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ORIGINAL ARTICLES

The Effect of Photodynamic Therapy on Microbial Reduction in Diabetic Rats with Induced Periodontitis

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ABSTRACT

Aim: to compare the outcome of conventional mechanical therapy with or without adjunctive photodynamic therapy (a PDT) on the number of Porphyromonas gingivalis (P.g.) and Aggregatibacter actinomycetemcomitans (A.a.) bacteria in experimentally induced periodontitis in diabetic and non-diabetic rats**.** **Materials and methods:** The animals were divided randomly into 2 groups of 30 rats each: In the non-diabetic group, only experimental periodontitis was induced, while in the diabetic group, both experimental periodontitis and diabetes were induced. Both diabetic and non-diabetic groups were further divided into two subgroups with 15 rats in each subgroup. In the non-diabetic group 15 randomly chosen rats were treated with SRP in addition to PDT, while the other 15 rats received only SRP treatment. The same treatment protocol was carried in the diabetic group. **Results:** The use of aPDT resulted in statistically significantly lower mean number of P.g. and A.a. in the non-diabetic groups than SRP alone through all time intervals. In the diabetic animals, the use of a PDT resulted insignificantly lower mean number of P.g. than SRP alone only after 1 and 3 months, while in case of A.a., this treatment resulted in significantly lower mean number of A.a. than SRP alone through all time intervals. **Conclusion**: Both SRP and SRP +PDT decreased the number of pathogens in both groups, however results showed a PDT demonstrated significant lower mean numbers of P.g. and A.a.than SRP alone. This effect was more pronounced in the non- diabetic than the diabetic animals.

Key words: Photodynamic therapy, microbial reduction, diabetic, periodontitis

Introduction

Diabetes mellitus (DM) includes a number of disease resulting from the malfunction of insulin-dependent glucose homeostasis (Soskolne and Klinger, 2001).The association between diabetes and periodontal disease (PD) is widely accepted (de Almeida *et al*, 2008b). While DM is a known risk factor of PD which may complicate the severity of diabetes, PD has been reported as the sixth complication of diabetes (Donahue and Wu, 2001, Bascones-Martinez *et al*, 2011and Janet *et al*, 2005).

According to the classification issued in 1997 by the American Diabetes Association, there are several causes of diabetes which do not fit into type 1, type 2, or gestational diabetes. Induced diabetes in experimental animals belongs to this category (Janet *et al*, 2005).

Periodontitis is a bacterially induced chronic inflammatory disease that results in destruction and degradation of the periodontal tissues causing tooth loss (de Almeida *et al*, 2007 and Parn*et al*, 2009).The initiation and progression of periodontal disease (PD) are caused by the presence of pathogenic microorganisms that can invade the host causing direct and indirect damage to periodontium through activation of several responses (de Almeida *et al*, 2008b). Numerous species ofperiodontopathogenic microorganisms residing in the plaque biofilm such as Porphyromonas gingivalis (P.g.) and Aggregatib acteractinomycetemcomitans (A.a.), previously Actinobacillus actinomycetemcomitans are responsible for the induction and maintenance of periodontal inflammation (de Almeida *et al*, 2007). These species are often referred to as key pathogens and suppression or elimination of these pathogens is the main treatment goal (Kamma *et al*, 2000).

Scaling and root planning (SRP) has been used as the gold standard treatment, however mechanical therapy alone may be ineffective at eliminating pathogenic bacteria, which are frequently inserted into both soft and hard tissues and even in areas inaccessible to periodontal instrumentation (Kamma *et al*, 2000, Garcia *et al*, 2011). Different adjuvant therapies to SRP including antibiotics are advocated in diabetic patients with periodontitis. (Caton *et al*,2001, Al-Mubarak *et al*, 2002). However this therapy has undesirable side effectsand contributes to the development of drug resistance (van Winkelhoff *et al*,2000). Also disruption of the oral

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microflora and the difficulty in maintaining therapeutic concentrations of antimicrobials in the oral cavity are also problems associated with the use of these agents (Fontana *et al*., 2009).

