Research Article

RED BACTERIAL ZONE IN DIABETES DISSOLVED BY FENUGREEK - A RANDOMISED CONTROL TRIAL

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ABSTRACT

Aims: To see the effect of fenugreek as an adjuvant to subgingival scaling and root planning (SRP)on the quantitative assessment of red complex microorganisms and compare it with the group treated with SRP and metformin alone.

Material and Methods: In this study 80 patients were included which was further divided in to two groups. Each group consisted of 40 patients. Group 1 included 40 chronic generalised periodontitis patients with uncontrolled Type 2 DM who received SRP and metformin. Group 2 included 40 chronic generalised periodontitis patients with uncontrolled Type 2 DM, who received SRP, Metformin plus Fenugreek powder. Periodontal parameters like gingival index, Plaque index, bleeding on probing, Pocket depth and clinical attachment levels were assessed at baseline and one month after non-surgical periodontal therapy. Blood samples were collected to assess the levels of glycemic status using FBS and HbA1c. Subgingival plaque samples were also collected to assess the quantitative measurement of red complex microorganisms (Porphyromonas gingivalis, Tanerella forsythia and Treponema denticola) at baseline and after nonsurgical periodontal therapy.

Results: The results showed statistically significant reduction in all the clinical parameters in both the groups. The glycemic status showed statistical significant changes in FBS for both the groups and in HbA1c for group 2 patients alone (P <0.001) when Intra group comparison of Red complex organisms was done between group 1 and group 2 patients, statistical significant reduction was seen for Treponema denticola and Tanerella forsythia and not for Porphromonas gingivalis in group 1 patients whereas there is statistical significant reduction was observed for all the three organisms in group 2 patients(P <0.001).

Conclusion: This study shows that fenugreek powder can be used in diabetic patients to reduce the microbial load and also as an adjuvant to nonsurgical periodontal therapy in chronic generalised periodontitis patients.

INTRODUCTION

Periodontal disease is caused by gram-negative anaerobic periodontopathic subgingival microflora. [1, 2] In recent years, much interest has focused on this subgingival microflora and its role in destructive periodontal disease. The gram-negative putative periodontopathic bacteria (Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola and Aggregatibacter actinomycetemcomitans) are known causative agents for periodontitis[2] and identification of these putative pathogens can act as marker for onset of the periodontal diseases.[3] The presence of gram-negative periodontopathic bacteria and its relation with periodontal disease is widely studied in Type 2 diabetic population.[4] Chronic hyperglycemia in diabetes mellitus (DM) is associated with an increased risk of development of systemic complications over the years, including microangiopathy, neuropathy, nephropathy, microvascular disease, and delayed wound-

healing.[5] Moreover, patients with DM have worse periodontal conditions compared with their non-diabetic counterparts.[6] Persons with poorly controlled DM also present more severe periodontal disease than do those with well-controlled DM. [7] Several mechanisms have been proposed to explain the periodontal etiopathogenesis in patients with DM.[8] Significant differences may be present in the periodontal microbiota between controlled and uncontrolled diabetic with chronic periodontitis.[9] However, there are conflicting findings which have shown that poorly controlled DM individuals may have elevated levels of microbiota,[10] reduced prevalence of pathogenic species, or even a microbiota which is similar to that of non DM individuals.[11] Differences among studies may be explained by the metabolic control used. Scaling and root planing (SRP) therapy, in combination with or without antimicrobial agents, might be effective in reducing periodontal pathogens in persons with DM,[12] although others have reported that SRP alone may not be capable of

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eliminating or reducing Tannerella forsythia and Porphyromonas gingivalis in those individuals. In general, a reduced level of those pathogens is crucial for a good response to periodontal therapy.[13] Analysis of data in the literature shows that it is unclear whether the subgingival microbiota in persons with DM is significantly affected by SRP. In our study, we used fenugreek as an adjunct to nonsurgical periodontal therapy in uncontrolled diabetic patients to see the antidiabetic effect of fenugreek powder on these patients and how this antidiabetic effect is influencing the microbiological changes showing good clinical response to nonsurgical periodontal therapy.

