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Effect of Non-Surgical Periodontal Therapy on Porphyromonas gingivalis and Filifactor alocis among Gestational Diabetes mellitus and Non-Gestational Diabetes mellitus subjects with chronic periodontitis.

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Abstract

Aim: To evaluate the microbiological counts of Porphyromonas gingivalis and Filifactor alocis clinical parameters among GDM and NGDM subjects with chronic periodontitis before and after non-surgical periodontal therapy.

Methods and methodology: A total of 40 subjects were divided in to 2 groups of 20 subjects each. Group 1 consisted of 20 subjects with GDM and chronic periodontitis and group 2 comprised of 20 subjects with NGDM and chronic periodontitis. Plaque samples were collected using paper points at baseline and 3 months after NSPT and were subjected for conventional PCR analysis. PI, GI, BOP, PPD and CAL were recorded at baseline and 3 months after NSPT.

Results: All the parameters in both the groups showed a significant clinical improvement after non-surgical periodontal therapy. However, only plaque index and microbiological counts of Filifactor alocis showed a statistically significant reduction after NSPT in both the groups.

Conclusion: Non-surgical periodontal therapy plays a very important role among GDM and NGDM subjects to reduce the severity of periodontitis. Also, the reduction in the colony counts of Porphyromonas gingivalis and Filifactor alocis after NSPT has helped to improve the periodontal status among the study subjects. Hence, NSPT can be a safe and effective line of treatment among pregnant women. Also, this study highlights the need of interdisciplinary treatment plan between dentists, gynaecologists and endocrinologists.

Keywords: Gestational diabetes mellitus, Non-gestational diabetes mellitus, Chronic periodontitis, Non-surgical periodontal therapy, Conventional PCR, Porphyromonas gingivalis, Filifactor alocis.

Introduction

The development of periodontal disease is a highly communicative and interactive process between pathogenic components in the dental plaque, the host tissues, the vasculature, immune systems, the connective tissue cells and their matrix[1]. It is believed that complex interactions between genetic predisposition, accumulation of advanced glycation end-products in periodontal tissues, alterations in host immune responses and collagen metabolism, and changes in gingival crevicular fluid and microflora may increase the prevalence and severity of periodontal disease in pregnant women with diabetes. Porphyromonas gingivalis is an anerobic , gram negative bacterium and a causative agent of chronic periodontitis that is associated with several systemic sequelae including

pregnancy complications. Even at low colonization levels, P.gingivalis employs a variety of strategies to control the commensal microbiota and direct disease progression[2]. Most studies has shown a positive correlation with Porphyromonas gingivalis between GDM and chronic periodontitis. Filifactor alocis is an anaerobic , gram positive bacterium that has recently been identified as a pathogen associated with chronic periodontitis , aggressive periodontitis and endodontic lesions[15]. F. alocis is an emerging pathogen causing high rate of infection but is an understudied pathogen.. Since there is only limited interventional studies done pertaining to P. gingivalis and F. alocis in GDM patients with chronic periodontitis, this study was conducted to evaluate the effect of NSPT on P. gingivalis and F. alocis in GDM patients with chronic periodontitis.

Materials and methodology

The study was conducted in Department of Periodontology, Faculty of Dental Sciences, Ramaiah University of Applied Sciences, Bengaluru, India for a period of 9 months(January 2018- September 2018). Patients were recruited from out-patient Department of Obstetrics and Gynaecology, Ramaiah Medical College, Bengaluru, India. Ethical clearance was obtained from ethical committee of the institution.

Inclusion criteria

1. Pregnant women between the age group 20 - 35 years.
2. Pregnant women diagnosed with chronic periodontitis with of pocket depth ≥ 5mm and clinical attachment loss ≥ 4mm.
3. Pregnant women diagnosed with gestational age of ≥ 12 weeks.
4. Pregnant women with a minimum of 12 teeth present.

Exclusion criteria

1. Pregnant women with a history of any other systemic diseases other than GDM.
2. Pregnant women with history of smoking or alcohol consumption during pregnancy.
3. Pregnant women who had been treated with antibiotic or other drugs 3 months prior to the study.
4. Subjects with preexisting type 1 or type 2 diabetes.