In this context alternative methods of bacterial reduction such as Photodynamic therapy (PDT) could be a complementary method to conventional periodontal treatment (de Almeida *et al*, 2007). Laser light therapy has been introduced in periodontal therapy in an attempt to improve the effectiveness and efficiency of root surface debridement and bacterial elimination (Polansky *et al*, 2009).

PDT involves the use of low power lasers with appropriate wavelength to kill cells or microorganisms previously treated with a photosensitizer drug. The excited photosensitizer reacts with the substrate, mostly oxygen or water, to produce highly reactive oxygen species, as free radicals and/or singlet oxygen. These compounds cause injury and death of microorganisms (Hayek *et al*, 2005). The major advantages of PDT are as its specificity for target cells; exerting no collateral effect, and supporting no resistant bacterial species selection. Thus, PDT may represent a promising alternative for reducing the bacterial load or eradication of certain periodontal pathogens .Recently studies showed satisfactory results with PDT in animals and humans without systemic disease (Kömerik *et al*, 2003, Sigusch *et al*, 2005, de Almeida *et al*, 2007, and Andersen *et al*, 2007). Other findings failed to demonstrate any additional effects when PDT was used as an adjunct to conventional periodontal treatment (Chondros *et al*, 2008, Polansky *et al*, 2009).

Because of the conflicting results regarding the adjunctive effect of PDT and because introduction of PDT as an adjuvant periodontal treatment in diabetic conditions is lacking in the literature, the aim of the present study was to compare the outcome of conventional mechanical therapy with or without adjunctive PDT on the number of P.g. and A.a. in experimentally induced periodontitis in diabetic and non-diabetic rats.

Materials and Methods

Animals:

The study protocol was approved by the Ethical Committee of the National Research Center, Cairo, Egypt. This study was conducted on 60 male albino rats (100-120g). The animals were kept in plastic cages with access to food and water ad libitium in a temperature-controlled room with a standard 12/12h light- dark illumination cycle. The cages were kept under the same hygienic conditions and away from any source of chemical contamination according to the animal house regulations. Before the study procedures, all the animals were allowed to acclimatize to the laboratory environment for a period of 5 days.

Experimental design:

The animals were divided randomly into 2 groups of 30 rats each:

In the first group, the non-diabetic group, onlyexperimental periodontitis was induced, while in the second group, the diabetic group, both experimental periodontal disease and diabetes were induced. Both diabetic and non-diabetic groups were further divided into two subgroups: resulting in a total of 4 subgroups with 15 rats in each group. In the non-diabetic group 15 randomly chosen rats were treated with SRP in addition to PDT, while the other 15 rats received only SRP treatment. The same treatment protocol was carried in the diabetic group, where 15 rats were treated withboth SRP and PDT and the other 15 rats were treated by SRP alone.

Protocol of experimental diabetes mellitus induction:

Initially, blood was collected from all animals via the caudal vein under ether anesthesia to determine the glycemic level. After a 24-hour fasting period, except for water ad libitium, diabetes was induced by intraperitoneal injection of Streptozotocin (Sigma, St Louis, MO, USA). Streptozotocin was used in multiple low doses (40 mg/kg for 3 days) which resulted in a slow immune-mediated form of type 1 diabetes (Brondum *et al*, 2005). Streptozotocin was freshly prepared immediately before injection. Animals in the non-diabetic, group (n=30) received a 1ml saline solution by intravenous injection. Feeding was resumed 12 hours after the administration of Streptozotocin and saline solution. Seventy two hours after Streptozotocin injection, glucose solution was administered to the diabetic animals to prevent secondary hypoglycemia which is fatal (José *et al*, 2005 and Coskun *et al*, 2005).Glucose level measurements were done using the glucose-oxidase method (Accu-Check Advantage System, Roche Diagnostics, Indianapolis, IN) to confirm the establishment of diabetes. Blood samples were recollected from the retro- orbital plexus of veins under ether anesthesia after 72 hours and again at the end of the experiment 3 months later before animal sacrifice to determine the glycemic rate. Animals that had glycemic levels above 300 mg/dL or higher were considered diabetic and were used in the present study (de Almeida *et al*, 2008b). Experimental diabetes was induced in both diabetic subgroups before induction of periodontal disease.

