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Effect of Photodynamic Therapy Versus Diode Laser Therapy on the Treatment of Localized Aggressive Periodontitis

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Abstract

Background: Localized Aggressive periodontitis (LAP) is a severe form of periodontal disease, that affects younger people, so it is a challenging disease, that needs rapid intervention. Photodynamic therapy (PDT) has been proposed as a new avenue to overcome the limitations of other treatment modalities. The benefits of PDT include instant suppression of causative periopathogenic bacteria and minimum antibiotic resistance.

Material and methods: Forty-five sites in fifteen patients with LAP were randomly selected and received the three modalities of treatment: SRP alone, laser therapy (LT) in repeated times (0, 7, 21days) and PDT using indocyanine green dye in repeated times also. All participants received full mouth SRP. At baseline, three and six months, clinical parameters were recorded. Gingival crevicular fluid (GCF) samples were collected and analyzed using ELISA test for quantitative measurements of MMP-8. Plaque samples were collected and analyzed using Rt-PCR for quantitative and qualitative measurement of Aggregatibacter Actinomycetecomitans.

Results: Results showed statistical significance in the probing pocket depth (PPD) and clinical attachment level (CAL) in LT and PDT than SRP. In addition to, Immunological and microbiological test that showed significant reduction in LT and PDT till the end of study period than SRP, that indicate the potent anti-bacterial effect of laser and photodynamic therapy, but there was no statistically significant difference between LT and PDT.

Conclusion: Based on the clinical results of the present study, all treatment modalities result in significant clinical improvement with a clear superiority of PDT procedures that may be a new avenue for future treatment of AgP. Immunological and microbiological results showed significant reduction in both PDT and LT rather than SRP till the end of study period at six months.

Keywords: Localized Aggressive periodontitis, Aggregatibacter Actinomycetecomitans, photodynamic therapy, Indocyanine green photo sensitizer dye, matrix metalloproteinase 8 (MMP-8), scaling and root planing (SRP).

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Introduction:

Periodontal disease is a complex multifactorial disease of a highly pathogenic biofilm that targets an immune/inflammatory host response, leading to the destruction of supportive periodontal tissues and eventual tooth loss⁽¹⁾. Periodontitis is a microbially driven host mediated disease, which is associated with dysbiotic plaque biofilm of the periodontium ⁽²⁾, that have a range of periodontal diseases (chronic periodontitis, aggressive periodontitis, periodontitis as a manifestation of systemic diseases, necrotizing periodontal diseases, abscesses of the periodontium and periodontitis associated with endodontic lesions)⁽³⁾.

Aggressive periodontitis (AgP) is a disease of the periodontium happening in an otherwise healthy adolescent, which is featured by a rapid loss of alveolar bone around more than one tooth of the dentition⁽⁴⁾. This destructive periodontal disease results in severe bone loss, tooth

mobility and eventual tooth loss (5).

Aggressive periodontitis was classified into localized and generalized forms according to American Academy of Periodontology in 1999⁽⁶⁾. According to the latest classification, localized aggressive periodontitis can be classified as stage III of grade B unless it is associated with risk factor it would be classified as stage III of grade C ⁽⁷⁾, where Stage of periodontitis is defined based on severity of periodontal breakdown and complexity of management, while grade of periodontitis is estimated with direct or indirect evidence of risk factor, where a risk factor is used as grade modifier⁽⁸⁾.

Localized aggressive periodontitis is a complex disease that has multifactorial etiology and in which the effects of various etiological factors are modified, which include microbial, immunological, genetic and environmental factors⁽⁹⁾. Aggregatibacter Actinomycetecomitans (A.a) has been thought to be the major pathogen in aggressive periodontitis, especially in its specific localized form ⁽¹⁰⁾.

The goal of treatment is directed toward the reduction of bacterial load and creation of a clinical healthy condition that aims to retaining the integrity of the periodontium with long term maintenance⁽¹¹⁾.

The backbone of periodontal treatment consists of mechanical debridement scaling and root planing to remove plaque, endotoxin, calculus and plaque-retentive factors (12). But some pathogens can invade into the tissue, so mechanical treatment is sometimes inadequate (13). Based on the literature, AgP responds with good clinical results to scaling and root planing (SRP) in the short term (up to 6 months). However, relapse and disease progression have been reported after 6 months despite frequent visits to the physician and strengthening oral hygiene (11), so the success of periodontal treatment need both mechanical and others as chemotherapeutic approaches to eliminate the microbial dental plaque biofilm, local drug delivery and lately laser and photodynamic therapy (11).