The beneficiary effect of dietary fibres in the management of diabetes have been well recognised. Seeds of fenugreek (Trigonella foenum graecum) are a rich source of fibre. Fenugreek is one of the oldest medicinal plant originating in india and northern Africa. The leaves and seeds of fenugreek are used either as extracts or powder form for medicinal use. Therefore there has been a greater source of awareness of the antidiabetic properties of fenugreek. Several studies have shown the antidiabetic and hypolipedemic effect of oral fenugreek seed powder in experimentally induced diabetic rats and humans trials. Fenugreek also possess antimicrobial property So in this study in addition to antidiabetic effect, the change in the composition of microbiota was also studied by using fenugreek as an adjuvant to nonsurgical periodontal therapy in Type 2 diabetic patients.

Detection of anaerobic bacteria by culture methods are challenging because of their specific growth requirements like anaerobic environment and certain technical barriers.Advances in molecular biology such as polymerase chain reaction (PCR) have enabled the identification of specific bacteria in large number of periodontitis cases. The PCR is a relatively simple, sensitive and rapid test for successful detection of oral anaerobic bacterial pathogens. Therefore, the aim of the present study was to investigate the effect of SRP after one month along with the administration of fenugreek in addition to metformin on the composition of the subgingival microbiota in individuals with Type 2 DM controlled and uncontrolled patients.

MATERIALS AND METHODS

The subjects were randomly selected from the outpatient clinic of the department of periodontics, Thai Moogambigai Dental College and Hospital, Maduravoyal, Chennai. Written consent was taken from each subjects. All participants completed the study. The protocol of this study was approved by the ethical committee of Dr.M.G.R University, Maduravoyal, Chennai, India, according to the declaration of 1975, which was revised in 2000.

This study consists of 80 subjects which is divided in to

Group 1: 40 subjects with chronic generalized periodontitis patients with uncontrolled type 2 Diabetes mellitus treated with metformin alone.
Group 2: 40 subjects with chronic generalized periodontitis patients with uncontrolled type 2 Diabetes mellitus treated with metformin along with fenugreek powder as an adjuvant to scaling and root planing. In the selected patients, detailed medical history was recorded. The treating physicians consent and details of the patients, regarding diabetes control were also obtained. The uncontrolled diabetes mellitus was defined based on HBa1C values more than 8mg/dl. The history of

these diabetic patients selected for the study were more than five years. All the clinical parameters, blood samples were obtained from these subjects at baseline and one month after non-surgical periodontal therapy. Subgingival plaque samples was collected from the deepest pocket at baseline and after one month of non-surgical periodontal therapy using sterile Gracey curette. The duration of the study to procure plaque samples from 80 patients was two months.

Inclusion criteria

- Subjects selected in this study should have chronic periodontitis, with uncontrolledType 2 diabetes mellitus.
- They should have atleast 30% of the sites with clinical attachment level (CAL) ≥4mm, Probing depth (PD) of ≥5mm and Bleeding on Probing (BOP).

Exclusion criteria

- Patients who had undergone periodontal treatment in the past six months
- Those with a history of antibiotic and antiinflammatory drugs administration with in the last three months
- Those with less than 20 remaining natural teeth,
- Subjects who are pregnant
- Subjects with a history of smoking and tobacco consumption were excluded in the study.