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Protocol of experimental periodontal disease induction:

General anesthesia was administered by a combination of ketamine 0.4 ml/kg and xylazine 0.2 mL/kg via intra-muscular injection. Animals of both groups were assigned randomly to receive the cotton ligature which were tied on the necks of one mandibular first molar on one side in the submarginal area and kept in position to promote microbial dental plaque accumulation and inflammation resulting in periodontal tissue breakdown and induction of experimental periodontitis. The contralateral mandibular first molar (right side) received neither a ligature nor any treatment. The ligatures were repeatedly inspected during the course of the study; because the molars move in an occlusal direction as a result of continuous tooth eruption and the ligatures tend to become loose or lost after 1 to 2 weeks (Page and Schroeder 1982, Nociti *et al*, 2000 and de Almeida *et al*, 2007). After the 7th day of periodontitis induction signs of inflammation such as redness, edema and bleeding start to appear. The ligatures were then removed in all animals and the bacterial samples were taken.

Bacterial sampling procedures:

After the ligatures removal bacterial samples were taken at baseline (control samples) for all animals. Theanimals were randomly allocated, using a computer-generated table, to the treatments of SRP and PDT. For better standardization, animal 1 was the first choice, followed by 2 and 3, respectively. Thus, the animals of each group (diabetic and non-diabetic) were randomly assigned to one of the two treatments (30 animals/ treatment). Subgingival plaque samples from the left molars were taken using sterile filter papers (3-4 filter papers for each sample) and preserved in epindorphe tubes containing basic nutritive transport (Thioglycolate broth transport medium).The left molars were submitted to SRP in both group with specific manual curettes through 10 distal–mesial traction movements by the same experienced operator and this was followed by irrigation with 1 ml of saline solution.. Subsequent bacterial samples were taken 48 hours, 1 month and 3 months intervals following the different methods of treatments.

PDT procedures:

In the PDT groups the photosensitizer formulation used consisted of 0.01% Methylene Blue (MB) in a phosphate buffered system which was applied using an insulin syringe without a bevel, then left in the pocket for three minutes prior to laser system Irradiation. The light source was a non-thermal low intensity diode laser system with a wavelength of 660nm and a power output of 100mW.Laser light was delivered via a fiber optic cable attached to the hand piece. The time of exposure was 60 seconds per clinical site. The optical fiber assembly was delivered with a sweeping stroke in an apico- coronal direction and activated in the deepest part of the periodontal pocket. Laser treatment was performed once for the selected samples. In those samples the mesial area was irrigated with 1ml MB after SRP was performed and after 1 minute laser irradiation was done using the same parameters above. Methylene blue is a typical thiazinium dye, with an absorption peak of 664nm in water solution. It was chosen for this study because of its own bactericidal action and high efficiency on the different strains of bacteria (Ovchinnikov *et al*, 2001).

Laboratory procedures:

In this study the TaqMan assay which has been developed for quantitative detection of DNA was performed using oligonucleotide primers and probes specific for Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans. This Real-time PCR analyses was used for quantitative measurements of the numbers of these periopathogenic bacteria in subgingival plaque samples according to Yoshida *et al*, 2003.

Statistical Analysis:

Comparisons between diabetic and non-diabetic groups as well as between SRP and SRP + PDT were performed using Student's t-test. Repeated-measures Analysis of Variance (ANOVA) was used for comparison between different time periods. Tukey's post-hoc test was used for pair-wise comparison between mean values when ANOVA test is significant. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with PASW Statistics 18.0® (Predictive Analytics Software) for Windows

Results:

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I-Table 1: Mean and standard deviation values of P.g. and A.a. units copies/mL through different time periods.

