics lower the pH so that bacteria cannot form dental plaque and calculus that causes the periodontal disease. They make an excellent maintenance product because they produce antioxidants. Antioxidants prevent plaque formation by neutralizing the free electrons that are needed for the mineral formation.

Current literature reports very few studies on the use of synbiotics for periodontal health [2]. Studies have shown that certain gut bacteria can exert beneficial effects in the oral cavity by inhibiting pathogenic species. The concept of periodontal replacement therapy, first proven by Teughels, et al. [4] consisted of applying beneficial oral bacteria subgingivally to prevent recolonization of periodontal pockets by pathogens after scaling and root planing. Given the emergence of antibiotic resistance and the lack of non-antibiotic treatment options, A "Guided Pocket Recolonization" (GPR) approach may provide a valuable addition or alternative to the armamentarium of treatment options for periodontitis. With this objective in mind, the present study was carried out to assess and evaluate the use of a synbiotic preparation as a guided pocket recolonisation (GPR) procedure in patients with chronic periodontal disease.

The synbiotic preparation used in this study was BIFILACR which is a preparation consisting of (*Streptococcus faecalis*, *Clostridium butyricum*, *Bacillus mesentericus*, and *Lactobacillus sporogenes*).

MATERIAL AND METHODS

The study which was carried out for a period of 1 year; included 180 patients with chronic periodontitis (localized or generalized) visiting the outpatient department of Periodontics, at a Dental institute in Bangalore, India. Ethical clearance was obtained from the ethical committee of the Institute as well as informed consent from the patients participating in the study.

Inclusion Criteria

- Patients who were systemically healthy
- Patients of both sexes aged between 25-45 years
- Patients with chronic periodontitis (mild to moderate) with presence of true periodontal pocket; 4-6 mm in depth
- Presence of any systemic or debilitating diseases
- Pregnant or Lactating women
- A recent history or presence of any acute or chronic infections
- Patients with history of any drug intake including antibiotics, analgesics or any other drugs three months prior to study
- Patients who had undergone periodontal therapy in the last six months
- Patients who were smokers/paan/tobacco/betel nut users
- Patients who were physically or mentally challenged

Clinical Assessment

The following clinical parameters were recorded.

- Gingival index (GI) by Loe and Silness [12]
- Plaque index (PI) by Loe [13]
- Probing pocket depth (PPD)
- Probing pocket depth (PPD) was recorded by using Williams's periodontal probe by noting the distance from gingival margin to base of the pocket

The 180 patients were randomly divided into the following three groups:

Control group: Sites/pockets in this group were treated by scaling and root planing only.

Test group 1: Sites/pockets in this group were treated by scaling and root planing and GPR procedure (intrapocket synbiotic application) at baseline only.

Test group 2: Sites/pockets in this group were treated by scaling and root planing and GPR procedure (intrapocket synbiotic application) at baseline (0), 1 week, 2 weeks and 4 weeks.

The clinical parameters (viz. GI, PI, PPD) were assessed at baseline (0), 3 months and 6 months postoperatively. All patients were subjected to scaling and root planing with the help of Gracey curettes at baseline. Plaque and GCF samples were collected prior to scaling and root planing.

Patients were recalled after 3 months and 6 months during which plaque and GCF samples were again collected and clinical parameters were recorded.

Microbiologic Assessment

Plaque samples in all three groups were collected from the deepest part of the pocket with the help of a curette at

baseline and at 3 months and 6 months postoperatively and stored in Eppendorf tubes containing sterile distilled water and stored at -40°C and were later assessed by anaerobic culture.

After incubation for 30 minutes, the plaque samples were inoculated onto blood agar plates. The plates were then arranged in the rack that was later immediately placed in the anaerobic jar. The jar was then kept in the incubator at 37°C for 3 days and was opened after 3 days. Organisms were provisionally identified based on their colony characteristics and the colony counts were recorded.

The micro-organisms assessed were:

- *Aggregatibacter actinomycetemcomitans*
- *Porphyromonas gingivalis*
- *Tanerella forsythia* and
- *Prevotella intermedia*

Biochemical Evaluation

Gingival Crevicular (GCF) fluid samples from the selected sites in all three groups were collected using micro-capillary pipettes at baseline, 3 and 6 months postoperatively and kept frozen at -40°C until analysis. Interleukin-6 (IL-6) and Alkaline phosphatase (ALP) were analysed in these samples using ELISA kit and photo cytometry respectively.

