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The effect of ozone gel as adjunct treatment in patients of periodontitis according to bleeding on probing and platelet-activating factor (A split-mouth randomized controlled clinical trial)

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Abstract:

Background: Ozone gel (deriving from a chemical reaction between ozone and pure plant extracts to form an oil or a jelly-like product). This gel not only represents the antimicrobial effect but can express safe cytocompatibility with host tissues and provide advantageous effects is improving.

Objective: bleeding on probing and platelet-activating factor measurements to compare traditional scaling with root planning only and scaling with root planning and ozone application for treatment of periodontal disease

Materials and methods: total sample composed of 58 periodontal pockets was divided into two groups. The control group had 28 periodontal pockets treated with scaling and root planning only, while the test group had 30 periodontal pockets treated with scaling and root planning and ozone gel. Two applications of the gel were applied at baseline and one month after the treatment .platelet activating factor (biomarker) and clinical parameters of bleeding on probing are measured at baseline, after one month, and after three months. In the second visit of periodontal therapy (after one day), GCF samples were obtained from patients by gently inserting periocol paper into the selected depth of the pocket when resistance was encountered. The periocol paper was then left in position for 30 seconds before being removed and weighed on a chemical balance. Each paper strip was placed in a tube keeping o.3ml of buffer phosphate saline, then transferred and stored at -40C. Calculated the difference between the weight of periocol paper before and after exudate absorption of gingival crevicular fluid. After sample storage for five minutes, the samples were centrifuged at 13,000 rpm.

Results: Test sites showed a greater reduction in bleeding on probing and there was a significant difference in the first month only when compared to control sites (Fisher exact=**0.010)** but no significant difference at three months. The platelet-activating factor was reduced significantly by ozone gel in the first month with only a p-value (0.000) but no significance at one and three months.

Conclusion: In comparison to SRP alone, treatment with ozone gel as an adjuvant therapy yields no statistically significant improvement after three months.

Keywords: ozone gel, bleeding on probing, and platelet-activating factor.

Introduction

Periodontitis is a multifactorial disease that result in tooth loss. The complicated pathogenesis of periodontitis suggests the involvement of a host that is susceptible and a bacterial challenge [1]. An inflammatory effusion called gingival crevicular fluid emanates from the periodontal pocket or gingival sulcus. Volumes are normally small (μL), and they tend to rise along with periodontal and gingival tissue irritation. Gingival crevicular fluid is a complicated mixture of substances from blood and locally produced mediators of inflammation and tissue turnover/breakdown. Additionally, it includes bacterial components as well as cellular elements like neutrophils. Through both a physical protective impact (bacteria and their byproducts are diluted, plus there is fluid output), as well as the delivery of antibacterial chemicals into the pocket, gingival crevicular fluid protects against host-bacteria interactions [2,3].

Gingival crevicular fluid analysis has the benefit of providing site-specific information regarding the state of inflammation at any given periodontal site, GCF is the principal target in research for proteomic biomarkers discovery of periodontal diseases [4]. Several approaches, such as gingival washing techniques, absorbent filter periocol paper, and capillary tubes or micropipettes, have been used to collect gingival crevicular fluid [5]. They have shown a strong correlation between GCF volume and the level of gingivitis or periodontitis-related periodontal inflammation. In particular, it has been observed that successful periodontal treatment has a favorable relationship with clinical characteristics such as probing pocket depth and GCF volume [6]. Numerous studies have demonstrated that when compared to healthy controls, individuals with periodontitis have higher levels of inflammatory response biomarkers [7,8]. One of the important agents and adaptable proinflamtory mediators discovered in human beings is the platelet-activating factor (PAF). A wide range of cells, particularly active inflammatory cells including macrophages, thrombocytes, and lymphocytes, can make and release it [9,10].

The connection between periodontal inflammation and PAF has not been fully explored by many studies. PAF secreted by inflammatory periodontal tissues is measurable in the GCF and decreases during periodontal treatment. In a prior investigation by our team, levels of PAF in serum and gingival crevicular fluid (GCF) from periodontitis patients were found to significantly positively correlate with

periodontal parameters [11]. The progression of the illness can be stopped by an anti-infective therapy that significantly lowers or eliminates these pathogens such as (*T.* denticola, T. forsythia, and P.intermedia). However, ozone functions as an antioxidant and an antibacterial. The term "ozone gel" (figure 1) refers to Pure olive oil that has been ozonized using a steady flow of ozone-oxygen mixture in the ratio of 5:95% till the oil transforms from a greenish liquid to a whitish gel condition[12]. Ozone's ability to both oxygenate the air and kill germs makes tissue regeneration more likely. This is in addition to the way that the omega 3 in ozone gel works, which may be useful in the adjunctive care of periodontitis as a host modulatory agent [13]. A potent oxidizing agent with a high antibacterial potency against pathogens of the oral cavity, ozone therapy is becoming more and more popular as a contemporary, non-invasive technique of treatment, Oral antiseptics can be substituted with ozone. Its utility to treat infectious illnesses of the mouth is suggested by a high level of biocompatibility with cementoblasts, epithelial cells, and fibroblasts [14].