Laser irradiation overcomes the resistance of subgingival biofilm communities to antibiotics conventionally used in dental practice and it exerts an antibacterial action⁽¹⁴⁾. In several published clinical studies, the authors stated that, the diode laser group showed a trend of some clinical benefits, compared to the control groups^{(15),(16)}. Photodynamic therapy (PDT) has been suggested as a novel noninvasive antibacterial treatment modality for eradicating microorganism. PDT depend on using a photosensitizer (photoactivatable agent) that absorbs light and can be concentrated in a target tissue^{(17),(18)}. Application of PDT to periodontal infection could prove to be a valuable adjunct to mechanical procedures, if the photosensitizer has broad spectrum activity against bacterial pathogens and selectivity for prokaryotic cells⁽¹⁹⁾.

Due to its high antibacterial potential with increased and long lasting decontamination of the periodontal pockets ,PDT has been used in the treatment of "periodontitis, peri-implantitis "('')'that lead to detectable bone mineralization and regeneration(20). A systematic review conducted by Chatzopoulos and Doufexi(21) concluded that PDT may exhibit a significant role in the therapy of aggressive periodontitis after repeated applications. However, there is still insufficient evidence to maintain the superiority of PDT in periodontal treatment compared to scaling and root planing alone or as adjunct⁽¹⁷⁾, (18).

Indocyanine green photosensitizer has positive specific effect on A.a.

as it has a role on outer membrane protein (OMP100 of A.a),which is important for export of leukotoxin from A.a as concluded by Pourha-jibagher and Bahador, (22) according to the results, anionic indocyanine green tends to interrelate with OMP 100 of A.a during PDT. So the aim of this study was to evaluate and compare the effect of diode laser alone versus photodynamic therapy using indocyanine green as an adjunctive to scaling and root planing for management of localized aggressive periodontitis, this study had been conducted to estimate whether or not which treatment modality would improve the clinical, microbial and immunological outcomes.

Subject & Methods:

- 1) Diode laser*, 808 nm +/- 10 nm wavelength, 300μm fibero.3W Power output. (fig1,2).
- 2) Photosensitizing agent" Indocyanine Green"**. (fig3)
- 3) Collagen plugs sheets***. (fig.5)
- **4)** ELISA "Enzyme linked immunosorbent assay kit (Quantikine) "for determination of Matrix Metalloproteinase (MMP-8) concentration in Gingival Crevicular Fluid Fig (4)****.
- 5) 5- Real time PCR kit ***** for detection of Presence of A.a in plaque sample(Fig 6).
- **6) Sterile paper points** (Absorbent colour coded, Ref A 022R) for collecting GCF samples for immunological analysis. This study had approval from research ethical committee (REC) in faculty of dentistry, Tanta university at February 2017 and from Cochrane library trial number PACTR202007628100458. The purpose of the present study was explained to the patients and informed consents were obtained.
- * GaAlAs diode (Elexxion claros nano compact class IV dental laser, Radolfzell, Germany).
- **Perio Green.
- *** Colla cone
- ***R& D Systems, Inc.
- ****Invitrogen primer design, thermofisher



Fig (1): showing the elexxion diode laser machine.



Fig (2): showing the set of fiber with different diameter.





Fig (4): showing perio Green dye after dilution.



Fig (6): showing ELISA kit for MMP-8 marker.



Fig (3): showing patch of perio Green dye



Fig (5): showing collagen sponge (colla cone)





Fig (8): Panoramic x-ray confirming diagnosis.

A total of forty-five sites in fifteen patients diagnosed with localized aggressive periodontitis⁽²³⁾ were selected who fulfilled the following criteria: Their age ranged from 18 to 35 years old of both genders, and optimal compliance as evidenced by no missed treatment appointments and a positive attitude toward oral hygiene. we excluded from this study patients with risk factors (e.g.- smoking, pregnancy or any other systemic disease that may alter the course of periodontal therapy), history of antibiotics or anti-inflammatory drugs in the previous six months, acute condition in the mouth and history of periodontal surgery in the last year in the selected sites. patients with hypersensitivity toward photosensitizer dye as evident by sensitivity test.

