

Comparison of 1-Periodontal Indices and Cultural *Porphyromonas Gingivalis* Colony Count in Aggressive Periodontitis Patients Treated by Scaling and Rootplanning with or Without Metronidazole Gel

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Abstract

Objective: Systemic antibiotics and locally applied antimicrobial agents have been suggested to enhance clinical parameters. Patients exhibiting aggressive periodontitis in particular benefit from adjunctive antibiotic therapy. The purpose of this investigation was to evaluate the effect of local antibiotic therapy with metronidazole adjunctively to scaling and root planning (SRP) in the treatment of aggressive periodontitis.

Materials and Methods: Twenty patients diagnosed with aggressive periodontitis were placed in a split mouth design. Microbial specimens were taken from the deepest pocket of the teeth. The sites that had positive results of *Porphyromonas gingivalis* (P.g) were located randomly to receive SRP treatment in the control group and SRP plus metronidazole gel in the test group. Pocket probing depth (PPD), clinical attachment level (CAL) and bleeding on probing (BOP) parameters and numbers of P.g. colony were taken at baseline, 6 weeks and 12 weeks later. All data were collected and analyzed and tested by Wilcoxon and paired t test.

Results: The case group patients had significantly better results in BOP, PPD and the number of P.g colony count reduction in comparison with the control group ($p < 0.05$). According to the measurements of CAL, the statistical difference was non significant ($p > 0.05$).

Conclusion: In non-surgical periodontal treatment of aggressive periodontitis adjunctive metronidazole gel therapy has a better effect on the reduction of *porphyromonas gingivalis* content of pockets.

Key Words: Local Antibiotic Therapy; Microbial Culture of *Porphyromonas Gingivalis*; Metronidazole; Aggressive Periodontitis; Periodontal Nonsurgical Treatment; Elyzol

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INTRODUCTION

Aggressive periodontitis was seen as a specific type of periodontitis in the 1999 International Workshop in which microbiological criteria

were generally included as elevated proportions of *Actinobacillus actinomycetemcomitans* (A.a) and, in some populations, *Porphyromonas gingivalis* (P.g) [1].

It has long been recognized that the interactions between these bacterial pathogenic microflora and the inflammatory responses of a susceptible host produces the progressive destruction of periodontal tissues [2]. However, adequate oral hygiene instructions, supragingival and subgingival plaque and calculus removal by scaling and root planing (SRP) are essential to reduce the amount of subgingival bacteria, but it is unlikely that mechanical debridement will eradicate and permanently clear these pathogens from a diseased site [3]. In particular, as probing depth (PD) increases, the effectiveness of SRP decreases, leaving subgingival plaque and calculus on the root surfaces [4].

Therefore, systemic antibiotics and locally applied antimicrobial agents have been suggested to enhance the clinical parameters. This is especially worthy as some bacteria strains have the ability to invade the soft tissues and escape from the mechanical debridement access [5]. Patients exhibiting aggressive periodontitis in particular benefit from adjunctive systemic antibiotic therapy. Especially, the combination of mechanical therapy and systemic application of amoxicillin and metronidazole together has been shown to resolve periodontal inflammation effectively in these patients [6].

However, administration of systemic antibiotics such as metronidazole, which require recommendation of large dosages to obtain suitable concentrations at the site of disease, have the risk of developing drug interactions, need the patient compliance for use and may lead to significant risk of nausea, headache, anorexia, vomiting and gastro-intestinal adverse reactions.

To avoid these limitations of systemic administration, a different approach has been introduced that uses local delivery systems that contain antibiotic or antiseptic agents. The objective of this study was to evaluate the efficacy of metronidazole dental gel as an adjunct to subgingival debridement compared to

subgingival debridement alone in the treatment of aggressive periodontitis.