The clinical, microbiological and biochemical parameters in all the groups were statistically analysed by Students'' test using SPSS version 19.0 software. Descriptive analysis that included mean, standard deviation and percentages were found for each parameter in three groups and were used for analysis.

1. Within each group, paired t- test was performed to compare post treatment changes from baseline
2. For comparison of variation between groups unpaired t-test was performed
3. A 'P' value of 0.05 or less was considered statistically significant

RESULTS

Clinical Parameters

Gingival index (GI): There were statistically significant differences within all the three groups (intragroup) in the GI scores ($p<0.05$), however, no significant differences were observed among all the 3 groups (intergroup) at the end of 3 months and 6 months ($p>0.05$) (Tables 1 and 2).

Table 1 Intra group comparison between various clinical parameters

Groups	Time Intervals	GI			PI			Probing Depth		
		mean	SD	p-value	mean	SD	p-value	mean	SD	p-value
Group 1	Baseline	0.65	0.28819	0.00	2.0167	0.62073	0.00	5.373	0.5455	0.00
	3 months	0.5167	0.21524		1.8167	0.4736		5.147	0.3964	
	Baseline	0.65	0.28819	0.00	2.0167	0.62073	0.00	5.373	0.5455	0.00
	6 months	0.45	0.13654		1.533	0.3428		4.78	0.412	
	3 months	0.5167	0.21524	0.01	1.8167	0.4736	0.00	5.147	0.3964	0.00
	6 months	0.45	0.13654		1.533	0.3428		4.78	0.412	

Group 2	Baseline	0.65	0.28819	0.00	2.0167	0.62073	0.00	5.373	0.5455	0.00
	3 months	0.5167	0.21524		1.8167	0.4736		5.147	0.3964	
	Baseline	0.65	0.28819	0.00	2.0167	0.62073	0.00	5.373	0.5455	0.00
	6 months	0.45	0.13654		1.533	0.3428		4.78	0.412	
	3 months	0.5167	0.21524	0.01	1.8167	0.4736	0.00	5.147	0.3964	0.00
	6 months	0.45	0.13654		1.533	0.3428		4.78	0.412	
Group 3	Baseline	0.6833	0.26786	0.00	2.05	0.65871	0.00	5.487	0.4827	0.00
	3 months	0.5333	0.20308		1.8167	0.4736		5.227	0.3324	
	Baseline	0.6833	0.26786	0.00	2.05	0.65871	0.00	5.487	0.4827	0.00
	6 months	0.4667	0.12577		1.533	0.3428		4.833	0.3977	
	3 months	0.5333	0.20308	0.01	1.8167	0.4736	0.00	5.227	0.3324	0.00
	6 months	0.4667	0.12577		1.533	0.3428		4.833	0.3977	

Table 2 Intergroup comparison of various clinical parameters

Clinical Parameters														
Time period	**Groups**	**Groups compared to**	**Gingival Index**				**Plaque Index**				**Probing Depth**			
			mean	**SEM**	**p -value**	**inf**	**mean**	**SEM**	**p -value**	**inf**	**mean**	**SEM**	**p -value**	**inf**
At 3 months	1	2	0	0.03857	1	Not significant	0	0.08647	1	Not significant	0	0.0687	1	Not significant
		3	-0.01667	0.03857	0.902		0	0.08647	1		-0.08	0.0687	0.476	
	2	3	-0.01667	0.03857	0.902		0	0.08647	1		-0.08	0.0687	0.476	
At 6 months	1	2	0	0.02429	1		0	0.0626	1		0	0.0744	1	
		3	-0.01667	0.02429	0.772		0	0.0626	1		-0.0533	0.0744	0.754	
	2	3	-0.01667	0.02429	0.772		0	0.0626	1		-0.0533	0.0744	0.754	

Correlation assessment showed significant negative correlation of GI scores with PG counts in group 1 and IL-6 levels at baseline ($p<0.05$); and with PI counts and IL-6 levels at 6 months ($p<0.05$). On the contrary, significant positive correlation was observed with PI counts and ALP levels at 3 months ($p>0.01$) and also ALP levels at 6 months ($p<0.05$). With group 2, significant negative correlation was observed with IL-6 at baseline; PG and PI counts and IL-6 levels at 3 months as well as PI counts and IL-6 at 6 months ($p<0.05$). In contrast, significant positive correlation with ALP at baseline, 3 months and 6 months was observed ($p<0.01$). In group 3, significant negative correlation was found with IL-6 levels and ALP levels at baseline and 3 months ($p<0.05$); and PG counts and IL-6 levels at 6 months ($p<0.05$). However, significant positive correlation was seen with PI counts at 3 months ($p<0.05$) (Table 3).