Time	Treatment	Diabetes	P.g.		A.a.	
			Mean	SD	Mean	SD
Before treatment	SRP	Non Diabetics	545.6	32.1	276.6	7.2
		Diabetics	577.2	14.3	286.9	6.9
	SRP + PDT	Non Diabetics	542.0	33.4	276.6	5.6
		Diabetics	583.2	12.3	285.8	6.5
48 hours	SRP	Non Diabetics	475.6	32.9	241.5	8.6
		Diabetics	484.5	34.5	253.0	17.6
	SRP + PDT	Non Diabetics	429.0	41.5	216.3	9.8
		Diabetics	483.8	32.1	239.6	8.3
1 month	SRP	Non Diabetics	405.9	14.7	214.1	9.8
		Diabetics	437.1	26.0	223.9	11.2
	SRP + PDT	Non Diabetics	347.3	31.0	176.9	12.8
		Diabetics	389.9	27.4	195.6	11.2
3 months	SRP	Non Diabetics	337.6	6.8	168.4	3.6
		Diabetics	357.6	20.5	172.8	6.5
	SRP + PDT	Non Diabetics	279.3	18.7	139.3	7.7
		Diabetics	306.3	25.4	150.9	10.2

II- Repeated measures ANOVA results:

The results showed that treatment, diabetes and time had a statistically significant effect on mean number of P.g. and A.a. units copies.

Table 2: Results of repeated measures ANOVA .

Microorganism	Source of variation	Sum of Squares	Df	Mean Square	*P*-value
P.g.	Treatment	10586.9	1	10586.9	<0.001*
	Diabetes	10344.3	1	10344.3	<0.001*
	Time	1280018	3	426672.7	<0.001*
A.a.	Treatment	4467.9	1	4467.9	<0.001*
	Diabetes	1152.9	1	1152.9	<0.001*
	Time	334901.7	3	111633.9	<0.001*

*df: degrees of freedom, *: Significant at P ≤ 0.05*

III-Detailed comparisons between diabetics and non-diabetics:

A- After treatment with SRP + PDT:

As regards P.g., non-diabetics showed statistically significantly lower mean number of P.g. than diabetics through all periods.As regards A.a., non-diabetics showed statistically significantly lower mean number of A.a than diabetics through all periods except after 1 month where there was no statistically significant difference between non-diabetics and diabetics.

Table 3: The mean, SD values and results of comparison between number of P.g. and A.a. units copies/mL in diabetics and non-diabetics treated with SRP + PDT.

Microorganism	Time	Non-diabetics		Diabetics		*P*-value
		Mean	SD	Mean	SD	
P.g.	Before treatment	542.0	33.4	583.2	12.3	<0.001*
	48 hours	429.0	41.5	483.8	32.1	<0.001*
	1 month	347.3	31.0	389.9	27.4	<0.001*
	3 months	279.3	18.7	306.3	25.4	<0.001*
A.a.	Before treatment	276.6	5.6	285.8	6.5	0.003*
	48 hours	216.3	9.8	239.6	8.3	<0.001*
	1 month	176.9	12.8	195.6	11.2	0.027*
	3 months	139.3	7.7	150.9	10.2	0.010*