The sample size has been estimated using the software GPower v. 3.1.9.2. Considering the effect size to be measured (d) at 80%, power of the study at 80% and the margin of the error at 0.05% for one-tailed hypothesis, the total sample size needed is 40. So, each study group [with GDM & without GDM] will comprise of 20 samples.

The study comprised of a total of 40 patients which was divided in 2 groups of 20 patients each and was subjected to computer-based randomization within each group. Group 1 consisted of GDM patients with chronic periodontitis and group 2 consisted of non GDM patients with chronic periodontitis. All the patients were native Indians from Bengaluru with a mean age difference of 27.5 ± 7.5 years (range was from 20-35 years). A detailed case history was recorded and a complete periodontal examination was performed. The teeth which had the deepest periodontal probing depth was selected in the patient for sample collection. The written informed consent was obtained from all the patients.

Methodology

The deepest periodontal pockets were selected for sampling in patients. After removing supragingival plaque, the area was isolated with cotton pellets and sterile paper points were inserted in to periodontal pocket for 20 seconds for 3 times to obtain adequate plaque quantity. The paper points were then transferred in to an aliquot containing Tris EDTA (TE) media. The collection of samples were done by examiner 1. All samples were coded and sent to laboratory for processing within 72 hours after sample collection. These samples were further analyzed using conventional PCR to detect the quantity

and quality of Porphyromonas gingivalis and Filifactor alocis using respective forward and reverse primers of these pathogens.

PCR was done in 3 steps:

1) First step was to extract DNA of Porphyromonas gingivalis and Filifactor alocis from the plaque sample using the reagents lysis buffer I and II by centrifugation and vortexing. The DNA was stored in -20 degree Celsius.

2) In second step, PCR analysis was done using the PCR mixture and PCR thermal cycler.

Premixture for Pg was prepared using Ampliquon master mix, forward and reverse primers of Pg followed by addition of water. Premixture for Fa was prepared using Ampliquon master mix, forward and reverse primers of Fa followed by addition of water.This prepared pre-mixture was then added to the vials containing extracted DNA samples and then the DNA samples was kept in PCR thermal cycler under specific temperatures particular to Porphyromonas gingivalis and Filifactor alocis for 15 minutes each for pathogen.

For Amplification of P.gingivalis, the thermal cycling conditions were as follows: Initial denaturation was done at 95^0 C for 5 minutes followed by 35 cycles of denaturation at 95^0 C for 1 minute, annealing at 62^0 C for 1 min 30 sec and extension at 72^0 C for 1 minute. Final extension was carried out at 72^0 C for 5 minutes.

For Amplification of F.alocis, the thermal cycling conditions were as follows: Initial denaturation was done at 95^0 C for 5 minutes followed by 36 cycles of denaturation at 94^0 C for 30 seconds, annealing at 55^0 C for 1 min and extension at 72^0 C for 2 minute. Final extension was carried out at 72^0 C for 5 minutes.

Porphyromonas gingivalis : Amplification product size= 404 base pair

Filifactor alocis : Amplification product size= 594 base pair

3) Third step in conventional PCR was Agarose gel electrophoresis, were 2% agarose gel was prepared using agarose powder and Tris Acetate EDTA (TAE) buffer in to which comb was placed. After setting of agarose gel, comb was removed and the mold containing gel was kept in electrophoresis unit. 20 µl of amplified product was loaded in to wells including DNA template as a marker in the last well. Electrode was fixed and the gel was allowed to run for 2 hours at 75 V.

4) The image of bands under UV light transilluminator was recorded using gel documentation system. The amplified product of size 175 base pair was identified with the help of DNA ladder which was run simultaneously with the samples in each run.

5) Further quantification was done using total lab software (United kingdom). The gel image was analysed by the software. Band intensity relative to DNA ladder was measured for both Porphyromonas gingivalis and Filifactor alocis and quantification was obtained for both the organisms.

The subjects were recalled after 3 months. The plaque samples were collected from the same sites as in baseline using paper points and was transferred in to vial containing TE buffer media. These vials were coded and sent for processing using conventional PCR. The processing was done in a similar way explained above.