Plaque index (PI): Statistically significant differences in PI scores ($p<0.05$) at all-time intervals i.e. from baseline to 3 months, baseline to 6 months and from 3 months to 6 months were observed in all the 3 groups. However, no significant differences were observed in the PI scores among all the groups at the end of 3 months and 6 months ($p>0.05$) (Tables 1 and 2).

Correlation assessment of PI scores in group 1 showed significant positive correlation with PG counts at baseline ($p<0.05$). In group 2, significant negative correlation was observed with ALP levels at baseline and 3 months ($p<0.05$); whereas significant positive correlation was found with PG counts at baseline ($p<0.001$) only. In group 3, significant negative correlation was observed with PG counts at 3 months and 6 months ($p<0.05$); and positive correlation with PI counts at 6 months ($p<0.001$).

Probing pocket depth (PPD): Statistically significant differences in PPD ($p<0.05$) at all-time intervals i.e. from baseline to 3 months, baseline to 6 months and from 3 months to 6 months were observed in all 3 groups. However,

Table 3 Clinical parameters correlated with microbiological parameters and biochemical parameters

Clinical Parameters			Gingival Index					Plaque Index					Probing Pocket Depth				
Groups	Time intervals		P.G	P.I	IL-6	ALP	Inf	P.G	P.I	IL-6	ALP	Inf	P.G	P.I	IL-6	ALP	Inf
1	Baseline	r	-0.360**	0.028	-0.348**	0.238	***: Negatively correlated and significant ****: Positively correlated and Significant	0.285*	0.105	-0.090	0.170	***: Negatively correlated and significant ****: Positively correlated and Significant	0.033	0.049	0.368**	-0.079	***: Negatively correlated and significant ****: Positively correlated and Significant
		p	0.005***	0.834	0.006***	0.067		0.027****	0.426	0.493	0.193		0.804	0.712	0.004****	0.546	
	3 months	r	0.006	0.365**	-0.223	0.486**		-0.193	-0.065	0.233	0.215		0.165	0.188	-0.118	0.092	
		p	0.963	0.004	0.087	0.000****		0.140	0.622	0.073	0.099		0.209	0.149	0.370	0.485	
	6 months	r	0.143	-0.270*	-0.306*	0.285*		-0.004	-0.043	0.242	0.062		0.171	0.435**	-0.085	-0.089	
		p	0.276	0.037***	0.017***	0.027****		0.975	0.747	0.063	0.635		0.191	0.001****	0.520	0.497	
2	Baseline	r	0.002	-0.069	-0.247	0.484**	***: Negatively correlated and significant ****: Positively correlated and Significant	0.476**	0.195	-0.214	-0.378**	***: Negatively correlated and significant ****: Positively correlated and Significant	-0.346**	-0.089	-0.342**	-0.419**	*** Negatively correlated and significant ****: Positively correlated and Significant
		p	0.988	0.600	0.057***	0.000****		0.000****	0.136	0.101	0.003***		0.007***	0.501	0.008***	0.001***	
	3 months	r	-0.339**	-0.310*	-0.233	0.554**		0.223	-0.130	-0.191	-0.425**		-0.098	-0.189	-0.453**	-0.176	
		p	0.008***	0.016***	0.073	0.000		0.086	0.322	0.144	0.001***		0.456	0.148	0.000***	0.178	
	6 months	r	-0.178	-0.261*	-0.383**	0.329*		0.041	-0.196	-0.232	-0.051		-0.105	-0.098	-0.151	-0.030	
		p	0.173	0.044***	0.002***	0.010****		0.757	0.134	0.075	0.699		0.423	0.458	0.250	0.818	
3	Baseline	r	0.043	0.173	-0.449**	-0.317*	***: Negatively correlated and significant ****: Positively correlated and Significant	-0.147	0.187	0.092	0.100	***: Negatively correlated and significant ****: Positively correlated and Significant	-0.013	0.041	0.108	0.285*	***: Negatively correlated and significant ****: Positively correlated and Significant
		p	0.742	0.185	0.000***	0.013***		0.262	0.152	0.482	0.447		0.923	0.758	0.411	0.027****	
	3 months	r	-0.146	0.310*	-0.422**	-0.274*		-0.346**	0.066	0.135	-0.128		-0.016	0.035	0.059	0.342**	
		p	0.265	0.016****	0.001***	0.034***		0.007***	0.614	0.302	0.328		0.904	0.790	0.652	0.007****	
	6 months	r	-0.246	-0.024	-0.346**	0.094		-0.303*	0.468**	0.033	-0.003		0.059	0.081	0.153	-0.340**	
		p	0.059***	0.853	0.007***	0.477		0.019***	0.000****	0.804	0.984		0.657	0.538	0.245	0.008***	