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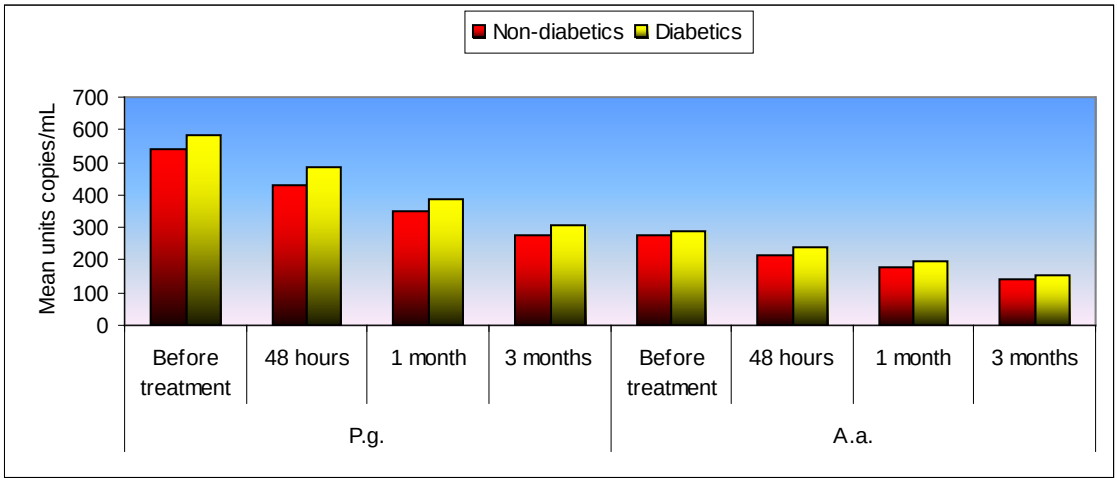

Fig. 1: Detailed comparisons between non-diabetics and diabetics treated with SRP+ PDT.

B- After treatment with SRP:

As regards P.g., non-diabetics showed statistically significantly lower mean number of P.gthan diabetics through all periods except after 48 hours where there was no statistically significant difference between the two groups. As regards A.a., , there was no statistically significant difference between non-diabetics and diabetics after 48 hours, 1 month and 3 months when treated with SRP alone.

Table 4: The mean, SD values and results of comparison between number of P.g. and A.a. units copies/mL in diabetics and non-diabetics treated with SRP.

Microorganism	Time	Non-diabetics		Diabetics		*P*-value
		Mean	SD	Mean	SD	
P.g.	Before treatment	545.6	32.1	577.2	14.3	0.011*
	48 hours	475.6	32.9	484.5	34.5	0.562
	1 month	405.9	14.7	437.1	26.0	0.004*
	3 months	337.6	6.8	357.6	20.5	0.009*
A.a.	Before treatment	276.6	7.2	286.9	6.9	0.004*
	48 hours	241.5	8.6	253.0	17.6	0.080
	1 month	214.1	9.8	223.9	11.2	0.053
	3 months	168.4	3.6	172.8	6.5	0.077

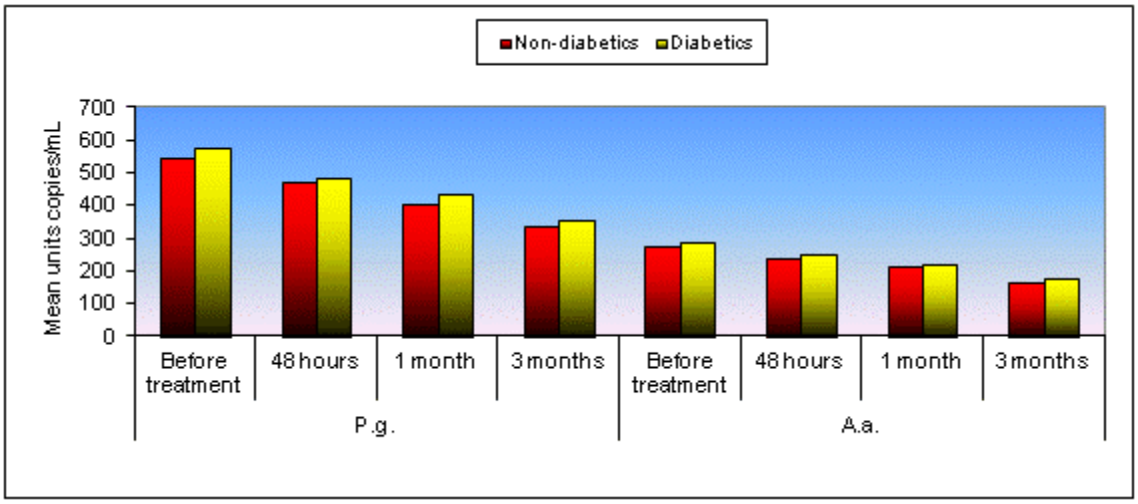

Fig. 2: Detailed comparisons between non-diabetics and diabetics treated with SRP.