All the clinical parameters (PI, GI, BOP, PPD and CAL) was measured in all the subjects at baseline and after NSPT. The deepest pocket was selected in each patient. Plaque index and gingival index was assessed among all subjects in index teeth 16,12,24,36,32,44. Plaque index was measured using index scores given by silness and loe in 1964. Gingival index was measured using the index scores given by loe and silness in 1963.

Ethical consent

Ethics committee number: UECHT/2016-18/PGDT/03

Results

A total of 40 subjects were recruited from November 2017 to August 2018.To avoid bias first examiner collected subgingival plaque samples followed by second examiner (blinded) who provided non-surgical periodontal therapy and measured clinical parameters among group 1 (GDM women with chronic periodontitis) and group 2(non GDM with chronic periodontitis) subjects. After 3 months all the subjects were recalled followed by collection of subgingival plaque samples (first examiner) and re-assessment of clinical parameters (second examiner).

Plaque index

On inter group comparison between GDM and NGDM group at baseline, showed a mean value of 0.29 in GDM group and 0.28 in NGDM group with a standard deviation of 0.29 and 0.28 respectively (table1). This difference was found to be statistically significant. On intergroup comparison between GDM and NGDM group after 3 months, showed a mean value of 0.53 in GDM group and 0.75 in NGDM group with a standard deviation of 0.37 and 0.25 respectively (table 2). This showed a statistically significant difference using independent student t test.

Gingival index

On inter group comparison between GDM and NGDM group at baseline, showed a mean value of 2.59 in GDM group and 1.81 in NGDM group with a standard deviation of 0.30 and 0.42 respectively (table1). This difference was found to be statistically significant. On intergroup comparison between GDM and NGDM group after 3 months, showed a mean value of 0.83 in GDM group and 0.77 in NGDM group with a standard deviation of 0.42 and 0.28 respectively (table 2). But, however the results did not show any statistically significant difference.

Bleeding on Probing

On intergroup comparison between GDM and NGDM group at baseline, showed a reduction in BOP scores from 100% to 55% with X2 value of 1.616(table3). But, however this was not statistically significant. On intergroup comparison between GDM and NGDM group after 3 months, showed a reduction in BOP scores from 100% to 35 % with x2 value of 1.616(table3).But, however, this reduction did not show a statistically significant reduction.

Probing pocket depth

On inter group comparison between GDM and NGDM group at baseline, showed a mean value of 6.75 in GDM group and 6.05 in NGDM group with a standard deviation of 0.85 and 0.60 respectively (table1). This difference was found to be statistically significant. On intergroup comparison between GDM and NGDM group after 3 months, showed a mean value of 4.10 in GDM group and 4.05 in NGDM group with a standard deviation of 1.07 and 0.39 respectively (table 2). But, however the results did not show any statistically significant association.

Clinical attachment level

On inter group comparison between GDM and NGDM group at baseline, showed a mean value of 4.80 in GDM group and 4.30 in NGDM group with a standard deviation of 0.77 and 0.66 respectively (table1).. This difference was found to be statistically significant. On intergroup comparison between GDM and NGDM group after 3 months, showed a mean value of 2.05 in both GDM group and NGDM group with a standard deviation of 0.00 (table 2). But, however the results did not show any statistically significant difference.

Porphyromonas gingivalis

On intergroup comparison between GDM and NGDM group at baseline, showed a mean value of 3.02x108 in GDM and 2.42x108 in NGDM group with a standard deviation of 1.22x109 and 9.23x108 respectively (table4). On intergroup comparison between GDM and NGDM group after 3 months, showed a mean value of 1.21x104 in

GDM and 874.1 in NGDM group with a standard deviation of 3.52x104 and 1572.79 respectively (table 5). Both the values however were not statistically significant.

Filifactor alocis

On intergroup comparison between GDM and NGDM group at baseline, showed a mean value of 1.41x109 in GDM and 2.76x109 in NGDM group with a standard deviation of 2.76x109 and 3.76x108 respectively (table4). This showed a statistically significant difference. On intergroup comparison between GDM and NGDM group after 3 months, showed a mean value of 6.34x105 in GDM and 272.4 in NGDM group with a standard deviation of 2.19x106 and 374.56 respectively (table 5).. This showed a statistically significant difference.