Periodontal Treatment and Clinical Measurements

All patients were subjected to a periodontal examination performed in six sites per tooth excluding third molar. Periodontal parameters like

Plaque Index (Silness and loe 1964),
Gingival Index (Loe and silness 1963),
Bleeding on probing (Muhlemann and son 1971),

Pocket depth and clinical attachment level were evaluated. Collection of blood samples were done after a minimum of 10hour of overnight fasting for all individuals at baseline and one month after treatment. After documenting the periodontal status, patients were given oral hygiene instructions and underwent full mouth non-surgical periodontal treatment under local anaesthesia. Group 1 patients were advised to take their regular treatment protocol, that is, metformin tablets as per the instruction of the physician whereas, Group 2 patients were instructed to take prepared fenugreek seed powder (12.5 gms) two times daily before breakfast and lunch along with the regular metformin tablets. After the periodontal treatment, patients were advised to maintain their oral hygiene with proper brushing and flossing technique. [15]

Collection of plaque sample

Subgingival plaque samples was collected in both the groups of patients from the deepest pocket at baseline and after one month of non-surgical periodontal therapy using sterile Gracey curette.The selected sites and the adjacent teeth were isolated with cotton rolls to prevent contamination of the samples with saliva or supragingival plaque. Gracey curettes (Hu-Friedy, Chicago, USA) no 7/8, 9/10 and 11/12 were used. The plaque samples was immediately transferred to sterile tubes containing 500 µl of sterile phosphate buffered saline (pH 7.8). The samples were then transferred with necessary precautions to the Quality diagnostic lab, Chennai and stored at −20° till assay.

Microbial assessment

The red complex organisms (Porphyromonas.gingivalis, Treponema denticola and Tanerella forsythia) were identified and assessed using PCR in both the groups at baseline and one month after therapy.

Quantitative Real- time polymerase Chain Reaction (q-RT-PCR)

Kit components

Kit is ready to use cocktail containing all components (except primers and template) for the detection and amplification of DNA in qPCR.

intergroup comparison was done after treatment.[Table-2]. The glycemic status was measured using FBS and Hba1C values. When intragroup comparison was done for FBS and HbA1c before and after nonsurgical periodontal treatment, there was statistically significant changes seen in both the groups for FBS (P < 0.001) [Table 3] and for HbA1c in Group 2 patients alone. Similarly, when intergroup comparison was done for FBS and HbA1c, individually, there was statistical significance observed only for FBS after treatment (P = 0.004) [Table 4]. When Intra group comparison of Red complex organisms was done at baseline and after treatment between group 1 and group 2 patients, statistical significant reduction

Table1 Intra group comparison of Clinical parameters at Baseline and one month after Treatment

	Plaque index Mean ± SD	Gingival index Mean ± SD	Bleeding on probing	Pocket depth Mean ± SD	CAL Mean ± SD
Group 1					
Mean value at Baseline	2.11 ± 0.44	2.66 ± 0.37	2.16 ± 0.53	4.04 ± 1.02	4.03 ± 0.47
Mean value after one month	0.94 ± 0.08	0.67 ± 0.21	1.02 ± 0.31	1.88 ± 0.54	2.73 ± 1.12
	P Value- 0.0039 **sig**	P Value - 0.0001 **sig**	P Value- 0.0075 **sig**	P Value-0.0121 **sig**	P Value- 0.0363 sig
Group 2					
Mean value at Baseline	1.66 ± 0.55	2.46 ± 0.77	2.16 ± 0.68	4.80 ± 0.57	4.36 ± 0.67
Mean value after one month	0.60 ± 0.20	0.94 ± 0.34	0.92 ± 0.38	1.99 ± 0.71	3.06 ± 1.05
	P Value- 0.0052 **sig**	P Value- 0.0046 **sig**	P Value- 0.0089 **sig**	P Value-0.0003 **sig**	P Vaue-0-018 **sig**

Sig – Significance NS- Non significance
SD- Standard deviation   CAL - Clinical attachment level

✓ The KAPASYBR FAST q PCR kit is supplied as a 2X master mix with integrated antibody – mediated hot start, SYBR Green I fluorescent dye, MgCI2, d NTPs , and stabilizers.