Hypothesis:

H_0: The ozone gel adjunct to non-surgical treatment (SRP) is not significantly effective when compared with non-surgical treatment alone

H_1: The ozone gel adjunct to non-surgical treatment (SRP) is significantly effective when compared with non-surgical treatment alone.

AIM:

This clinical trial aims to evaluate the effect of using local Ozone gel as an adjunct to non-surgical treatment on bleeding on probing and platelet-activating factor level in GCF.

Objectives:

Measuring the following periodontal clinical parameter bleeding on probing and biological marker platelet-activating factor before application of ozone treatment and after treatment at one and three months.

Material and methods

Study population

This study was conveyed at the Department of Periodontology/Teaching Hospital/College of Dentistry/University of Babil, from March 2022 to September 2022. The study was approved by the ethical committee/College of Dentistry/ University of Baghdad at reference no.524 on 17/4/2022 subordinate the guidelines of Helsinki and Tokyo for humans, (NCT:05905445)

People were registered volunteers to the study after signing the informed consent sheetto share in research and submitted to a questionnaire comprising their name, age, gender, medical history, dental history, and smoking followed by a complete examination of periodontal parameters. Exclusion criteria included subjects taking antibiotics or other medications in the last 3 months, Smokers, and Pregnant or lactating women. Inclusion criteria systemically healthy patients, patients have periodontitis according to Tonitti [15] Interdental CAL is detectable at ≥2 non-adjacent teeth, or Buccal or oral CAL ≥3 mm with pocketing >3 mm is detectable at ≥2 teeth.

Trial design

Twenty-one subjects participated in this study. The age ranged from 35 to 60 years old, with a mean ±SD of 50.5±7.72.

Ten subjects (48%) were 35 to 47 years old, and eleven (52%) were 48 to 60. The subjects were 18 males (86%) and 3 females (14%). A randomized split-mouth, blinded design was followed to evaluate the anti-inflammatory effect of ozone gel. In the upper and lower jaw, two sites have been selected from each patient, the pocket depth must be equal to or more than 4mm. the total sample composed of 58 periodontal pockets was divided into two groups, The control group had 28 periodontal pockets treated with scaling and root planning only, while the test group had 30 periodontal pockets treated with scaling with root planning and ozone gel (When patients visit the clinic with three pockets, they may undergo two tooth controls and one tooth test, whereas another may undergo one tooth control and two teeth tests).

Measurement of the periodontal parameter (bleeding on probing) and biological marker in GCF (platelet-activating factor) were done at baseline, at first recall, and second recall at three-month follow-up.

At baseline, measuring the periodontal parameter BOP, in the next day collected GCF sample (to avoid contamination of periocol paper) and full mouth supragingival scaling and oral hygiene instructions.The application of ozone gel to the test site. At first recall after one month was include GCF sample collection and measuring bleeding on probing, then application of ozone. At second recall after three months included also GCF sample collection and measuring the bleeding on probing.

Sample size calculated:

To calculate sample size, will be Using G power 3.1.9.7 (Program written by Franz-Faul, Universitat Kiel, Germany) with the power of study=85%, alpha error of probability=0.05 two-tailed, from an article written by (Marco Colombo et al., 2021) depending on CAL for ozonated and CHX gels on mean±SD on 3 months period are 4.43±0.47 and 4±0.56 respectively making Cohen's D effect size is 0.8317 thus sample size is about 25 sites for each group.

Randomization and blindness

The sites that were taken into consideration for each patient were randomly assigned to the test and control groups using the coin toss method. The study's blindness was attained in the following ways: The researcher noted baseline clinical periodontal parameters. While post-intervention examinations (1 and 3 months later) were carried out by an experienced periodontist with blind calibration. Furthermore, periodontal procedures for all the sites were performed by an experienced calibrated periodontist who was blind for site assignment. While the Ozone application was carried out by the researcher.

Clinical intervention:

The control site was treated with SRP only. In contrast, the test site was treated with SRP and the administration of ozone gel by using a disposable syringe and a blunt 25-ga needle as shown in figure (2) The chosen teeth were carefully isolated with cotton rolls, dried completely, and then the gel was carefully placed sub-gingivally and interproximal until access gel was visible from the gingival margin. To treat every tooth, this process was repeated. Patients were asked to refrain from eating, drinking, or rinsing for at least 30 minutes after the excess gel was removed with a cotton roll, this procedure done at baseline and first month [14].