Discussion

Periodontal disease is a mixed infection primarily caused by periodontal pathogens existing within subgingival plaque[3]. Porphyromonas gingivalis is proposed to play a prominent role in modulating the dynamics of the host immune response and composition and structure of the dental biofilm in order to persist in oral tissues[5]. Another emerging opportunistic pathogen, Filifactor alocis has recently become a subject of great interest due to its association with chronic periodontitis.

Many observational Studies in literature has evaluated the relationship between GDM and chronic periodontitis as by A.P Dasanayake et al in 2008, Xiong et al in 2006 and Ruitz et al in 2011.Findings, from this study has shown a higher frequency of periodontitis among women with GDM as compared to NGDM group. This could be due to the effect of diabetes on periodontium or the effect of subgingival microbiota on the systemic immune response or can be due to the genetic factor which has an effect on both diabetes and periodontitis.

An observational study by Xiong et al in 2009, also showed higher mean PPD, CAL and BOP scores among GDM women with chronic periodontitis. Other observational studies by Gogeneni et al in 2015 and Nitin Dani et al in 2016 showed mean higher scores of plaque index, gingival index, PPD and CAL in GDM women with periodontitis. The present study showed a higher mean scores of plaque index, gingival index, BOP, PPD and CAL among GDM group with chronic periodontitis at baseline. This could be due to the effect of hormonal alteration seen during pregnancy and the effect of insulin hormone on subgingival microbiota among GDM women with chronic periodontitis.

An interventional study by Navano sanchez et al in 2007 showed improvement in mean scores of plaque index, BOP and CAL among diabetic patients with chronic periodontitis 3 months after SRP. Another interventional study study by Ralee spooner et al in 2017 showed a significant improvement in mean scores of plaque index, bleeding index, PPD and CAL 3 months after SRP among type 2 diabetic patients with chronic periodontitis.An interventional study by Omneya et al in 2018 also showed a significant improvement in plaque index, gingival index, BOP, PPD and CAL with a significant reduction in TNFα levels 2 months after SRP among GDM subjects with chronic periodontitis. Similaraly, In the present study, there was a significant improvement in plaque index, gingival index, BOP, PPD and CAL 3 months after SRP which was more evident in GDM group with chronic periodontitis. This could be due to the effect of SRP on subgingival microflora which in turn leads to better metabolic control of diabetes and hence improves the clinical parameters.

Porphyromonas gingivalis is the “key stone” pathogen and is a part of red complex bacterium[2]. The virulence factors secreted by Pg can cause extensive destruction by invading in to deeper cells of the periodontium[5]. Filifactor alocis is a new emerging pathogen found to be associated

with chronic periodontitis[4]. Evidence on the interaction between F. alocis and other periodontal pathogens is limited, however recent evidence suggests the pathogenicity of F. alocis is likely potentiated by P. gingivalis[12] . The specific interaction of P. gingivalis and F. alocis is a newly studied phenomenon and the growth of one relative to another may be a key indicator of conditions within the dental biofilm[12]. An observational study by Gogeneni et al in 2015 showed an increased infection of Porphyrominas gingivalis and Filifactor alocis in saliva samples of GDM women with gingivitis. Also another observational study by Nitin Dani et al in 2016 showed a significant higher gram negative rods in subgingival plaque samples of GDM women with chronic periodontitis. Also, another observational study by Cristiane goncalves et al in 2016 showed an increased microbial count of Filifactor alocis in subgingival plaque samples of chronic periodontitis subjects. An interventional study by Ralee spooner et al in 2016 on interaction and growth activity between Porphyromonas gingivalis and Filifactor alocis showed an increase in numbers of actively growing Porphyromonas gingivalis and Filifactor alocis in deeper pocket of > 4mm at baseline before NSPT showing an inter-species correlation among subgingival plaque samples of chronic periodontitis subjects. This present study, showed an increase in microbial counts of both Porphyromonas gingivalis and Filifactor alocis in subgingival plaque samples of GDM women with chronic periodontitis at baseline. The increase in microbial counts of Porphyromonas gingivalis and Filifactor alocis could be due to the influence of pathogenicity of microbial complexes on host system which further exaggerates the disease. In the literature, only observational studies are done on the effect of Porphyromonas gingivalis and Filifactor alocis and interventional studies were not found so far in literature search on the effect of same among GDM and NGDM subjects with chronic periodontitis. Hence, more such studies are required to identify the effect of other periodontopathogens among GDM and NGDM subjects, to identify the effect of Porphyromonas gingivalis and Filifactor alocis on pregnancy outcomes as well as the effect of other regimes of NSPT like LDD among GDM and NGDM subjects.