Site grouping:

The Forty-five sites among the patients were randomly classified into

two groups using sealed envelopes. The patients treated with one of the following modalities as follow:

- Group I: sites received only SRP.
- Group II: sites received SRP + LT in repeated times (0, 7, 21days).
- Group III: sites received SRP +PDT in repeated times (0, 7, 21days).

Treatment steps:

- **Group I:** Complete SRP was done for all patients. and follow up after three and six months.
- **Group II:** (laser therapy): Sites received combined therapy of SRP plus LT which was done after SRP. According to the laser machine manufacturer's instructions, the pockets were irradiated by a 300μm fiber using diode laser 808 nm wavelength, the laser light 1.0 W and 20 kHz for about 30 seconds in repeated application (0,7days,21 days).



Step A: Trans-gingival irradiation had been performed by bleaching hand piece (glass rod) in a non-contact mode for 10 seconds.



Step B: pockets had been irradiated using a 300-um fiber in an undulating vertical pattern for 30 seconds using contact mode for granulation tissue removal.

Fig (9): Steps of treatment in laser group II

– Group III: (PDT): Sites received combined therapy of SRP plus PDT where the photosensitizer injected into the periodontal pocket. All pockets irradiated by a 300μm fiber using diode laser 808 nm wave-

length, 0.3 W power output and 0.05 kHz in a circular pattern for about 40 seconds according to laser machine manufacturer's instructions in repeated application (0,7days,21 days).



Step A: the dye was injected into the periodontal pocket in an apical to coronal direction using a blunt needle straight application



Step B: Trans-gingival irradiation had been performed by bleaching hand piece (glass rod) in a non-contact mode for 10 seconds for activation of the dye.



Step c: pockets had been irradiated using a 300-um fiber in an undulating vertical pattern for 30 seconds using contact mode for granulation tissue removal

Fig (10): Steps of treatment in photodynamic group (III)





Fig (11): Packing of collagen sponge in pocket



Fig (12): Periodontal pack application after treatment

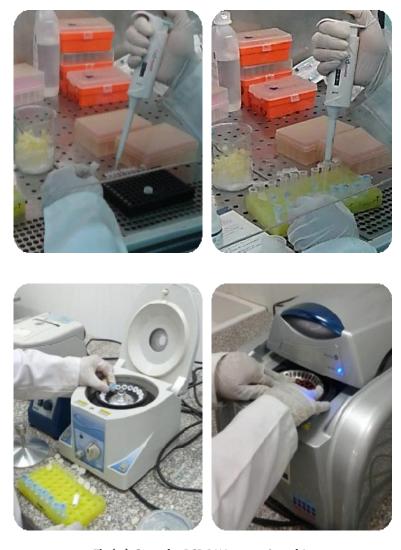


Fig (13): Steps for PCR DNA extraction of A.a



Fig (14): Microplate reader for measuring the absorbance and the intensity of MMp-8

- After treatment in all groups the explorer was inserted at base of pocket to induce bleeding that carry all growth factors and stimulate angiogenesis, then collagen plug sheet was used to fill the depth of the treated pocket to enhance hemostasis in contact with blood to stabilize the blood clot. As it will help to maintain and enhance regeneration. Then the area was sealed with periacryl as periodontal protocol of Lares research institute in wavelength optimized periodontal therapy.
- After treatment, the patients were instructed to follow their usual

oral hygiene regimens including flossing, brushing, and mouth rinsing, but flossing would have been delayed until collagen sponge resorbed **Collection of GCF samples:**

The GCF samples were taken from sites of selected teeth. For each site, (GCF) sample was collected after removal of the plaque (to prevent site contamination) using paper points, which had been inserted into the pockets until resistance felt and kept there for 30 seconds. The samples were diluted in phosphate buffer saline (PBS) up to 0.2ml. After 15 min., the paper points were removed, and the samples frozen at -20 C for analysis. Blood contaminated samples were discarded. Immu-