IV-Detailed comparison between SRP and SRP + PDT:

A- In non-diabetics:

In non-diabetics group, as regards, SRP + PDT showed statistically significantly lower mean number of P.g.

and A.athan SRP alone through all time intervals.

B- In diabetics:

In diabetics group, as regards P.g., SRP + PDT showed statistically significantly lower mean number of units copied than SRP alone only after 1 and 3 months.As regards A.a., SRP + PDT showed statistically significantly lower mean number of units copied than SRP alone through all time intervals.

Table 5: The mean, SD values and results of comparison between number of P.g. and A.a. units copies/mL after treatment with SRP alone or SRP + PDT.

Group	Microorganism	Time	SRP		SRP + PDT		*P*-value
			Mean	SD	Mean	SD	
Non-diabetics	P.g.	Before treatment	545.6	32.1	542.0	33.4	0.809
		48 hours	475.6	32.9	429.0	41.5	0.012*
		1 month	405.9	14.7	347.3	31.0	<0.001*
		3 months	337.6	6.8	279.3	18.7	<0.001*
	A.a.	Before treatment	276.6	7.2	276.6	5.6	1.000
		48 hours	241.5	8.6	216.3	9.8	<0.001*
		1 month	214.1	9.8	176.9	12.8	<0.001*
		3 months	168.4	3.6	139.3	7.7	<0.001*
Diabetics	P.g.	Before treatment	577.2	14.3	583.2	12.3	0.328
		48 hours	484.5	34.5	483.8	32.1	0.963
		1 month	437.1	26.0	389.9	27.4	0.001*
		3 months	357.6	20.5	306.3	25.4	<0.001*
	A.a.	Before treatment	286.9	6.9	285.8	6.5	0.719
		48 hours	253.0	17.6	239.6	8.3	0.043*
		1 month	223.9	11.2	195.6	11.2	<0.001*
		3 months	172.8	6.5	150.9	10.2	<0.001*

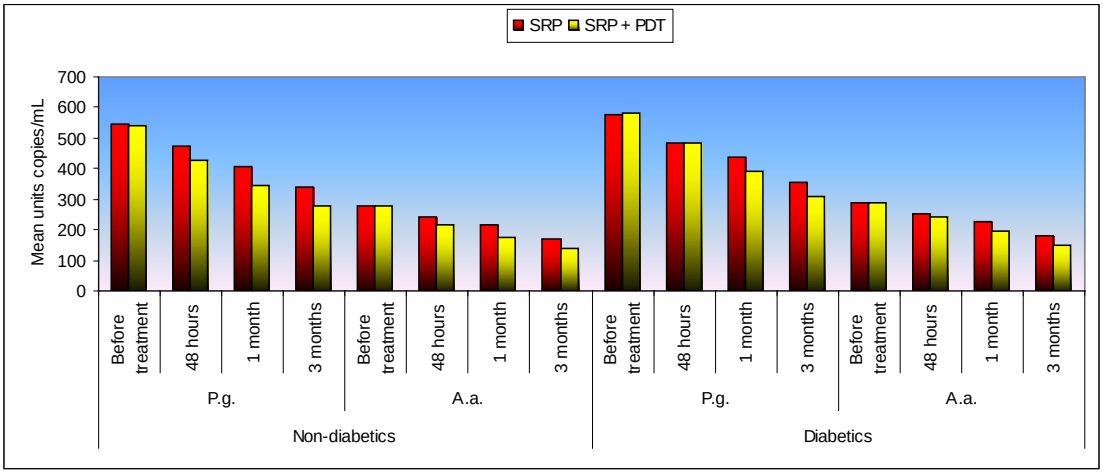

Fig. 3: Detailed comparisons between SRP alone and SRP + PDT.

Discussion:

The present study evaluated the antibacterial effect of photodynamic therapy (PDT), as an adjunctive treatment to scaling and root planning (SRP) in diabetic and non-diabetic rats.