Conclusion

Non-surgical periodontal therapy plays a very important role in reducing the microbiota contained in the complex biofilm. The improvement in periodontal condition is due to the reduction in Porphyromonas gingivalis and Filifactor alocis and hence NSPT can be considered as safe and effective line of treatment among GDM and NGDM subjects with chronic periodontitis. So, more such interventional studies are required to understand the pathogenesis hidden behind every unique periopathogens and its interaction with systemic diseases leading to potentially fatal pregnancy outcomes. This study highlights the interdisciplinary treatment plan among dentists, gynaecologists and endocrinologists regarding the awareness of oral hygiene among pregnant women inspite of systemic complications involved. Also, there should be increased awareness among rural and urban population on the pregnancy outcomes of various systemic complications and also the importance of professional dental care which could enhance the health of pregnant mothers and their newborns.

Acknowledgement

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Legends Figure and Table

Table 1: Comparison of clinical parameters between GDM and NGDM groups at baseline

Comparison of mean values of Clinical Parameters between 2 groups at Baseline using Independent Student t Test							
Parameters	Group	N	Mean	SD	Mean Diff	t	P-Value
PI	GDM	20	2.48	0.29	0.64	7.098	<0.001*
	NGDM	20	1.85	0.28			
GI	GDM	20	2.59	0.30	0.78	6.736	<0.001*
	NGDM	20	1.81	0.42			
PPD	GDM	20	6.75	0.85	0.70	2.999	0.005*
	NGDM	20	6.05	0.60			
CAL	GDM	20	4.80	0.77	0.50	2.213	0.03*
	NGDM	20	4.30	0.66			

This table shows the comparison of clinical parameters between 2 groups at baseline and all the 4 clinical parameters showed a statistical significance with $p < 0.005$.

Table 2: Comparison of mean values of clinical parameters between GDM and NGDM group after 3 months

Comparison of mean values of Clinical Parameters between 2 groups after 3 Months using Independent Student t Test							
Parameters	Group	N	Mean	SD	Mean Diff	t	P-Value
PI	GDM	20	0.53	0.37	-0.22	-2.175	0.04*
	NGDM	20	0.75	0.25			
GI	GDM	20	0.83	0.42	0.06	0.528	0.60
	NGDM	20	0.77	0.28			
PPD	GDM	20	4.10	1.07	0.05	0.196	0.85
	NGDM	20	4.05	0.39			
CAL	GDM	20	2.05	0.83	0.00	0.000	1.00
	NGDM	20	2.05	0.39			

This table shows the comparison of mean values of clinical parameters between 2 groups after 3 months where only plaque index showed a statistically significant association.

Table 3: Comparison of BOP scores in each study group at baseline and after 3 months

Comparison of Bleeding on Probing between baseline and 3 months in each study group using McNemar's Test						
Groups	Category	Baseline		3 Months		P-Value
		n	%	n	%	
GDM	Present	20	100%	11	55%	0.03*
	Absent	0	0%	9	45%	
NGDM	Present	20	100%	7	35%	0.01*
	Absent	0	0%	13	65%	

This table shows the comparison of BOP scores in each study group at baseline and after 3 months and showed statistically significant results with $p<0.005$.