no significant differences were observed in the PPD values among all the groups at the end of 3 months and 6 months ($p>0.05$) (Tables 1 and 2).

Correlation assessment of PPD in group 1 showed significant positive correlation with IL-6 levels at baseline and PI counts at 6 months ($p<0.005$). In group 2, significant negative correlation was observed with PG counts, IL-6 and ALP levels at baseline ($p<0.05$) and with IL-6 levels at 3 months ($p<0.001$). In group 3, significant negative correlation was seen in ALP levels at 6 months ($p<0.01$) whereas significant positive correlation was found in ALP levels at baseline and 3 months ($p<0.05$) (Table 3).

Microbiological parameters: It was attempted to assess *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tanerella forsythia* and *Prevotella intermedia* but *Aggregatibacter actinomycetemcomitans*, and *Tanerella forsythia* were not detected in any of the samples in all the groups. Hence statistical analysis was carried out only for *Porphyromonas gingivalis* (PG) and *Prevotella intermedia* (PI) (Tables 4 and 5).

This difference in PG CFU and PI CFU was found to be statistically significant ($p<0.05$) at all-time intervals i.e. from baseline to 3 months, baseline to 6 months and from 3 months to 6 months in all 3 groups. However, no significant differences were noted in the *P. gingivalis* CFU and *P. intermedia* CFU among the 3 groups at the end of 3 months and 6 months ($p>0.05$).

Biochemical parameters: The difference in IL-6 levels and ALP levels were found to be statistically significant ($p<0.05$) at all-time intervals i.e. from baseline to 3 months, baseline to 6 months and from 3 months to 6 months. However, intergroup comparisons revealed significant differences in group 2 and 3 with regard to IL-6 levels ($p<0.001$) at 3 and 6 months and in group 3 only with regard to ALP levels at 3 months ($p<0.001$) and 6 months respectively ($p<0.05$) (Tables 4 and 5).

Table 4 Intra group comparison between microbiologic and biochemical parameters

Groups	Time Intervals	*P. gingivalis*			*P. intermedia*			Interleukin-6			Alkaline Phosphatase		
		mean	SD	p -value	mean	SD	p -value	mean	SD	p -value	mean	SD	p -value
Group 1	Baseline	66666.67	14695.401	0.00	33333.33	21286.318	0.00	2.7407	0.42663	0.00	3.1753	1.15514	0.00
	3 months	43066.667	12103.882		21000	14884.869		2.064	0.43246		2.4307	1.01339	
	Baseline	66666.67	14695.401	0.00	33333.33	21286.318	0.00	2.7407	0.42663	0.00	3.1753	1.15514	0.00
	6 months	22200	4860.076		11104	7209.5575		1.3813	0.38802		1.55	0.82291	
	3 months	43066.667	12103.882	0.00	21000	14884.869	0.00	2.064	0.43246	0.00	2.4307	1.01339	0.00
	6 months	22200	4860.076		11104	7209.5575		1.3813	0.38802		1.55	0.82291	
Group 2	Baseline	65666.67	15117.244	0.00	33333.33	21286.318	0.00	3.4333	1.02425	0.00	2.922	0.50375	0.00
	3 months	41966.67	13567.742		21133.33	14746.981		2.6087	0.97615		2.198	0.54625	
	Baseline	65666.67	15117.244	0.00	33333.33	21286.318	0.00	3.4333	1.02425	0.00	2.922	0.50375	0.00
	6 months	21933.33	9878.699		10333.33	5473.098		1.7287	0.81049		1.4787	0.46249	
	3 months	41966.67	13567.742	0.00	21133.33	14746.981	0.00	2.6087	0.97615	0.00	2.198	0.54625	0.00
	6 months	21933.33	9878.699		10333.33	5473.098		1.7287	0.81049		1.4787	0.46249	
Group 3	Baseline	63940	14956.108	0.00	33333.33	21286.318	0.00	3.7673	0.6116	0.00	3.752	0.70594	0.00
	3 months	39646.67	11863.967		21666.67	15274.513		2.866	0.84763		2.8653	0.78876	
	Baseline	63940	14956.108	0.00	33333.33	21286.318	0.00	3.7673	0.6116	0.00	3.752	0.70594	0.00
	6 months	17446.67	6478.006		9100	4411.848		1.7447	0.74057		1.86	0.76373	
	3 months	39646.67	11863.967	0.00	21666.67	15274.513	0.00	2.866	0.84763	0.00	2.8653	0.78876	0.00
	6 months	17446.67	6478.006		9100	4411.848		1.7447	0.74057		1.86	0.76373	