	Scaling and Root planing	Laser therapy	Photodynamic therapy	Kruskal Wallis Test	
		(Baseline)			
Min-Max	6.00-10.00	5.00-8.00	5.00-8.00	$\chi^{2}_{\text{(df=2)}} = 2.604$ p=0.272NS	
Median (IQR)	6.00 (6.00-7.00)	6.00 (5.00-7.00)	6.00 (5.00-7.00)		
		(3 Months)		,	
Min-Max	5.00-9.00	4.00-7.00	3.00-7.00	$\chi^2_{(df=2)} = 11.567$ p=0.003*	
Median (IQR)	6.00 (6.00-6.00)	5.00 (4.00-5.50)	5.00 (4.00-6.00)		
		(6 Months)			
Min-Max	5.00-9.00	4.00-7.00	3.00-7.00	$\chi^2_{(df=2)} = 14.358$ p=0.001*	
Median (IQR)	6.00 (6.00-6.50)	5.00 (4.00-5.00)	5.00 (4.00-5.50)		
Friedman Test					
Chi-Square χ ² (df=2)	13.400	27.395	25.400		
р	0.001*	0.000*	0.000*		

Table (1): Pocket depth of the three studied groups and different time of assessment

nological assessment (24) for the quantitative measurement of matrix metallo-proteniease-8 (MMP8) concentration in the gingival crevicular fluid using (Enzyme-Linked Immuno-Sorbent Assay) was made.

Collection of plaque samples:

Supra-gingival plaque was removed with a sterile scaler and cotton gauze. The selected site was dried and isolated with sterile cotton rolls as a precaution to prevent contamination of examined site. Sub-gingival plaque samples were obtained by inserting sterile curette into a sub-gingival site for 10 s. Samples were discarded, if contaminated with blood or saliva during collection. Sub-gingival plaque samples were transferred immediately into 200 µl of phosphate buffered saline (PBS) for microbiological assessment for the detection of A.a by RT-PCR.

Treatment evaluation:

The following clinical parameters, Probing pocket depth (PPD) and Clinical attachment loss (CAL)), immunological parameter (Enzyme-Linked Immuno-Sorbent Assay (ELISA) for the quantitative measurement of MMP-8 concentration in the GCF) and microbiological parameter for the detection of A.a in plague sample by RT-PCR were measured at baseline (before treatment), 3 months and 6 months (after treatment).(fig.13,14)

Results:

I- Clinical results: Table 1,2.

There was significant reduction in PPD and CAL in laser group and photodynamic group. There was significant difference between both (laser group and SRP) and (photodynamic group and SRP) at three months, while at six months there was significance between photodynamic group and SRP.

Microbiological results: Table 4

Using Rt-PCR index, the results showed significant reduction in count of A.a in laser group and photodynamic group between (baseline and six months) and (three months and six months). However, there was significant difference at six months between (SRP and LT) and (SRP and PDT), but at three months the significance was between (SRP and LT). This means the effectiveness of anti-bacterial effect of laser therapy on aggressive periodontitis.

	Scaling and Root planing	Laser therapy	Photodynamic therapy	Kruskal Wallis Test
		(Baseline)		
Min-Max	6.00-11.00	5.00-10.00	5.00-8.00	$\chi^{2}_{(df=2)} = 3.611$ p=0.164NS
Median (IQR)	7.00 (6.00-8.00), p=0.071 NS	6.00 (5.00-8.00), p=0.001*	6.00 (5.00-8.00) p=0.029*	
		(3 Months)		
Min-Max	5.00-10.00	4.00-9.00	3.00-8.00	χ ² _(df=2) = 9.185 p=0.010*
Median (IQR)	7.00 (6.00-7.00), p=0.004*	5.00 (4.00-6.50), p=0.006*	5.00 (4.00-6.00), p=0.200 NS	
		(6 Months)		
Min-Max	5.00-10.00	4.00-9.00	3.00-8.00	$\chi^{2}_{(df=2)} = 11.256$ p=0.004*
Median (IQR)	7.00 (6.00-7.00), p=0.030*	5.00 (4.00-6.00), p=0.008*	5.00 (4.00-6.00) , p=0.200 NS	
		Friedman Test		
Chi-Square χ ² (df=2)	13.400	27.395	25.400	
р	0.001*	0.000*	0.000*	

n: Number of patients

*: Statistically significant (p<0.05)

Min-Max: Minimum - Maximum

NS: Statistically not significant (p ≥0.05)

CI: Confidence interval

KW: Kruskal Wallis Test

F: Friedman Test IQR: Inter-quartile range

Table (2): CAL index of the three studied groups and different time of assessment

Immunological results: Table3

ELISA test demonstrated significant improvement in MMP-8 levels regarding all groups, but at three months the statistically significant was in laser group and photodynamic group that indicate the rapid anti-inflammatory effect of laser or photodynamic therapy. Additionally, in the inter-group comparison, there was significant difference between both (SRP and PDT) and (SRP and LT) at six months.