Table 4: Comparison of Pg and Fa between GDM and NGDM at baseline

Comparison of mean values of P.Gingivalis & F. Alocis between 2 groups at Baseline using Mann Whitney U Test							
Organisms	Group	N	Mean	SD	Mean Diff	Z	P-Value
Pg	GDM	20	$3.02x10^8$	$1.22x10^9$	$5.96x10^7$	-0.027	0.98
	NGDM	20	$2.42x10^8$	$9.23x10^8$			
Fa	GDM	20	$1.41x10^9$	$2.76x10^9$	$1.28x10^9$	-2.340	0.02*
	NGDM	20	$1.34x10^8$	$3.76x10^8$			

This table shows the comparison of Pg and Fa between GDM and NGDM groups at baseline and Fa showed a statistically significant association with $p<0.005$.

Table 5: Comparison of Pg and Fa between GDM and NGDM after 3 months

Comparison of mean values of P.Gingivalis & F. Alocis between 2 groups after 3 months using Mann Whitney U Test							
Organisms	Group	N	Mean	SD	Mean Diff	Z	P-Value
Pg	GDM	20	$1.21x10^4$	$3.52x10^4$	$1.12x10^4$	-0.298	0.77
	NGDM	20	874.1	1572.79			
Fa	GDM	20	$6.34x10^5$	$2.19x10^6$	$6.34x10^5$	-3.889	<0.001*
	NGDM	20	272.4	374.56			

This table shows the comparison of mean values of Pg and Fa between GDM and NGDM after 3 months and only Fa showed a statistically significant association with $p<0.005$.

