Journal of Research in Medical and Dental Science
2021, Volume 9, Issue 2, Page No: 30-35

Available Online at: www.jrmds.in
eISSN No. 2347-2367: pISSN No. 2347-2545

Vascular Endothelial Growth Factor Estimation Before and After Various Treatment Modalities in Patients with Periodontal Disease (Clinical and Immunological Study)

Ismaeel Ahmed Ismaeel*, Alaa Omran Ali AL-Mosawi

Department of Periodontics, College of Dentistry, University of Baghdad, Baghdad, Iraq

ABSTRACT

Background: Periodontal diseases are the most common inflammatory oral conditions that affects the periodontium following dental caries, and may ultimately lead to tooth loss if left untreated. With recent advancement in the field of periodontics, application of adjunct methods and/or devices have been advocated to further improve healing in periodontally affected teeth. Vascular endothelial growth factor (VEGF) is considered the most potent angiogenic factor that is associated with both health and disease, many studies have researched the upregulation of vascular endothelial growth factor in association with wound healing and tumor growth. This study focuses on the expression levels of vascular endothelial growth factor within gingival crevicular fluid (GCF) before and after treatment.

Materials and methods: A clinical trial was conducted on 30 subjects with periodontitis (16 male and 14 female). Subjects participating in the clinical trial were divided into three groups, each group including 10 individuals. 1st group were treated by scaling and root planing (SRP), 2nd group were treated by scaling and root planing with adjunct chlorhexidine, and 3rd group were treated by scaling and root planing with adjunct laser irradiation. Clinical periodontal parameters were obtained at baseline and post-treatment to assess improvement in periodontal health status clinically, parameters included were plaque index (PII) and gingival index (GI). Gingival crevicular fluid samples were collected from each subject using filter paper strips inserted deep inside periodontal pocket for 30 seconds, collection was carried out before treatment on the 2nd visit and after treatment on 3rd visit with 2-weeks interval between visits. Concentration of vascular endothelial growth factor within collected gingival crevicular fluid samples was analyzed via the use of enzyme-linked immunosorbent assay (ELISA).

Results: Improvement in clinical parameters were recorded following treatment for all subjects, the mean of vascular endothelial growth factor concentration was decreased after treatment in all subject with two of the study groups having statistically significant decrease in concentration following treatment.

Conclusion: Results from this study indicate that all study groups showed clinical improvement in periodontal parameters, and decrease in VEGF conc. following treatment and no clear-cut advantage could be established between the three different treatment modalities.

Key words: Periodontal disease, Clinical and immunological study, Vascular endothelial growth factor, Chlorhexidine

HOW TO CITE THIS ARTICLE: Ismaeel Ahmed Ismaeel, Alaa Omran Ali AL-Mosawi, Vascular Endothelial Growth Factor Estimation Before and After Various Treatment Modalities in Patients with Periodontal Disease (Clinical and Immunological Study), J Res Med Dent Sci, 2021, 9 (2): 30-35.

Corresponding author: Ismaeel Ahmed Ismaeel

e-mail✉: ali.mario28@yahoo.com

Received: 02/01/2021

Accepted: 19/01/2021

INTRODUCTION

Periodontal diseases are the 2nd most common inflammatory oral conditions that affect teeth and surrounding structures following dental caries, amounting up to 10.6% of world population in 2017 [1], periodontal diseases could ultimately lead to tooth loss in aggressive cases or if the condition left untreated. Recent studies suggested that periodontal disease is initiated by synergy of different and/or specific gene combination that lead disruption to periodontal tissue homeostasis and commensal bacterial leading to alteration in host response that cause tissue destruction, this etiological concept is known as "polymicrobial synergy and dysbiosis" (PSD) [2]. Periodontal therapy consists of surgical and non-surgical approaches depending on the condition each with their own advantages and disadvantages, surgical approach is mainly reserved for hard-to-reach

periodontal defects or large defects that cannot be repaired without surgical intervention and/ or reconstructive procedures. Scaling and root planing (SRP) has long been considered the "gold standard" of non-surgical periodontal therapy to which all other treatment modalities are compared to, however SRP alone may not be sufficient for removal of bacterial biofilm and infected periodontal tissue, hence adjunct materials and devices to be used in combination with SRP were advocated. Following removal of bacterial biofilm and infected tissue by SRP, granulation tissue fibrous tissue and subsequent long-junctional epithelium will fill periodontal defect space in what is known as "repair". Rate of repair in periodontal tissue is highly dependent on revascularization of affected site by angiogenesis, the higher the revascularization the faster the repair rate of the periodontal defect.