	Scaling and Root planing	Laser therapy	Photodynamic therapy	Kruskal Wallis Test
		(Baseline)		
Min-Max	0.20-1.50	0.60-1.50	0.20-1.50	χ² _(df=2) = 2.496 p=0.287NS
Median (IQR)	0.80 (0.60-1.20), p=0.200 NS	1.00 (0.80-1.30) , p=0.177 NS	1.00 (0.80-1.30) , p=0.200 NS	
		(3 Months)		
Min-Max	0.30-1.30	0.10-1.30	0.10-1.20	χ ² _(df=2) = 0.019 p=0.991NS
Median (IQR)	0.70 (0.40-0.80), p=0.200 NS	o.6o (o.5o-o.9o), p=o.20o NS	o.6o (o.3o-o.9o), p=o.20o NS	
		(6 Months)		
Min-Max	0.30-1.00	0.10-0.60	0.10-0.60	χ ² _(df=2) = 10.843 p=0.004*
Median (IQR)	o.6o (o.3o-o.8o) , p=o.2oo NS	0.30 (0.10-0.40), p=0.073 NS	0.40 (0.10-0.50), p=0.200 NS	
		Friedman Test		
Chi-Square χ² (df=2)	9.120	28.107	28.737	
р	0.010*	0.000*	0.000*	

 Table (3): ELISA index of the three studied groups and different time of assessment

	Scaling and Root planing	Laser therapy	Photodynamic therapy	Kruskal Wallis Test
		(Baseline)		
Min-Max	1.0-1.5	11.0-2.0	1.0-2.0	χ ² _(df=2) = 6.008 p=0.049
Median (IQR)	1.1 (1.0-1.3) , p=0.000*	1.3 (1.2-1.5) , p=0.040*	1.3 (1.1-1.5) p=0.200*	
		(3 Months)		
Min-Max	0.9-1.4	0.9-1.8	0.8-1.3	χ ² _(df=2) = 6.611 p=0.037*
Median (IQR)	1.1 (1.1-1.2) p=0.001*	1.3 (1.1-1.4), p=0.017*	1.2 (1.2-1.3), p=0.002*	
		(6 Months)		
Min-Max	0.9-1.2	0.5-1.0	0.0-1.0	χ ² _(df=2) = 19.557 p=0.000*
Median (IQR)	1.1 (1.0-1.1) , p=0.027*	1.0 (0.9-1.0), p=0.000*	o.9 (o.8-1.0) , p=o.000*	
		Friedman Test		
Chi-Square χ ² (df=2)	1.805	18.667	19.069	
р	o.406 NS	0.000*	0.000*	

Table (4): Rt-PCR index of the three studied groups and different time of assessment

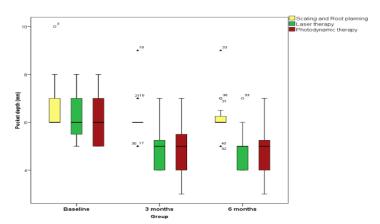


Figure (1a): Box and whisker graph shows the measurement of PPD score in each study period in all groups

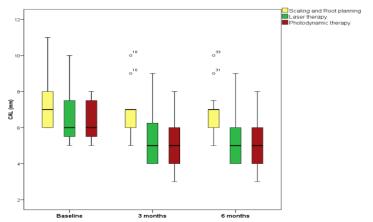


Figure (2a): Box and whisker graph shows the measurement of CAL score in each study period at three groups

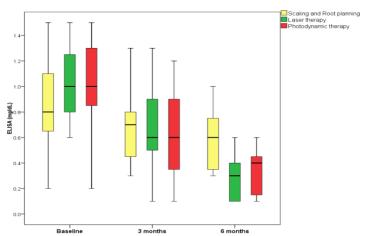


Figure (3a): Box and whisker graph shows the measurement of ELISA score in each study period at three groups.

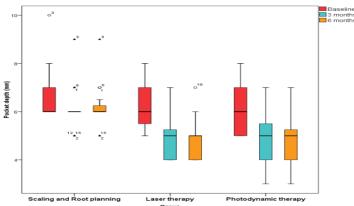


Figure (1b): Box and whisker graph shows the measurement of PPD in the three studied groups along the study period

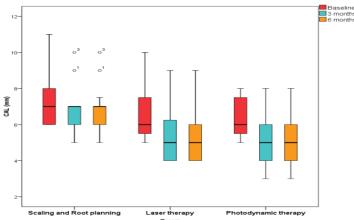


Figure (2b): Box and whisker graph shows the measurement of CAL in the three groups along the study period.