Table 5 Intergroup comparison of microbiologic and biochemical parameters

Time period	Groups	Groups compared to	*P. gingivalis*				*P. intermedia*				Interleukin-6				Alkaline Phosphatase			
			mean	SEM	p-value	inf	mean	SEM	p-value	inf	mean	SEM	p-value	inf	mean	SEM	p-value	inf
At 3 months	1	2	1100	2288.4767	0.881	Not significant	-133.3333	2733.2185	0.999	Not significant	-0.54467*	0.1437	0.001	*significant at 3 months group 2 and 3	0.23267	0.1471	0.256	*significant at 3 months group 3
		3	3420	2288.4767	0.296		-666.6667	2733.2185	0.968		-0.80200*	0.1437	0		-0.43467*	0.1471	0.01	
	2	3	2320	2288.4767	0.569		-533.3333	2733.2185	0.979		-0.25733	0.1437	0.176		-0.66733*	0.1471	0	
At 6 months	1	2	266.6667	1346.4918	0.979		770.6667	1061.4295	0.748		-0.34733*	0.12274	0.014	significant at 6 months group 2 and 3	0.07133	0.12799	0.843	*significant at 6 months group 3
		3	4753.33*	1346.4918	0.002		2004	1061.4295	0.145		-0.36333*	0.12274	0.01		-0.31000*	0.12799	0.043	
	2	3	4486.67*	1346.4918	0.003		1233.333	1061.4295	0.478		-0.016	0.12274	0.991		-0.38133*	0.12799	0.009	

DISCUSSION

Periodontitis is a multifactorial disease that encompasses the hard and soft tissue, microbial colonization (with or without invasion), inflammatory responses and adaptive immune responses. The complexity of the local tissue components, including bacteria and/or their products and virtually all aspects of host response mechanisms, has complicated our ability to elucidate the critical protective functions in the tissues and has continually provided evidence for the potential of host destructive factors as the ultimate causative parameters in the disease [14]. Conventional treatment modalities of periodontal disease include non-surgical and surgical management, which emphasizes mainly on mechanical debridement, often accompanied by antibiotics. These treatment modalities are aimed at eliminating the entire microflora irrespective of their pathogenicity. Due to the emergence of antibiotic resistance and frequent recolonization of treated sites with pathogenic bacteria, need for an alternate treatment paradigm eliminating the adverse effects of the above methods was felt. This was fulfilled by the introduction of synbiotics and bacterial replacement therapy in the field of periodontics [15].

The concept of bacterial replacement therapy also known as "probiotic therapy" was first introduced in periodontics by Teughels, et al. where it was referred to as guided pocket recolonisation (GPR) [4]. They reported that the subgingival application of a bacterial mixture including *Streptococcus sanguis*, *Streptococcus salivarius*, and *Streptococcus mitis* following scaling and root planing significantly suppressed the re-colonization of Porphyromonas gulae (canine *P. gingivalis*) and *P. intermedia* in a beagle dog model. Nackaerts, et al. [15] also observed that the subgingival application of beneficial oral bacteria (i.e. *Streptococcus sanguinis*, *Streptococcus salivarius* and *Streptococcus mitis*) delayed recolonization by periodontal pathogens, reduced inflammation, and improved bone density and bone levels in a beagle dog model thereby suggesting that this guided pocket recolonization approach may provide a valuable addition or alternative to the wide array of treatment options currently available for periodontitis. Synbiotics (mixtures of probiotics and prebiotics that beneficially affect the host) utilize naturally occurring bacteria to confer a healthy benefit when administered in adequate amounts.