MATERIAL AND METHODS

This study was a controlled clinical trial with Split Mouth Design. It was carried out among the patients referred to the periodontology department, Dental School of Tehran University of Medical Sciences. Twenty patients selected of all patients were referred to the periodontology department and diagnosed as aggressive periodontitis by the periodontist of this department in the last year. The inclusion criteria were as follows: (1) the presence of aggressive periodontal criteria (2), age between 20-45 years old (3), patient's proper cooperation (4) presence of bilateral symmetric greater than 5mm pockets positive in P.g.

The exclusion criteria were as follows: (1) presence of systemic disease (2), poor oral hygiene (O'leary plaque index higher than 35% after oral hygiene instruction) (3), smoking (4) periodontal treatment over the previous 6 months (5), antibiotic therapy over the last 8 months.

These patients had active pockets larger than 5mm with bleeding on probing in two quadrant of their teeth. Their low calculus and microbial plaque was not proportionate to their rapid bone loss shown in the radiographic images which were taken during their follow up periods in the last year. Diagnosis of aggressive periodontitis in these patients was confirmed by other periodontics specialists of the periodontology department and selected for the study.

The study was approved by the ethical committee of Tehran University of Medical Sciences. After explaining objectives of the study for each patient and obtaining written consent, pocket depth attachment and bleeding were recorded and supragingival plaque was removed by sterile cotton swabs prior to sampling. The samples were taken from the deepest bleeding pocket of the tooth in each qua-

drant by using a paper point no. 40 for 30 seconds. The paperpoints were transferred to a 1 microliter reduced transfer media and sent to the laboratory immediately.

Transfer media containing samples were vortexed for 30 seconds and using a bacteriologic loop, 100 microliter of the samples were aseptically cultured on Brucella agar enriched with sheep blood (hemin) and vitamin K1. The cultured plates were placed in an anaerobic jar containing a gaspack system and incubated for 3-4 days at 37°C. After incubation, black pigmented gram negative P.g colonies were identified and counted.

Following the arrival of the laboratory report, the clinical indices of clinical attachment level (CAL), pocket probing depth (PPD) and bleeding on probing (BOP) were recorded for pockets in which laboratory proof of positive P.g was documented. In each patient in a split mouth design a quadrant containing a pocket larger than 5mm was selected randomly for the test group by the clinician other than authors. Another quadrant was selected as the control group. In the test and control quadrant, the deepest pockets were selected for the test (test pockets) or control (control pockets) group of the pocket study. Each patient was put for full mouth subgingival and supragingival scaling and root planning (SRP) program. The test pocket group was treated with 25% metronidazole gel (Elyzol)¹ instantly by a periodontist other than the authors and the whole pocket was filled up with gel some of which was visible on the surface. This procedure (application of Elyzol in the test group) was repeated a week later by the same clinician other than authors.

Six weeks after primary treatment, the clinical indices including CAL, BOP and PPD were measured again in both test and control pockets. Besides, new samples of test and control pocket groups were recollected and sent to the

laboratory without hesitation as the same way described earlier and the P.g colonies were counted.

This procedure was also repeated 3 months after the initial treatment. Collected data were analyzed for paired condition using Wilcoxon signed ranks tests in the SPSS statistical program. It is mentioned that the oral hygiene instruction and maintenance was applied all throughout the study.

RESULTS

Twenty patients (13 females and 7 males) aged from 20-42 years (mean, 31.4) were selected. Totally, 92 pockets (46 in each of the test and control groups) were investigated.

The pockets average depth and standard deviation among test group were 3.02mm and 0.91mm, respectively 12 weeks after the primary treatment; whereas, the similar figures for the control group were 3.76mm and 1.21mm, respectively and the differences were significant (Table 1).

Table 2 shows the CAL changes over different times of treatment. The CAL average was initially the same in both groups. However, there was no significant difference between the test and control groups ($p=0.78$).

Table 3 shows a comparison of bleeding on probing (BOP) variation in the test and control groups over 3 stages of therapy. The BOP average was almost the same among the two groups at the beginning of treatment, but as the time passed on, there was more reduction in the test group compared with the control group which produced a statistical significant difference among the two sets ($p=0.005$). This showed clinical improvement in both groups with a more evident effect in the case group.