PCR Primers used in the study

In the present study, the following primers was selected

Description	Sequence (5'-3')	Size (bp)
Prophyromonas Gingivalis	Forward: 5'-AGG CAG CTT GCC ATA CTG CG-3'	172 bp
Tanerrella forsythus	Reverse: 5' – ACT GTT AGC AAC TAC CGA TGT – 3' Forward: GCG TAT GTA ACC TGC CCG CA	641 bp
Treponema denticola	Reverse: TGC TTC AGT GTC AGT TAT ACC T Forward: TAA TAC CGA ATG TGC TCA TTT ACA T Reverse: CTG CCA TAT CTC TAT GTC ATT GCT CTT	131 bp

Statistical analysis

Data are presented as mean ± standard deviation. Statistical analyses were performed using a software program (SPSS Version 16, IBM, Chicago, Illinois, USA) Comparison of variables within the groups was calculated by paired t-test. Comparison between two groups was analyzed using unpaired T test. Pearson correlation was done for pocket depth and bleeding on probing with red complex microorganisms

RESULTS

There was statistical significance observed in both the groups, when intragroup comparison was done for all the clinical parameters after treatment.[Table-1]. Whereas there was statistical significance seen only in plaque index score, when

was seen only for Treponema denticola and Tanerella forsythia and not for Porphromonas gingivalis in group 1 patients. Whereas there is statistical significant reduction was observed for all the three organisms in group 2 patients[Table-5]. When intergroup comparison of Red complex organisms was done at baseline and after treatment between group 1 and group 2 patients, statistical significant reduction was seen only for Treponema denticola [Table-6]. When Pearson Correlation was done between PD, BOP with red complex microorganisms for Group 1 patients at baseline and after treatment, correlation was observed between baseline pocket depth and P.gingivalis alone [Table-7]. When Pearson Correlation was done between PD, BOP with red complex microorganisms for Group 2 patients at baseline and after treatment, there was no correlation seen [Table-8].

Table 2 Intergroup comparison of mean and SD of clinical parameters after treatment

Clinical parameters	Groups	Mean SD	P .Value
Plaque Index	Group I	0.94 ±0.08	P=0.0031
	Group II	0.60±0.20	Sig
Gingival Index	Group I	0.67 ±0.21	P=0.1289
	Group II	0.94±0.34	NS
BOP	Group I	1.02 ±0.31	P=0.2140
	Group II	0.79±0.29	NS
PD	Group I	1.88 ±0.54	P=0.7688
	Group II	1.99±0.71	NS
CAL	Group I	2.73±1.12	P=0.6100
	Group II	3.06±1.05	NS

SD- Standard deviation BOP- Bleeding on probing   PD – Probing depth
CAL- Clinical attachment level   Sig – Significance NS- Non significance

Table 3 Intra Comparison of FBS and Hba1C at baseline and after one month treatment

Groups		FBS mg/dl		HBA1C mg/dl	
		Mean (SD)	P value	Mean (SD)	P value
Group-1	Baseline	178.2±11.2	<0.001	8.5±0.9	NS
	After Rx	150±0.9.8		7.3±0.6	
Group-2	Baseline	182.4±10.4	<0.001	8.9±1.1	<0.001
	After Rx	130.2±8.8		6.7±0.5	

P<0.05 is considered significant.
SD- Standard deviation FBS- Fasting blood sugar Hba1C- Glycosylated Haemoglobin

Table 4 Intergroup comparison of FBS and Hba1C at baseline and after one month treatment

Parameters Groups		Baselinel		After treatment	
		Mean (SD)	P value	Mean (SD)	P value
FBS	Group-1	178.2±11.2	NS	8.5±0.9	0.004
	Group-2	182.4±10.4		7.3±0.6	
HbA1c	Group-1	8.5±0.9	NS	8.9±1.1	NS
	Group-2	8.9±1.1		6.7±0.5	

P<0.05 is considered significant.
SD- Standard deviation FBS- Fasting blood sugar Hba1C- Glycosylated Haemoglobin

Table 5 Intragroup comparison of Red complex organisms at baseline and after treatment in group 1 and group 2 patients using paired t-test