Bifilac®* is a unique preparation containing both prebiotics and probiotics. It has been suggested that Bifilac® improves the patient's condition by performing the following functions:

- Maintaining a healthy balance of microflora in the intestine
- Inhibiting the growth of bacteria
- Providing good bacteria to the gut

*TABLETS (India) limited, Chennai in the form of sachets. Each sachet/capsule contained:

Streptococcus faecalis T-110-30 million (prebiotic), *Clostridium butyricum* TO-A-2 million (prebiotic), *Bacillus mesentericus* TO-A-1 million (prebiotic), *Lactobacillus sporogenes*-50 million (probiotic), Vitamin C-Echinacea extract: Preparation that normalizes gut organisms.

Prevents the occurrence of disbacteriosis, raises the immune reaction of the organism. *Lactobacillus sporogenes* and *Streptococcus faecalis* produce lactic acid and reduce the potentially pathogenic microbes. In addition, *Clostridium butyricum* and *Bacillus mesentericus* also produce butyric acid and acetic acid respectively and reduce the potentially pathogenic microbes.

BifilacR has been extensively researched in the medical field and has been effectively used in [16]

1. Infective diarrhoea/dysentery (viral, bacterial, protozoal)
2. Antibiotic associated diarrhoea
3. Inflammatory bowel disease (ulcerative colitis, chrons disease)
4. Irritable bowel syndrome
5. Travelers diarrhoea

However, their role in periodontics is still in infancy and a complete understanding of the broad ecological changes induced by synbiotics is essential to assess their long-term consequences for oral health and disease [17]. So far, probiotics have been evaluated as a mouth rinse and as tablets in human clinical trials [18,19]. No human study has evaluated its effects when applied within the periodontal pocket as a replacement therapy. Recent research has revealed that probiotics also have a positive influence on reducing the ALP levels and IL-6 levels when used as an adjunct to SRP in the management of chronic periodontitis [20,21]. ALP allows bone mineralization by releasing an organic phosphate and by hydrolysing inorganic pyrophosphate, a potent inhibitor of hydroxyapatite crystal formation and dissolution [22], ALP activity is valuable to clinicians because enzymatic modifications occur at the GCF level earlier than clinically evident modifications associated with tissue destruction [23]. IL-6 has important effects in the response to microbial insults, acting not only as an anti-inflammatory agent (eg, in the down-regulation of neutrophil recruitment and proinflammatory cytokine expression) [24,25] but also as a proinflammatory agent when the inflammatory process becomes chronic [26]. Variability in individuals' ability to synthesize and release IL-6 may affect the susceptibility, development, and progression of a number of autoimmune and inflammatory diseases, such as atherosclerosis, rheumatoid arthritis, and periodontitis, etc. [27]. This study was thus an attempt to clinically evaluate and compare the effectiveness of intrapocket synbiotic application as an adjunct to scaling and root planing in patients with chronic periodontitis as a guided pocket recolonisation (GPR) technique/bacterial replacement therapy. In addition, their effectiveness was evaluated through microbiological analysis (anaerobic bacterial culture) and biochemical assessment of interleukin-6 (IL-6) and alkaline phosphatase (ALP).

Within the limitations of the study, following statistical analysis of the results obtained, intragroup comparison showed a significant improvement in gingival index, PI and PPD scores within all the 3 groups from baseline to 3 month and 6 months. This is in accordance with various studies where it was concluded that probiotic therapy [4,28-30] and SRP [31] resulted in reduced gingival inflammation, plaque index and PPD.

Inter group comparison of GI scores, PI and PPD revealed no significant differences between the 3 groups at the end of 3 months and 6 months. This is in contrast to the findings of Martin Cabezas, et al. [28], Dhawan, et al. [29], Vivekananda, et al. [30] who concluded that probiotic therapy was more effective than SRP in reducing gingival inflammation.