Angiogenesis is a double-edged sword when it comes to periodontal health and disease as it both promotes regeneration of new blood vessels while healing and causes increased permeability and chemotaxis subsequently leading to tissue destruction in periodontal diseases. Vascular endothelial growth factor (VEGF) is considered the most potent angiogenic factor responsible for angiogenesis present in periodontal tissue within endothelial cells, plasma cells, macrophages and periodontal epithelium in both health and disease [3], VEGF not only directly induces angiogenesis but also enhances tissue permeability as well, which is said to be 50,000 times more potent that histamine hence it was formerly known as human vascular permeability factor (hVPF) [4], increased expression of VEGF in gingival crevicular fluid (GCF) was evident in cases of periodontitis and gingivitis [5]. Due to the episodic nature of periodontal diseases having periods of destruction and quiescence, fluctuation of VEGF expression could be observed in GCF samples as it is upregulated when the disease is in its "active" stage and VEGF levels drop when disease is "inactive" [6,7]. Further evidence of increased levels of VEGF expression in periodontal disease was noted by upregulation with tissue hypoxia and glucose starvation [8] which are synonymous with periodontal diseases.

MATERIALS AND METHODS

30 systemically healthy subjects with periodontitis (16 male and 14 female) were selected for this study, all in-patients from the Department of Periodontics/College of Dentistry, University of Baghdad. Subjects participating in the clinical trial were divided into three groups, each group including 10 individuals. Subjects of the 1st group were treated by scaling and root planing, subjects of the 2nd group were treated by scaling and root planing with adjunct chlorhexidine gel application into periodontal pocket, subjects of the 3rd group were treated by scaling and root planing with 30 seconds diode laser irradiation along the depth of periodontal pocket. Subjects were scheduled for morning appointments between 9-12AM to avoid possible effect of circadian rhythm on GCF flow and volume, GI and PlI indices were recorded for the 1st visit followed by impression taking and a full-mouth scaling by ultrasonic scaler, and subjects are then scheduled for a recall visit after 1 week (2nd visit), custom-made acrylic occlusal stent is then fabricated in the laboratory.

On the 2nd visit, subject's oral hygiene is assessed to evaluate commitment to the clinical trial, GI and PlI are recorded. Plaque is then gently removed from the site of periodontal pocket, site is then isolated by cotton pallets and dried with jet streams of air, saliva ejector is also used simultaneously to avoid contamination with saliva while obtaining GCF sample. A PerioCol absorbent paper strip is inserted inside the pocket until minimum resistance is felt, the absorbent paper strip is kept inside the pocket for 30 seconds and then removed carefully and preserved inside an already weighted Eppendorf tube which is filled with a 200µl of PBS. The Eppendorf tube containing the collected paper strip is then immediately weighted and kept in a cooler box for overnight storage. After collecting GCF sample subject is then treated according to their respective group, subjects in the 1st group are treated by SRP and instructed to maintain standard oral hygiene measures. Subjects in the 2nd group are treated by SRP and chlorhexidine digluconate: 0.2% paste (PerioKIN paste) is introduced into the depth of the pocket via a deep pocket applicator, subjects were prescribed a commercially available chlorhexidine mouthwash to be used twice daily for no more than 7 days, and recommended subjects to avoid eating and/or rinsing mouth with water to remove the chlorhexidine gluconate after

taste as it may reduce the effectiveness of chlorhexidine, subject were also instructed to maintain standard oral hygiene measures. Subjects in the 3rd group were treated by SRP followed by 30 seconds application of Epic™ X Biolase diode laser into the depth of the pocket, the tip is gently inserted parallel to the long axis of the root and manipulated along the pocket depth, subjects were instructed to maintain standard oral hygiene measures. All groups were scheduled for a 3rd visit 2-weeks following the 2nd visit so healing may not be disrupted during probing and/or GCF sample collection.