Figures

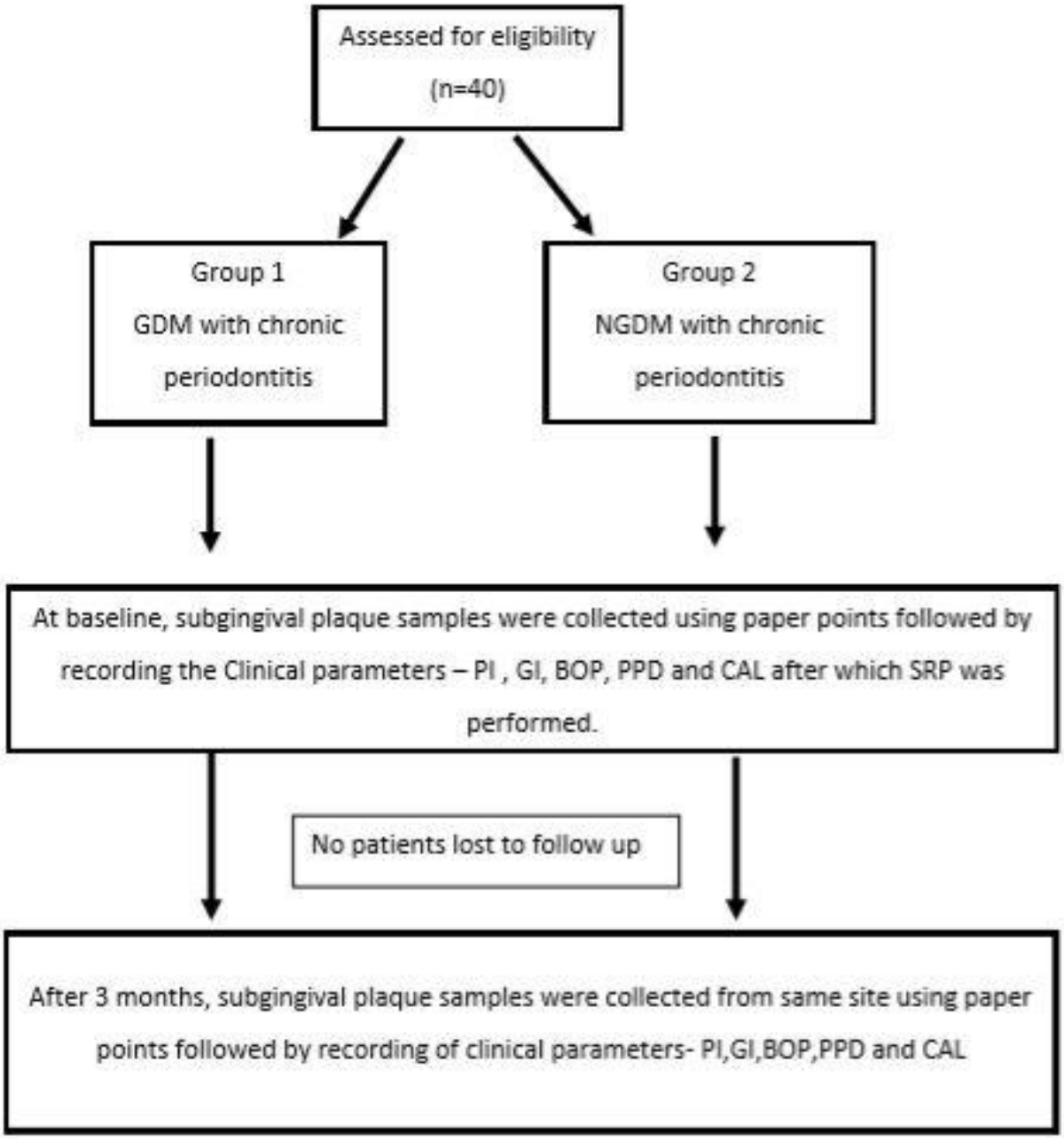

Figure 1: Consort Flow Chart

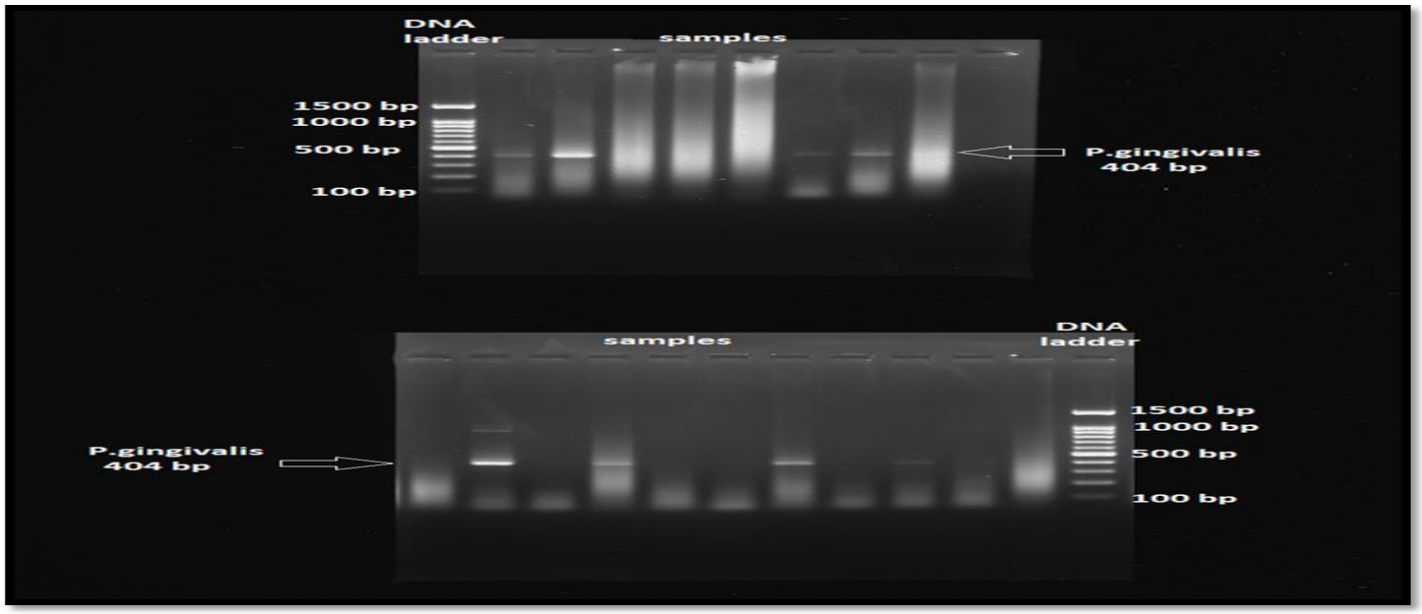

Figure 2: Gel electrophoresis