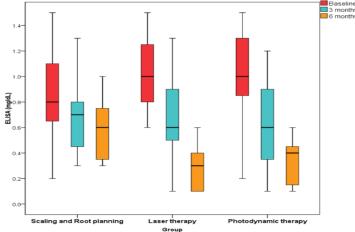


Figure (3b): Box and whisker graph shows the measurement of ELISA in the three groups

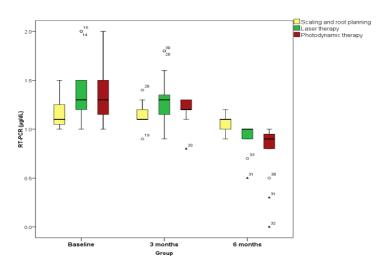


Figure (4a): Box and whisker graph shows the measurement of Rt-PCR score in each period at three groups

Discussion

In the present study, at baseline, no significant differences between the three treatment groups in all parameters were found indicating absence of any selection bias. Therefore, any difference during the study period among the groups at the assigned intervals would be due to the treatment modality used.

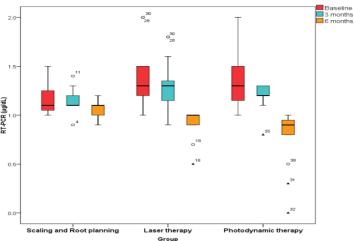
The results of the probing pocket depth index (PPD) and clinical attachment level (CAL) showed improvement in PPD and CAL which are important clinical outcome measurements to determine the success of the treatment.

The results of the current study showed a statistically significant reduction in the PPD and CAL for group II and III at three months, which was maintained till the end of the study (six months) with better result in favor to group II and III as compared with group I.

In inter group comparison, there was statistically significant improvement between SRP and both PDT and laser therapy at three and six months. This finding reflects the effectiveness of PDT and laser therapy till the end of study period.

Several studies corroborated with our study results as Matarese et al(25), who found that SRP+diode laser produced a significant improvement in probing depth and CAL values compared with SRP alone. Sreedhar et al(26), also confirmed the present study who compared SRP; (SRP + Diode Laser) and (SRP + PDT) in a split mouth design. They found statistically significant reduction in clinical parameters like PI, BOP, PPD and RAL (relative attachment level) which observed in laser therapy and PDT groups rather than SRP alone. Moreira et al(27), who concluded that difference in the mean PPD between SRP alone and SRP plus PDT was detected after application of PDT and gain in CAL that was significantly different from the control group at 90 days.

In contrary with the result of our study Arweiler et al.,(28) compared patients with AgP treated with SRP and antibiotic versus two episodes of PDT, the result showed a significant reduction in the antibiotic group compared with the PDT group. This can be speculated to



Figure(4b): Box and whisker graph shows the measurement of Rt-PCR in the three groups along the study period

different short wavelength and different photosensitizer type Phenothiazine chloride blue Photosensitizer and concentration decreased in the deep pockets due to washing of it with saline that decrease its absorbance by tissue. The systemic use of amoxicillin and metronidazole may have a powerful bactericidal effect on cell wall and DNA of A.a so give significant improvement.

In periodontitis, matrix metalloproteinase-8 (MMP-8) is considered the most collagenolytic enzyme in gingival crevicular fluid and regarded as a potential biomarker(29).

Concerning the immunological parameters, there was statistically significant improvement in group II and III at 3 months and at 6 months in MMP-8 level in GCF compared with baseline values. There was a significant difference between group I and both groups II, III at six months, which reflect the effectiveness of laser therapy and PDT until the end of the study.

This improvement in the MMP-8 levels can be explained by its relation with bacterial load and immune cells(30). Therefore, it may be hypothesized that improvement in the levels of MMP-8 could be the direct influence of the reduction in periopathogenic microbes in periodontal lesions through the three modalities of treatment(31).

In consistence with the present study, Marcaccini et al.(32) reported that higher levels of MMP-8 and MMP-9 form in the GCF of chronic periodontitis patients decreased at three months after periodontal therapy. Deumer et al(33) ,also in agreement with the results of our study who compared the effect of the antimicrobial photo-thermal therapy (PTT) in combination with conventional SRP.