On the 3rd visit GI and PlI are recorded to assess whether there is an improvement in clinical parameters and overall status of oral hygiene. Plaque was gently removed and site was isolated and prepared for GCF sample collection.

Statistical analysis

The data analyzed using Statistical Package for Social Sciences (SPSS) version 25. The data presented as mean, standard deviation and ranges. Categorical data presented by frequencies and percentages. Independent t-test and Analysis of Variance (ANOVA) (two tailed) was used to compare the continuous variables accordingly. Paired t-test was used to compare the continuous variables on admission and on discharge. Pearson's correlation test (r) was used to assess correlation between continuous variables accordingly A level of P–value less than 0.05 was considered significant.

RESULTS

Subjects in the 1st group who were treated by scaling and root planing showed significant reduction in gingival index at the end of treatment (3rd visit) in comparison to baseline (1.0 versus 2.20 at baseline, P=0.001). Subjects of the 2nd group who were treated by scaling and root planing and adjunct chlorhexidine application within periodontal pocket showed significant reduction in gingival index at both 2nd and 3rd visits compared to baseline (1.7 versus 2.3, P=0.005; and 1.0 versus 2.3, P=0.001 respectively). Subjects in the 3rd group who were treated with scaling and root planing and 30 seconds of laser irradiation along the pocket depth have also showed significant reduction in gingival index at both 2nd and 3rd visits compared to baseline (1.7 versus 2.4, P=0.01; and 1.1 versus 2.4, P=0.001 respectively). Subjects of the 2nd group showed significant reduction in plaque index at both 2nd and 3rd visits compared to baseline (1.5 versus 2.2, P=0.025; and 1.1 versus 2.2, P=0.002 respectively). Subjects of the 3rd group have also showed significant reduction in plaque index in both visits as compared to baseline (1.9 versus 2.4, P=0.015; and 1.1 versus 2.4, P=0.001 respectively). The reduction of inflammation after treatment was evident by the reduction of GCF samples volume obtained from the periodontal pocket by filter paper strips, VEGF concentration was shown to be decreased in 1st group after 2 weeks following treatment as compared to baseline, however this decrease in concentration was not statistically significant (238.04 pg/mL versus 283.47 pg/mL, P=0.067). concentration of vascular endothelial growth factor was shown to be significantly reduced following treatment in both 2nd and 3rd group (194.8 pg/mL versus 302.27 pg/mL, P=0.014; and 194.75 pg/mL versus 335.2 pg/mL, P=0.001 respectively) (Tables 1-Table 6).

Table 1: Comparison between GI in each study group at 2nd and 3rd visits with baseline level.

Study group	GI				
	Baseline	2nd visit	P-Value	3rd visit	P-Value
	Mean ± SD	Mean ± SD		Mean ± SD	
SRP	2.20 ± 0.42	1.20 ± 0.42	-	1 ± 0	0.001
SRP+CHX	2.30 ± 0.67	1.70 ± 0.48	0.005	1 ± 0	0.001
SRP+Laser	2.40 ± 0.51	1.70 ± 0.48	0.01	1.10 ± 0.31	0.001

Table 2: Comparison in percentage of change in GI between study group at 3rd visit.

Study Group	Percentage of change in GI	P-Value
	Mean ± SD	
SRP	53.33 ± 7.02	0.952
SRP+CHX	51.66 ± 19.95	
SRP+Laser	53.33 10.54	

Table 3: Comparison between PII in each study group at 2nd and 3rd visits with baseline level.