Groups	N	Mean	Standard deviation	P
Grp-1 Pg Baseline	40	19.8000	3.52136	0.04
After treatment	40	21.0000	3.33333	
Grp-1 Td Baseline	40	24.4000	2.11870	0.001
After treatment	40	27.4000	2.36643	
Grp-1 Tf Baseline	40	30.6000	2.11870	0.001
After treatment	40	32.8000	2.25093	
Grp-2 Pg Baseline	40	18.1000	2.07900	0.001
After treatment	40	19.9000	2.46982	
Grp-2 Td Baseline	40	23.2000	2.20101	0.001
After treatment	40	24.6000	1.77639	
Grp-2 Tf Baseline	40	29.6000	1.71270	0.001
After treatment	40	32.2000	1.81353	

Pg- Porphyromonas gingivalis, Td- Treponema denticola, Tf- Tanerella forsythia

Table 6 Intergroup comparison of Red complex organisms at baseline and after treatment in group 1 and group 2 patients using Independent t-test

Organisms	Groups	N	Mean	Standard Deviation	P
Pg Baseline	Group-1	40	19.8000	3.52136	0.20
	Group-2	40	18.1000	2.07900	
Td Baseline	Group-1	40	24.4000	2.11870	0.23
	Group-2	40	23.2000	2.20101	
Tf Baseline	Group-1	40	30.6000	2.11870	0.26
	Group-2	40	29.6000	1.71270	
Pg After treatment	Group-1	40	21.0000	3.33333	0.41
	Group-2	40	19.9000	2.46982	
Td After treatment	Group-1	40	27.4000	2.36643	0.004
	Group-2	40	24.6000	1.77639	
Tf After treatment	Group-1	40	32.8000	2.25093	0.518
	Group-2	40	32.2000	1.81353	

Pg- Porphyromonas gingivalis, Td- Treponema denticola, Tf- Tanerella forsythia

Table 7 Pearson Correlation between PD, BOP with red complex microorganisms for Group 1 at baseline and one month after treatment

Microorganism	Pearson correlation	Pocket depth	Bleeding on probing
Pg Baseline	Pearson correlation	-.833	-.633
	Sig. (2 tailed)	0.003	0.049
	N	40	40
Td Baseline	Pearson correlation	.114	-.091
	Sig. (2 tailed)	.755	.802
	N	40	40
Tf Baseline	Pearson correlation	-.516	-.424
	Sig. (2 tailed)	.126	222
	N	40	40
Pg After treatment	Pearson correlation	-.487	-.435
	Sig. (2 tailed)	.154	.209
	N	40	40
Td After treatment	Pearson correlation	-.133	-.262
	Sig. (2 tailed)	.755	.464
	N	40	40
Tf After treatment	Pearson correlation	.135	-.283
	Sig. (2 tailed)	.709	.428
	N	40	40

Pg- Porphyromonas gingivalis, Td- Treponema denticola, Tf- Tanerella forsythia

Table 8 Pearson Correlation between PD, BOP with red complex Microorganisms for Group 2 at baseline and one month after treatment

Microorganism	Pearson correlation	Pocket depth	Bleeding on probing
Pg Baseline	Pearson correlation	-.427	-.428
	Sig. (2 tailed)	.219	.217
	N	40	40
Td Baseline	Pearson correlation	-.047	-.216
	Sig. (2 tailed)	.898	.549
	N	40	40
Tf Baseline	Pearson correlation	.208	.085
	Sig. (2 tailed)	-.563	.815
	N	40	40
Pg After treatment	Pearson correlation	.293	-.097
	Sig. (2 tailed)	.411	.790
	N	40	40
Td After treatment	Pearson correlation	-.114	-.050
	Sig. (2 tailed)	.753	.890
	N	40	40
Tf After treatment	Pearson correlation	.505	-.082
	Sig. (2 tailed)	.136	.823
	N	40	40