By assessing the average of P.g count after 6- and 12-week intervals following treatment, there was statistically significant ($p=0.006$) improved results observed in the test group comparing the colony counts before and after

¹Elyzol 25% Dental Gel , Dumex Corp , Copenhagen , Denmark

the gel application in contrast to the follow up intervals in the control group (Table 4).

DISCUSSION

Our study was carried out on 20 patients selected from patients referred to the periodontology department, Dental School of Tehran University of Medical Sciences. The selection was based on initial diagnosis (clinical/radiological) carried out by department professors. Other tasks such as taking written agreement, oral hygiene control, SRP and also sampling were performed by clinicians who were blinded to the test and control area. All laboratory studies associated with the current study were carried out in the microbiology department, Faculty of Medicine, Shahed University and all the bacterial colony counting was performed by a designated member of staff.

In this study, we selected an aggressive form of periodontitis with documentation of high presence of P.g. Mombelli and coworkers showed persisting P.g and Actinobacillus actinomycetemcomitans in the pocket after mechanical therapy. They thought that local antibiotic therapy must be guided by microbiologic diagnosis of pocket microflora [7].

However, the administration of antibiotics has to be limited only for patients with advanced periodontitis and aggressive forms of periodontitis and local antibiotic therapy is not necessary in the chronic form to avoid microbial resistance [7].

In the "split mouth design" the degree of similarity between genetic, socioeconomic, health, defense and microbiological conditions of the samples are very high.

This is a valuable feature of our study particularly in the treatment of aggressive periodontitis, because the initial conditions are the same for the teeth.

This feature has repeatedly been used in many previous investigations and we believe that, it is the best plan for studying the effect of an antibiotic. In 1992, Pedrazzoli et al. used the same scheme in a multi centered research [8].

In addition, in 2000, Griffiths et al., pointed out the same scheme in a multi centered study [9].

Our study was specifically carried out on aggressive periodontitis; whereas, in other studies, the therapeutic effect of metronidazole gel and systemic metronidazole alone or with other antimicrobial agents were tested on patients with chronic periodontitis [10,11].

Table 1: Pocket Probing Depth (PPD) Variations at different times in the case and control groups

		Index							
		Variable	Mean	STD*	Range	SE†	CI‡	L	U
Pocket depth	Case	Before primary treatment	6.09	1.13	4	0.16	6.09 ± 0.32	5	9
		6 wks after primary treatment	3.39	0.98	4	0.14	3.39 ± 0.28	2	6
		12 weeks after primary treatment	3.02	0.91	4	0.13	3.02 ± 0.26	1	5
	Control	Before primary treatment	6.30	1.55	8	0.22	6.30 ± 0.44	4	12
		6 wks after primary treatment	4.04	1.21	6	0.17	4.04 ± 0.342	3	9
		12 weeks after primary treatment	3.76	1.21	7	0.17	3.76 ± 0.34	2	9

* STD: Standard deviation, † SE: standard Error, ‡ CI: Confidence Interval, L: Lower, U: Upper

In our study, the time of treatment was not a determinant factor, but successfully SRP was important in contrast to suggestions of Griffiths and Palmer [9,12]. They believed that the time of treatment is an important factor in the treatment of aggressive form pockets. In our opinion this variation depends on a series of mechanical and therapeutic factors and is also related to the skill of the operator, the patient's cooperation and the rate of calculus in the area. Griffiths proposed the performance of SRP on all quadrants for a maximum of 60 minutes [9]; whereas, Palmer recommended a fixed protocol for successive scaling in two weeks for 90 minutes each time [12]. There are different views on the therapeutic effect of metronidazole on clinical and microbiological parameters. Some clinicians have used metronidazole in therapeutic surgery and did not find better results [13,14]. In some studies, the systemic metronidazole as mono therapy did not produce encouraging results [15], while in others, this type of mono therapy was accompanied with good responses [16-18]. Some investigators have used a combination therapy composed of metronidazole and amoxicillin in the treatment of advanced periodontitis and have obtained promising results [10,11].