Study group	PII				
	Baseline	2nd visit	P-Value	3rd visit	P-Value
	Mean ± SD	Mean ± SD		Mean ± SD	
SRP	2.30 ± 0.67	1.60 ± 0.51	0.025	1 ± 0	0.001
SRP+CHX	2.20 ± 0.63	1.50 ± 0.52	0.025	1.10 ± 0.31	0.002
SRP+Laser	2.40 ± 0.51	1.90 ± 0.31	0.015	1.10 ± 0.31	0.001

Table 4: Comparison in percentage of change in PI between study group at 3rd visit.

Study group	VEGF		
	2nd Visit	3rd Visit	P-Value
	Mean ± SD	Mean ± SD	
SRP	283.47 ± 63.43	238.04 ± 73.37	0.067
SRP+CHX	302.27 ± 80.89	194.80 ± 63.52	0.014
SRP+Laser	335.2 ± 81.51	194.75 ± 91.10	0.001

Table 5: Comparison between VEGF in each study group at 2nd and 3rd visits.

Study Group	Percentage of change in VEGF	P-Value
	Mean ± SD	
SRP	15.03 ± 24.64	0.179
SRP+CHX	28.93 ± 35.03	
SRP+Laser	40.55 ± 29.31	

Table 6: Comparison in percentage of change in VEGF between study groups at 3rd visit.

Study Group	Percentage of change in PII	P-Value
	Mean ± SD	
SRP	51.6 ± 19.95	0.602
SRP+CHX	45 ± 24.90	
SRP+Laser	53.33 10.54	

DISCUSSION

Healing and/or regaining healthy periodontal tissue is the ideal outcome of periodontal therapy, however due the nature of periodontal disease having periods of active destruction and quiescence and subsequent loss of periodontal apparatus healing and regeneration may not be fully possible. Angiogenesis role in periodontal disease is well documented and the rate of angiogenesis depends on plethora of inflammatory mediators, however VEGF was considered to be the main and most potent angiogenic mediator within the periodontal tissue, contributing not only to angiogenesis when periodontal disease is active but also in maintaining healthy periodontal tissue as well.

This study focuses on the concentration of VEGF in GCF samples collected before and after various treatment modalities analyzed by ELISA, and on the clinical findings of periodontal parameters and whether any of used treatment modalities yielded superior results clinically. Subjects participating in this study were carefully selected and examined, patients with chronic inflammatory diseases and/or diseases that may affect periodontal health were excluded (e.g., hypertension, diabetes mellitus, rheumatoid arthritis, etc.). Patients who were smokers were excluded as well as smoking may increase the transient expression of certain cytokines and may affect the flow and volume of GCF, and female patients who either pregnant and/or lactating we also excluded to eliminate the possibility of hormonal changes effecting periodontal health. Regarding adjunct therapy and its effect as scored by clinical parameters, there were some mixed reviews regarding the use of diode laser as an adjunct to scaling and root planing with some investigators deeming it not significant to overall improvement in periodontal health status [9,10,11], while majority of investigators opting for its use as adjunct to SRP [12]. As for the role of adjunct chlorhexidine in periodontal therapy, it is well-established as the gold standard anti-plaque agent and there is world-wide consensus over the benefits of chlorhexidine in periodontal therapy [13,14]. Discrepancy in results of this study could be owed to interindividual variability (e.g., level of commitment to oral hygiene instructions and use of interdental aids)

that may higher impact on results especially in a small sample size.

This study found a statistically significant difference when comparing GI and PlI before and after treatment for all study groups. Subjects in the SRP+Laser group had higher percentage of change in GI and PlI out of all participants, However, no clear-cut advantage to any of the treatment modalities could be established as the percentage of change in clinical parameters was not statistically significant when comparing between study groups. As for the VEGF concentration in GCF Prapulla et al. found that VEGF drastically decreased for patients with gingivitis and periodontitis following treatment by scaling and root planing [5], Padma et al. also supported that claim and found strong correlation between level of VEGF and volume of GCF in patients with gingivitis and periodontitis in comparison to patients with healthy periodontium [15]. This study found a statistically significant difference between concentration at baseline and 2-weeks after treatment in all subjects with 22.2% decrease in concentration overall, however no statistically significant difference was found when comparing between the study groups with SRP+Laser group having highest reduction in VEGF levels (40.55%), followed by SRP+CHX group (28.93%) and SRP group with the least reduction in VEGF levels (15.03%). This study found a positive correlation between expression levels of VEGF and volume of collected GCF samples, supporting claims of both Padma et al. and Prapulla et al. and confirming upregulation of VEGF levels with marked inflammation which is almost always accompanied with increased volume of GCF [5,15].