Pg- Porphyromonas gingivalis, Td- Treponema denticola, Tf- Tanerella forsythia

DISCUSSION

The etiology of periodontal disease is a result of interaction between the plaque biofilm, microbial by products and the host response.[14] It is a proven fact that diabetes mellitus is a risk-factor for development of periodontitis in adults.[15] The association between periodontal disease and diabetes mellitus with respect to gram-negative anaerobes in adult population has been extensively studied.[16] The prevalence of periodontal disease is more severe in diabetic than in non-diabetic subjects. The chronic challenge of the periodontal pathogens may provide a constant source of proinflammatory cytokines. This may be associated with an increased tissue insulin resistance and poor glycemic control in subjects with diabetes mellitus.[17]

Patients with DM and poor glycemic control present more severe gingival inflammation and periodontal destruction

compared with individuals without DM or those with well-controlled DM.[18]A complex consisting of Porphyromonas gingivalis (Pg), Treponema denticola (Td), and Tannerella forsythia (Tf) was strongly related to clinical parameters of periodontitis and termed as Red Complex organisms and strongly associated with chronic periodontitis.[19]In this current study, those with diabetes and inadequate metabolic control presented significantly higher pocket depth and bleeding on probing compared to controlled diabetic patients at baseline. Nonsurgical periodontal treatment resulted in significant reduction in all clinical parameters compared to the baseline values, corroborating results from previous studies. [20]Porphyromonas gingivalis is considered one of the main agents causing different types of periodontal disease, including chronic periodontitis. The virulence of Porphyromonas gingivalis, a gram negative anaerobic bacterium, is attributed to its various surface components, such as fimbriae, lipopolysaccharides and proteases which makes its surface possible for the bacterium to interact with the external medium and facilitates its growth, colonization, and formation of a biofilm that protects it against the host's defences.[21] Intragroup comparison showed statistical significance in all the clinical parameters. Intergroup comparison of clinical parameters after treatment shows statistical significance for plaque index alone.

Periodontal disease may affect those with DM directly through chronic inflammatory alterations.[22] Clinical studies have demonstrated that improvement in metabolic control correlates with improvement in periodontal health.[23] In the current study, while the improvement in the periodontal condition observed in those with DM had some impact on glycemic control, and showed statistical significance. Moreover, glycemic control is influenced by other variables, such as diet, weight control, physical exercises, and use of medication to control glycemic level. There was no such alteration of those variables during the study, so the obtained effect might be the result of periodontal treatment.

In this study fenugreek seeds were given as an adjuvant to metformin in treating Group 2 patients. Fenugreek seeds exert hypoglycemic effects by stimulating glucose-dependent insulin secretion from pancreatic beta cells, as well as by inhibiting the activities of alpha-amylase and sucrase, two intestinal enzymes involved in carbohydrate metabolism. Therefore, a cosiderable improvement in glycemic control helps to improve the periodontal parameters significantly. The improvement in all the clinical parameters in this study was similar to a study done by Rodrigues DC and Taba MJ, *et al.* [24]

In our study, when intragroup comparison was done, there was a reduction in the FBS and for Hba1C values in group 2 patients alone. When intergroup comparison was done, there was statistical significance observed only for FBS after treatment. Periodontal Inflammation is reduced by periodontal treatment which helps to restore insulin sensitivity and also shows improvement in glycemic control.[25] In our study as an adjunct to SRP, fenugreek was given along with metformin to the group 2 patients. In many diabetic patients, blood glucose levels are not properly controlled by bonafide antidiabetic medicines and malnourished individuals take suboptimal doses of drug to prevent hypoglycemic episodes. As fenugreek is commonly used as a condiment in india, the beneficiary effect of fenugreek in controlling blood sugar and overall cholesterol levels would have a considerable practical implication. The

biochemical benefits of the fibers present in fenugreek facilitates insulin secretion as a result of 4-hydroxyisoleucine which helps to lowering the rate of glucose absorption in the intestines, thus controlling the blood sugar levels.[26] Besides 4-hydroxyisoleucine, arginine and tryptophan are the other amino acids having antidiabetic and hypoglycemic effect. Since fenugreek seeds are a source of protein, they can replace pulses in the diet of a diabetic patient. 25-50 g fenugreek in the diet of diabetic patients (taken daily) can be an effective supportive therapy in the management of diabetes. [27] The bioactive compounds include the galactomannan-rich soluble fiber, fraction of fenugreek which may be responsible for the antidiabetic activity of the seeds. [28] Fenugreek seeds contain 25% fiber that can slow the rate of postprandial glucose absorption. This may be a secondary mechanism for its hypoglycemic effect (Basch etal.2003). [29]