In periodontal therapy the use of local antimicrobial agent such as PDT has been recommended as an adjunct to SRP (de Almeida *et al*, 2008 b, Fernandez *et al*, 2009), and many studies indicated that clinical outcomes of conventional subgingival debridement in subjects with chronic periodontitis can be improved by the adjunctive use of PDT (Braun *et al*, 2008). However few investigations have explored the microbiological effects underlying these observed clinical benefits.

In the present study both SRP and SRP +PDT decreased the number of measured pathogens in both groups, however results showed that adjunctive use of PDT demonstrated statistical significant lower mean no of P.g. and A.a. units than SRP alone. This effect was more pronounced in the non- diabetic than the diabetic animals .

The microbiologic efficacy of PDT was demonstrated in several in vivo and in vitro publications, where there was a statistical significant decrease of periodontopathogenic bacteria in the laser-assisted therapy compared to conventional treatment (Gutknecht *et al* 2002 and Sigusch *et al* 2005, 2007). Dörtbudak *et al*, 2001reported a significant reduction of A.a, P.g. and Prevotellaintermedia following PDT, although complete

elimination of bacteria was not achieved. Polansky *et al*, 2009 however failed to demonstrate any significant difference in the microbial reduction between those subjects with chronic periodontitis treated with subgingival ultrasound alone or those that received additional treatment by PDT.

Some studies in animals have also shown satisfactory results with the use of a PDT for the treatment of experimental PD (Kömerick *et al*, 2003, de Almeida *et al*, 2008a, b and Fernandes *et al*, 2009. With respect to induced periodontitis in rat models antimicrobial PDT proved to be an effective adjunctive treatment to SRP for induced periodontitis in dexamethasone-inhibited as well as nicotine modified rats (Fernandes *et al*, 2009 and Garcia *et al*, 2011).

On the other hand Pfizner *et al*, 2004 demonstrated that it is possible to inactivate P. g., Fusiform nucleatum, and Capnocytophaga gingivalis completely using diode laser, while A. a and Eikenella corrodens responded only minimally to treatment.

Other studies demonstrated that gram positive bacteria are susceptible to photodynamic inactivation, but gram negative bacteria are significantly resistant to many photosensitizers used in PDT. Low level laser therapy action on the viability or activity of bacteria was said to be species independent (Chan *et al*, 2003) and is directly related to the absorption of the laser wavelength by endogenous porphyrins which are present in some bacteria like P.g. (Soukos *et al*, 2005).

While much research has focused on altered host immune –mediated inflammatory response to plaque in diabetic patients, the role of diabetes in modulating the periodontal microbiota remains unclear (Field *et al*, 2012).

To our knowledge, this is the first study that has attempted to compare between the efficacy of PDT on microbial reduction in diabetic and non-diabetic rats. Hence the discussion is originally focused on the results of this study.

Studies demonstrated periodontal sites in diabetic subjects to harbor a higher frequency of P.g, A.a and Campylobacter species than those without diabetes and the increased severity of periodontal disease in diabetics was said to reflect an alteration of the pathogenic potential of bacteria in diabetics compared to non-diabetics (Ebersole *et al*, 2008).This is supported by results from earlier rodent studies in which increased no of gram negative anaerobes were found in diabetic rats compared to non-diabetic rats where the changes in the gingival microflora was said to result from diabetes-induced alterations in the sulcular microenvironment (McNamara *et al*, 1982 and Takai *et al*, 1986). In other findings from human and rodent studies, the ultimate consequences of hyperglycemia in the periodontium was said to be impaired host defense upon pathogens and prolonged inflammatory response (Andersen *et al*, 2007).

The precise explanation for the lower number of bacteria in the non-diabetic group than diabetic group when a PDT was used cannot be elucidated, but it is reasonable to assume that the increased pathogenic potential or the impaired host defense in diabetes might have led to these results.In a study by Almeida *et al*, 2008 b the authors concluded that PDT may be an effective adjuvant treatment to conventional SRP in periodontal disease induced by bacterial plaque and systemically modified by diabetes, which is in line with results of the present study.