CONCLUSIONS

On the light of the clinical and immunological findings of this study the following can be concluded:

Improvement in periodontal health could be achieved with all treatment modalities with no noticeable advantage for any of the modalities used

VEGF expression increased in active periodontitis sites and expression decreased after treatment with disregard to treatment modality used.

REFERENCES

1. Vos T, Abajobir AA, Abate KH, et al. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990–2016: A systematic analysis for the Global Burden of Disease Study 2016. Lancet 2017; 390:1211-1259.
2. Hajishengallis G, Lamont RJ. Beyond the red complex and into more complexity: the polymicrobial synergy and dysbiosis (PSD) model of periodontal disease etiology. Molecular Oral Microbiol 2012; 27:409-419.
3. An Y, Liu WJ, Xue P, et al. Autophagy promotes MSC-mediated vascularization in cutaneous wound healing via regulation of VEGF secretion. Cell Death Dis 2018; 9:58.
4. Connolly DT, Olander JV, Heuvelman D, et al. Human vascular permeability factor. Isolation from U937 cells. J Biol Chem 1989; 264:20017-20024.
5. Prapulla DV, Sujatha PB, Pradeep AR. Gingival crevicular fluid VEGF levels in periodontal health and disease. J Periodontol 2007; 78:1783-1787.
6. Sapna G, Gokul S, Bagri-Manjrekar K. Matrix metalloproteinases and periodontal diseases. Oral Diseases 2014; 20:538-550.
7. Agis H, Watzek G, Gruber R. Prolyl hydroxylase inhibitors increase the production of vascular endothelial growth factor by periodontal fibroblasts. J Periodont Res 2012; 47:165-173.
8. Ebersole JL, Novak MJ, Orraca L, et al. Hypoxia-inducible transcription factors, HIF1A and HIF2A, increase in aging mucosal tissues. Immunology 2018; 154:452-464.
9. Dukić W, Bago I, Aurer A, et al. Clinical effectiveness of diode laser therapy as an adjunct to non-surgical periodontal treatment: A randomized clinical study. J Periodontol 2013; 84:1111-1117.
10. Saglam M, Kantarci A, Dundar N, et al. Clinical and biochemical effects of diode laser as an adjunct to nonsurgical treatment of chronic periodontitis: A randomized, controlled clinical trial. Lasers Med Sci 2014; 29:37-46.
11. Üstün K, Erciyas K, Sezer U, et al. Clinical and biochemical effects of 810 nm diode laser as an adjunct to periodontal therapy: A randomized split-mouth clinical trial. Photomed Laser Surg 2014; 32:61-66.
12. Qadri T, Javed F, Johannsen G, et al. Role of diode lasers (800–980 nm) as adjuncts to scaling and root planing in the treatment of chronic periodontitis: A systematic review. Photomed Laser Surg 2015; 33:568-575.
13. Jeffcoat MK, Bray KS, Ciancio SG, et al. Adjunctive use of a subgingival controlled-release chlorhexidine chip reduces probing depth and improves attachment level compared with scaling and root planing alone. J Periodont 1998; 69:989-997.

14. Jones CG. Chlorhexidine: Is it still the gold standard? Periodontology 1997; 15:55-62.

15. Padma R, Sreedhara A, Indeevar P, et al. Vascular endothelial growth factor levels in gingival crevicular fluid before and after periodontal therapy. J Clin Diagnostic Res 2014; 8:ZC75.