Some researchers also have used a serial of antibiotics which resulted in different findings. The basis of such treatment was based on the use of two bactericidal and bacteriostatic agents.

In a study by Kaner et al., SRP plus adjunctive systemic amoxicillin/metronidazole was compared with SRP plus adjunctive CHX chip placement for the treatment of generalized aggressive periodontitis [19]. There was significantly higher PD reduction and "gain" of CAL for SRP plus adjunctive systemic amoxicillin/metronidazole patients. Besides, interestingly, there was a further continuous decrease of supragingival plaque measures in the amoxicillin/metronidazole group only and they presented significantly less supragingival plaque after 6 months.

In our study, twenty aggressive periodontitis patients were treated who showed the effect of local 25% metronidazole gel (Elyzol) alone or with SRP. This has been investigated in many studies [8,20].

In the study by Griffiths et al., the clinical effects of subgingival scaling (SRP) with SRP plus subgingival application of 25% metronidazole gel, Elyzol were compared in patients with chronic adult periodontitis.

Table 2. Attachment Level (CAL) Variations at different times (before , 6 weeks and 12 weeks after)in case and control groups

		Index							
		Variable	Mean	STD*	Range	SE†	CI‡	L	U
Attachment level	Case	Before primary treatment	5.17	1.43	7	0.21	5.17 ± 0.41	3	10
		6 wks after primary treatment	7.20	1.75	9	0.25	7.20 ± 0.50	4	13
		12 weeks after primary treatment	7.72	1.89	9	0.27	7.72 ± 0.54	5	14
	Control	Before primary treatment	5.61	2.02	12	0.29	5.61 ± 0.58	1	13
		6 wks after primary treatment	7.43	2.44	12	0.35	7.43 ± 0.70	4	16
		12 weeks after primary treatment	7.83	2.51	12	0.37	7.83 ± 0.72	4	16

* STD: Standard deviation , † SE: standard Error, ‡ CI: Confidence Interval , L: Lower , U: Upper

The results showed that the combination therapy of SRP gel was superior to the conventional treatment of SRP alone regarding PPD, BOP and CAL and the differences were consistent for 9 months [9].

In a systematic review done by Arthur et al. in 2005, of the 11 studies of SRP plus locally delivered metronidazole, four yielded statistically significant reductions in PD. Three reported data showed a statistically significant difference in the net PD reduction that favored the treatment group. Another study reported that the net difference between the treatment and control groups was significant at 12 weeks ($p < 0.01$) and two studies reported statistically significant CAL gains; 0.66 mm at 6 weeks ($p < 0.01$) and 0.4 mm at 39 weeks ($p < 0.001$) [21].

According to our study; in the case group a considerable reduction in the number of P.g colonies 6 and 12 weeks after treatment was demonstrated which was significant statistically ($p < 0.05$) when compared with the control group (SRP). This finding was both clinically and statistically evident and significant, the therapeutic use of metronidazole gel can inhibit the bacterial growth in periodontal pockets.

In the present study the SRP was performed by one designated clinician and was the same for all the patients. The type of metronidazole therapy was consistent with those of Klinge et al. in 1992 who determined the most effective concentration of metronidazole when applying the drug in two successive weeks, one immediately after scaling and the second a week later [22]. This protocol has been used by many researchers [8,20,23]. However, in the study by Riep et al., in order to have a more constant influence on the subgingival microflora, they decided to apply metronidazole gel more often and in shorter intervals. The other remarkable fact was excluding the patients who had received antibiotic therapy within 6 months prior to the study [3].

In our study, the SRP was done immediately prior to the application of metronidazole gel. In the study by Salvi GE et al., one of the explanations for not achieving any changes in the microbiologic quantity and composition of the subgingival microbiotic after placing the antimicrobial agents has been stated the re-establishment of the subgingival biofilm after initial therapy that protects the microorganisms from the antimicrobial agents [24].