The more significant effect of adjunctive PDT in the non-diabetic group does not nullify the fact that the use of PDT as adjunct to SRP did result in a significant microbial reduction when compared with SRP alone. When the effect SRP alone was studied on the number of P.g and A.A, there was a statistically significantly lower mean number of P.g. in the non-diabetics compared to the diabetics through all periods except after 48 hours where there was no difference between the two groups. However there was no statistically significant difference between non-diabetics and diabetics as regards A.a. at all time periods, suggesting that both diabetic and non-diabetic groups are similarly susceptible to A.a. bacteria and as such SRP had a similar effect on A.a. in both groups.

Research into the role played by subgingival plaque in patients with diabetes has been rather more limited. While some studies concluded that significant difference existed in the subgingivalmicrobiota between diabetic and non-diabetic subjects (Ebersole *et al*, 2008, Zambon *et al*, 1988), others did not provide any evidence for differences in the subgingivalmicrobiota between periodontal subjects with or without diabetes (Lalla *et al*, 2006, Ebersole *et al*, 2008, Field *et al*, 2012).

Makiura *et al*, 2008 found the occurrence rates of P.g., Tannerella forsythensis, Treponema denticola and Prevotella intermedia to be reduced after subgingival debridement. Interestingly, P.g. was detected more frequently in subjects with increased glycated hemoglobin (HbA1c) values after periodontal treatment than in those patients with decreased HbA1c values.

In a study by Komiya Ito *et al*, 2010, no significant association was found between A.a. and the tested clinical parameters. Henderson *et al*, 2003 highlighted that although A.a. bacteria are mostly found in individuals with advanced gum disease, they are also found in healthy oral cavities, being an oral opportunistic pathogen, a fact that may relate to the insignificant difference in the number of A.a. when SRP was applied on both groups.

In contrast to our study Haffajeeand coworkers using DNA probes evaluated samples both pre-and post SRP for their content of subginival species and their results showed that only P.g., Tannerellaforsythus and

Treponema denticola were significantly decreased while A.a. was minimally affected by this therapy (Haffajee *et al*, 1997). Other investigations showed minimal long term effects of SRP on the subginival microbiota especially for species like A.a. (Mombelli *et al*, 2003, Nieminen *et al* 1995). The differences in the results of those studies with the present one could be due to the different sample populations and the technical limitations of the methods used in each study.

Noaves *et al*, 2011 found a PDT to be effective in decreasing the numbers of A.a than SRP, while SRP was more efficient than aPDT in reducing the presence of periodontal pathogens of the red complex. The study of Noaves *et al*, however was conducted on subjects with aggressive periodontitis who were otherwise healthy.

The insignificant difference between the non-diabetics and diabetic groups that resulted when the effect of SRP was measured after 48 hours could have been resulted from possible contamination of the samples or inaccurate successive positioning of the paper point in the exact location and depth to obtain the most accurate pre and post treatment samples (Mullins *et al*, 2007). We consider our results to be preliminary and recommend the conduction of quantitative and time-dependent approaches together with site specific analysis in order for conclusions to be extrapolated into the broader clinical picture. Furthermore ligature-induced tissue destruction does not accurately recapitulate in vivo pathogenesis (Freire *et al*, 2011), and therefore more in vivo studies are recommended to elucidate the action of PDT and to ascertain whether any significant difference truly exist in the subgingivalmicrobiota of diabetic subjects.

Conclusion:

Within the limitations of this study we can conclude thatboth SRP and SRP and PDT decreased the number of measured pathogens in both groups, however results showed that adjunctive use of PDT demonstrated statistical significant lower mean no of Pg and Aa units than SRP alone. This effect was more pronounced in the non- diabetic than the diabetic animals

Because PDT is a local therapy, it may be a valuable alternative for most indications in which antibiotic drugs have been administrated as in cardiovascular disease and diabetes, as it reduces the probability of side effects associated with the systemic administration of these drugs.

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