In this current study, when intragroup comparison was done in group 1 patients, there was significance seen for treponema denticola and Tanerella forsythia. Similarly when intragroup comparison was done in group 2 patients, there was significance seen for all the three organisms. Other studies reported a reduction in the prevalence of these pathogens, when nonsurgical periodontal therapy was used in combination with antimicrobials.[30] In this study fenugreek seed was given as an adjunct to nonsurgical periodontal therapy for group 2 patients, because of its antidiabetic property. In the present study, except for P.gingivalis, there was significant reduction in the other two organisms, which enabled a good clinical response in both the groups. In contrast, other studies observed a modest and non-significant reduction in the levels of these species after full-mouth subgingival debridement in individuals with DM. However, these authors used only one or two sessions in contrast to four sessions of SRP and a rigorous periodontal maintenance program followed in the present study.

When intergroup comparison was done, there was significance observed only for treponema tenticola. Mechanical periodontal therapy alone may not be capable of eliminating T. forsythia and P. gingivalis in individuals with DM. [31]As mentioned earlier there should be a differences in the microbiological profile in those patients with uncontrolled diabetes mellitus when compared to controlled diabetic patients due to the impaired inflammatory response, which can lead to favourable environment to these proteolytic species.[32] The results in this study which failed to establish statistical significance for microorganisms between groups could be due to various other factors. Many studies have suggested that factors other than organisms are needed to initiate periodontal disease. Studies done by Van winkelhoff *et al* has shown that mere presence of periodontal pathogens in subgingival plaque is not enough to initiate periodontal disease and apparently susceptible host is needed to initiate the disease.[33] Studies done by Cianciola *et al.* have shown that altered host immune response has a vital role in the development and progression of periodontal disease.[34] Results in the present study showed that the mean CT (Threshold cycles) value of p. gingivalis, treponema denticola and tanerella forsythia at baseline was higher and showed significant reduction after treatment for treponema denticola and tanerella forsythia in group 1 patients and for all the three organisms in group 2 patients. (CT value is inversely proportional to presence of organisms).

Pearson correlation was done between red complex microorganisms with pocket depth and bleeding on probing in both the groups at baseline and after treatment. Correlation was observed between baseline pocket depth and P.gingivalis alone, which shows that the pocket depth is directly correlated to the presence and load of p.gingivalis. In our current study, Fenugreek seeds were given as an adjunct to nonsurgical periodontal therapy for group 2 patients along with metformin as it has got a good antidiabetic property. There is reduction in the amount of proinflammatory cytokines and improved periodontal health as a result of improvement in glycemic control due to nonsurgical periodontal therapy. Since there was an improvement in the glycemic status after treatment, there was a change in the microbiological profile after treatment compared to the baseline.

CONCLUSION

In our current study there was a significant reduction in the microbial load of red complex organisms when compared to baseline values. Nonsurgical periodontal therapy along with fenugreek seed has enabled a good clinical response after treatment by showing an improvement in the glycemic status. This improvement was beneficial to see the changes in the microbiological profile after treatment compared to the baseline values. Fenugreek seed powder was not only helpful in treating Type 2 uncontrolled diabetic patients for its antidiabetic and antilipedimic properties, but has also proven that it can be used as an adjuvant to nonsurgical periodontal therapy and along with regular antidiabetic medicines.

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How to cite this article:

Gopalakrishnan S *et al* (2018) 'Red Bacterial Zone In Diabetes Dissolved By Fenugreek - A Randomised Control Trial', *International Journal of Current Medical And Pharmaceutical Research,* 04(8), pp. 3535-3541.

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