Table 3. PBI Variations at different times (before , 6 weeks and 12 weeks after) in case and control groups

		Index							
		Variable	Mean	STD*	Range	SE†	CI‡	L	U
Probing bleeding	Case	Before primary treatment	2.74	0.57	2	0.08	2.74 ± 0.16	2	4
		6 wks after primary treatment	0.087	0.28	1	0.04	0.087 ± 0.08	0	1
		12 weeks after primary treatment	0.15	0.51	3	0.07	0.15 ± 0.14	0	3
	Control	Before primary treatment	2.80	0.65	2	0.09	2.80 ± 0.18	2	4
		6 wks after primary treatment	0.54	0.78	3	0.11	0.54 ± 0.22	0	3
		12 weeks after primary treatment	0.52	0.52	3	0.12	0.52 ± 0.24	0	3

* STD: Standard deviation , † SE: standard Error, ‡ CI: Confidence Interval , L: Lower , U: Upper

Comparison of statistical values of PPD among the case and control groups shows that the P-value was initially 0.58 (insignificant) which turned to 0.002 (significant) and less than 0.001 (significant) following 6 and 12 weeks after primary treatment, respectively. So, there was a significant statistical difference between the case and control groups and that, the case group showed a higher PPD reduction compared to the control group.

In 1992, Pedrazzoli et al. obtained a PPD average of 1.14 mm after gel treatment and 0.88mm after scaling with no significant difference statistically, when compared with the initial conditions [8].

Palmer et al. (1998) also failed to show any clinical differences among the two groups and rejected the necessity of metronidazole application in routine periodontal therapies [12].

In another study by Knoll- Kohler (1999), it was concluded that the use of metronidazole gel as a therapeutic and mechanical substitution for adult periodontitis treatment is unsuitable [25].

It is worth mentioning that in Kohler's study two issues were not addressed. First, it was not clear whether metronidazole gel can affect other types of periodontitis, and second, the possibility of metronidazole gel plus SRP application as a therapeutic alternative. In 1992, Ainamo et al. reported that the pocket depth decreased to 1.2 mm and 1.5 mm in the case and control groups, respectively. However, the differences were significant statistically but clinically it was not important as there was 88% reduction of BOP in both groups [20]. Regarding the Split Mouth Design of our study and the similarity of inclusion and exclusion criteria, the same level of initial conditions were achieved throughout the study.

CONCLUSION

Considering the limited number of studies on the effect of metronidazole gel on aggressive periodontitis, the current study highlighted the importance of the clinical application of such an intervening procedure and produced a suitable basis to be used by all dentists and

Table 4. Porphyromonas Gingivalis Colony Count Variation at different times (before , 6 weeks and 12 weeks after) in case and control groups

		Index							
		Variable	Mean	STD*	Range	SE†	CI‡	L	U
P.g. Colony Count	Case	Before primary treatment	29862.59	147617.48	9997	21765.01	29862.59 ± 42659.42	3	10 ⁴
		6 wks after primary treatment	32.63	148.5 ^v	1000	21.9	32.63 ± 43.93	0	10 ³
		12 weeks after primary treatment	7.37	22.32	99	3.3	7.37 ± 6.45	0	100
	Control	Before primary treatment	6648.78	20532.18	99998	3027.30	6648.78 ± 5933.50	2	10 ⁵
		6 wks after primary treatment	365.17	1490.21	10 ⁴	219.71	365.17 ± 430.65	0	10 ⁴
		12 weeks after primary treatment	260.76	1475.43	10 ⁴	217.54	260.76 ± 426.37	0	10 ⁴

* STD: Standard deviation , † SE: standard Error, ‡ CI: Confidence Interval , L: Lower , U: Upper

researchers. Further studies on this field to examine more variations in therapeutic procedures are suggested.

In addition, by extending the period of study more reliable bacteriological and clinical findings may be obtained.

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