With regard to the microbiologic parameters only *P. gingivalis* and *P. intermedia* could bedetected in all the plaque samples whereas *A. acomitans* and *T. forsythia* could not be identified in any of the samples. There is conflicting

evidence with regard to identification of *A. acomitans* and *T. forsythia* in a given plaque sample. Variations may occur due to nature of the plaque sample and also sensitivity of microbiologic test employed [31,32]. Culture may not be a very sensitive option when compared to PCR [33,34]. Intra group comparison revealed a significant improvement in *P. gingivalis* and *P. intermedia* counts with in all the 3 groups from baseline to 3 months and 6 months. This is in accordance with various evidences which concluded that probiotic therapy [32,33] and SRP [34] results in effective reduction in pathogenic periodontal microflora.

Inter group comparison showed no significant differences in *P. gingivalis* and *P. intermedia* counts among the three groups at the end of 3 months and 6 months suggesting that scaling and root planing alone was as effective as single or multiple applications of synbiotics. This is in contrast with the findings of Teughels, et al. [4], Malathi, et al. [35], Suchetha, et al. [36] who concluded that probiotic therapy results in more efficient microbial reduction compared to SRP alone.

With regard to the Interleukin-6 levels, intra group comparison revealed a significant improvement with in all 3 groups from baseline to 3 months and 6 months. This is in line with evidences which concluded that synbiotic therapy [21] and SRP [37] results in improvement of IL-6 levels.

Intergroup comparison showed significant reduction in Interleukin-6 levels at the end of 3 and 6 months in the groups with single and multiple applications of synbiotics were done compared to the group treated with SRP only. These findings are in accordance with the findings of Zhao et al. [38] who concluded that synbiotic therapy results in improvement of IL-6 levels when compared to SRP alone; but contrary to the findings of Twetman, et al. [39].

With regard to the Alkaline Phosphatase levels, intra group comparison revealed a significant improvement in all the 3 groups from baseline to 3 months and 6 months which is in accordance with the evidence which concluded that synbiotic therapy [36] and SRP [40] results in improvement of ALP levels.

Inter group comparison showed significant improvement in Alkaline Phosphatase levels only in the group with multiple symbiotic applications at the end of 3 months and 6 months. This is in line with the findings of various authors [40-42] who concluded that synbiotic therapy results in more effective improvement of ALP values compared to SRP alone.

Reduction in gingival inflammation showed a strong positive correlation with reduced alkaline phosphatase levels at the end of 3 and 6 months in the control group (with only scaling and root planing) and in the group with single probiotic application. This is in accordance with Moosavinasab, et al. [20] who observed reduction in ALP levels and inflammation following probiotic application. Scaling and root planing alone also results in reduced ALP levels [43] as was also observed in our study. Reduction in gingival scores also positively correlated with reduced *P. intermedia* counts at the end of 3 months in the group with multiple probiotic applications. Additionally, reduction in plaque scores positively correlated with reduced counts of *P. intermedia* at the end of 6 months in the group with multiple synbitoic applications'. This is in line with evidence suggestive of the same [30].

Reduced probing depths also positively correlated with *P. intermedia* counts at the end of 6 months in the group treated with scaling and root planing alone which is in line with the findings [44] that reported reduced probing depths and microbial counts following non-surgical periodontal therapy. Additionally, reduced probing depths were also observed to positively correlate with alkaline phosphatase levels at the end of 6 months in the group treated with multiple synbiotic applications. Interestingly, this correlation is reported for the first time in our study as past literature only suggests the role of probiotics in improving periodontal parameters as also reduced alkaline phosphatase levels [45].

Contrary to the above findings, negative correlations were observed between the clinical and the microbial counts and biochemical parameters at some time intervals. These variations may be attributed to the inconsistencies among the patients with regard to oral hygiene and hormonal variations (in females).

Limitations and Future Considerations

Larger sample size involving a larger cross section of the population needs to be carried out for a longer duration of time. Additional microbiological and biochemical parameters could have been added to the study. More sophisticated and sensitive techniques like PCR could have been used since *Aggregatibacter actinomycetemcomitans* and *Tanerella forsythia* could not be detected in our study samples.

CONCLUSION

Within the limitations of the present study it can be concluded that synbiotic therapy may have additional benefit and may contribute to the improvement of clinical, microbiological and biochemical parameters in patients with chronic periodontitis. Although, promising results have been seen with its use in the medical field, further long term cross sectional studies on larger populations could probably provide better insights into credibility of this treatment modality in periodontal practice.

DECLARATIONS

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Conflicts of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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