image of Pg before and after NSPT

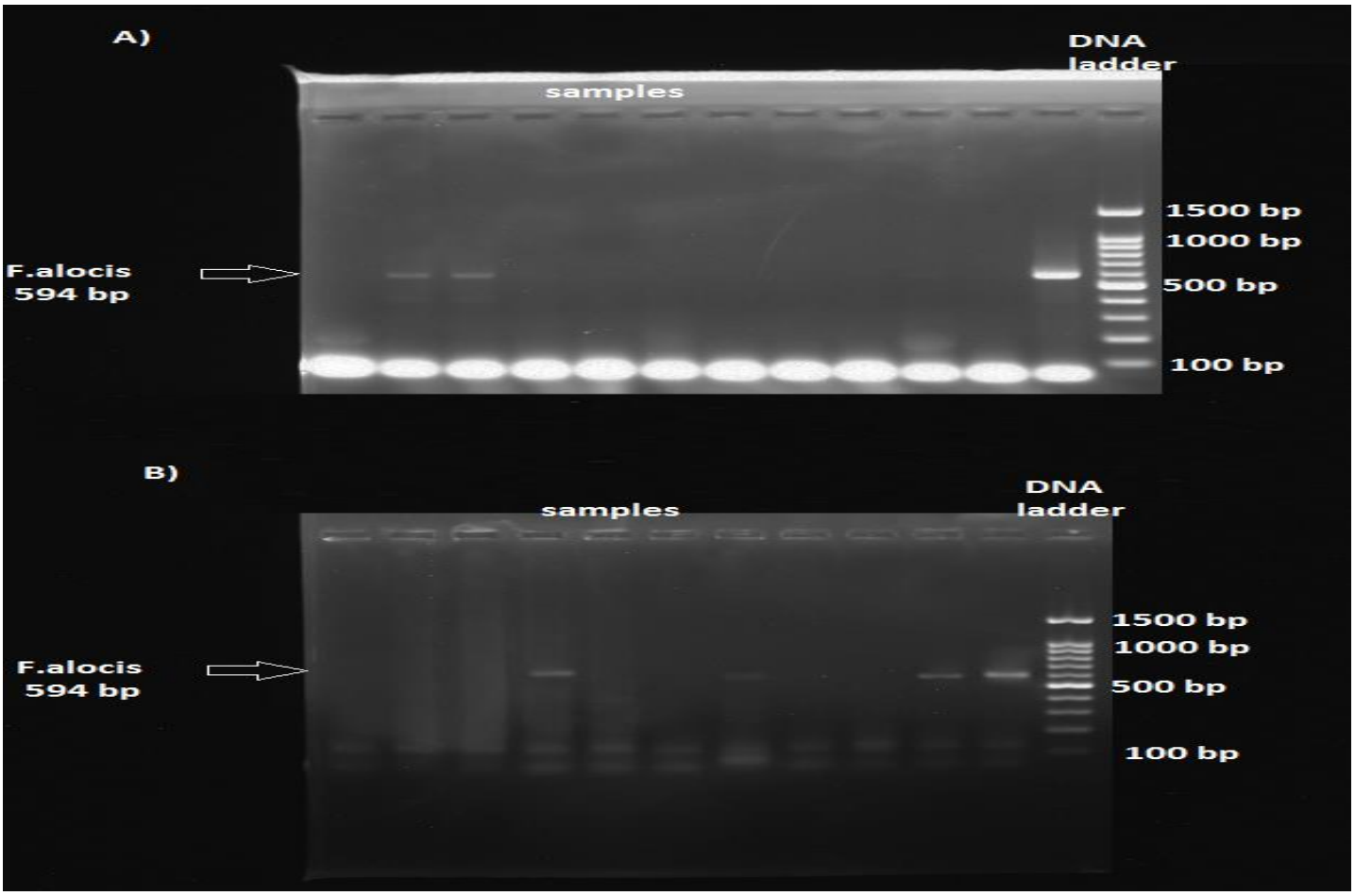

Figure 3: Gel electrophoresis image of Fa before and after NSPT