Moreover, the reduction in MMP-8 level in laser group is in agreement with numerous studies that have found that diode laser exhibits anti-inflammatory action with improved periodontal wound healing in aggressive periodontitis(34),(31). Qadri et al(34), reported that the treatment with laser therapy reduced the periodontal inflammation, as assessed by the gingival index, PPD, GCF volume, and MMP-8 levels On contrary of our study Skurska et al(35), suggested that in patients

with AgP, treated with SRP with adjunctive systemic administration of amoxicillin and metronidazole, is statistically significant in reducing GCF MMP-8 levels than the adjunctive use of PDT. This could be speculated to short wavelength and photosensitizer type Phenothiazine chloride HELBO® Blue Photosensitizer and concentration affected its absorbance by tissue. Certain dyes may reduce specific inflammatory mediators since the dyes react with certain mediators and make them more exposed to the applied radiation(36, 37).

Real-time PCR is considered a good, precise tool for accurate quantification of the periodontal pathogen in periodontal plaque sample(38, 39). Hence, this study used Rt-PCR due to its not only specificity but also sensitivity for the detection of bacterial pathogens(40).

Concerning the microbial parameters, it was observed that group I (SRP+ control) showed a reduction in A.a count at all evaluation periods. This can be explained by the assumption that a single-session of full mouth SRP might influence the host-microbial equilibrium a qualitatively increase in local and systemic acute immune response that protects the host periodontal tissues and has an effect on the bacterial biofilm. In addition, one stage SRP prevented bacterial translocation from untreated sites to the treated ones(41). This result was repeatedly reported in Yashima et al.(42), who demonstrated that SRP causes a shift in bacterial flora and improves the periodontal condition in the early stages.

Regarding group II (SRP+LT) and group III (SRP+PDT), results showed a significant reduction in A.a count until the end of the evaluation period. Also, the results showed statistically significant improvement in group II and group III at (baseline and six months) and at (three and six months). In the intergroup comparison, there was a significant difference between groups I and group II at three-month periods. There was a statistically significant reduction at six months between (group I and group III) and between (group II and group I), which reflect the effectiveness of photodynamic therapy and laser therapy till the end of the study.

There are many studies that augment our study as Pourhajibagher et al(43) ,who stated that high doses of methylene blue MB-PDT could potentially exhibit antimicrobial activity as an essential virulence factor of A.a is reduced in cells surviving PDT with MB as detected in Rt-PCR. Another study to Pourhajibagher et al(44),are in accordance with the present study, who concluded that PDT with ICG could reduce cell survival and the virulence of A.a when compared with control group in AgP patients using 125 $\mu g/mL$ ICG, diode laser irradiation that could reduce the expression of A.a approximately 6-fold.

On reflection of advantage of PDT, Sreedhar et al., (26) reported that a PDT in multiple application in AgP could be a valuable tool for treating A.a infections in which there was reduction in CFUs of A.a and BPB(black pigmented bacteria) at 3 months that attributed to the beneficial effects multiple application of PDT over single application .

Based on the clinical results of the present study, all treatment modalities result in significant clinical improvement in laser and PDT than SRP with slight improvement of PDT procedure that may be a new avenue for future treatment of AgP. But immunological and microbiological result showed significant reduction in both PDT and LT rather than SRP till the end of study period.

However, it is important to emphasize that the clinical conditions such as time of performance and tissue photosensitizer concentration, pH change, exudate presence, and gingival fluid in the sub-gingival environment can influence the effectiveness of therapy(45). Thus, the comparison between different studies is challenged by various laser parameters, photosensitizers' concentrations, and changes in periodontal conditions and periodontal treatments.

In no account an insufficiency in our study size samples due to the incidence and prevalence of AgP isn't higher in comparison with chronic periodontitis that induce difficulty to more enroll subjects in our study, that could affect to prove definitive preference between laser and PDT groups in comparison of both techniques. This could need long term study screening periods.

Conclusion:

Based on the clinical results of the present study, all treatment modalities result in significant clinical improvement with a clear superiority of PDT procedures that may be a new avenue for future treatment of AgP. Immunological and microbiological result showed significant reduction in both PDT and LT rather than SRP till the end of study period.

Conflicts of Interest

The authors declare no conflict of interest with the products utilized in the present study.

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