Additionally, patients were advised

not to use interdental appliances, brush close to the area that had received gel treatment, or chew on sticky or hard foods. At the application location, instructions for supra-gingival brushing were given.

The site was protected from saliva contamination with cotton rolls. The clinical parameter of both sites (BOP) were measured before the start of the treatment to create a baseline record for future investigation and clinical improvement tracking. After a month, the patient was initially called back to measure the clinical parameters and for a second gel application. To prevent disrupting the healing process, the gel was administered at the beginning of the pocket rather than up to the base.

The second recall appointment was to measure clinical parameters three months after the baseline visit. The intra-crevicular approach was used to collect the sample of GCF at baseline and the first and second follow-ups (Periocol, Oraflow, USA).

The site must be dry and saliva-free to ensure optimal GCF collection. Before recording clinical periodontal data, gingival crevicular fluid (GCF) was collected from the gingival sulcus. If there was contamination from bleeding during recording or saliva stimulation, it should be discarded. Before placing the periocol paper, cotton rolls were inserted around the relevant places, light pressure was applied to control bleeding, and the paper strips were placed deep into the pocket for thirty seconds. After that, the GCF sample is put into Eppendorf tubes and stored in an ultra-low-temperature freezer at -40°C until sample collection is complete.

Measuring of bleeding on probing:

If bleeding occurs within 30 seconds of the periodontal probe being inserted at the bottom of the periodontal pocket, the site is given a score of (1), while a score of (0) is provided for the non-bleeding site[16].

The procedure of Sampling of gingival crevicular fluid for analysis:

GCF samples were collected from patients after examination of the periodontium if there was periodontitis to avoid contamination of periocol paper postponed the patient to the next day of the sample. The plaque on the teeth should be completely removed with cotton carefully without harming the gingivae before the sampling. Before beginning to collect GCF, the teeth and gingiva were carefully dried. When resistance was felt, a previously weighed sample was carefully inserted into the chosen pocket depth, held there for 30 seconds, and then removed to be measured on a chemical scale (SARTORIUS scientific balance: four digits after zero, with a potential of 210 g and a sensitivity of 0.1 mg as shown in figure 3), Each periocol paper was put in an Eppendorf tube keeping o.3ml of buffer phosphate saline, Calculated the difference between the weight of periocol paper after and before exudate absorption of gingival crevicular fluid, then transferred and stored at -40C. After storage, the samples were centrifuged at 13,000 rpm for 5. then, periodontal therapy would be performed (after that patient had received supra gingival and subgingival scaling and polishing and received good oral hygiene instructions then applied ozone gel in one site and left another site without ozone).

Statistical analysis:

Both Descriptive Analysis and Inferential analysis. The statistics description includes Range, mean, median, and standard deviation (SD) while inferential analysis involved: The Shapiro-Wilk test, Friedman test, Mann-Whitney U test, Fisher exact test, Spearman rank correlation analysis, and Mcnemar test was used.

Results

Results in Table (1) show that all studied variables are normally distributed among groups and time using Shapiro Wilk test at p>0.05. Findings in Table (2) show that after one month 14 sites (66.67%) is still have BOP in the control group while 5 sites only contain BOP in the study group (23.81%) with the significant association and the effect size of BOP drop from baseline to after one month in the control group is larger than that in the study group used mcnemar test.

Results in the table (3) show that platelet-activating factor (PAF) declined from the baseline Mean=27.923, ±SD=1.678, than after one and three months of treatment in each group with significant change and the greater change finds in the study group than that in control and when comparing each time with each other there is a significant difference between them in each group, and although its value is lower in the study group than that in the control in each time (after one and three months from base line) these results are not significant difference after 3 months but the significant difference after one month only used Friedman test.

Results in Table (4) show that Gingival crevicular fluid (GCF in µL) declined from the baseline then after one and three months of treatment in each group with significant change and the greater change finds in the study group than that in the control and when compare each time with each other there is a significant difference between them in each group, and although its value is lower in the study group than that in the control in each time (after one and three months from base line) these results are not a significant difference.

Findings in Table (5) show that there is a weak positive significant correlation between PAF and GCF in each group and time except in the study group after one month, its result is a moderate positive significant correlation using Spearman rank correlation analysis.

Findings in Table (6) between PAF and BOP, these results are positive weak significant correlation in the control while not significant in the study and a weak negative not significant correlation in the study group between GCF and BOP.

Discussion:

In the control group, BOP is still present at 14 sites (66.67%) but only at 5 sites (23.81%) in the study group. This shows that only a one-month significant difference and not significant at three months. leeding on probing (BOP) indicates an inflammatory tissue response to bacterial infections. Soft or hard deposits are considered one of the leading causes of BOP [17]. However, ozone gel may have an antimicrobial effect and anti-inflammatory and antioxidant actions [18]

This study is consistent with the previous research of **Kshitish and Laxman (2010)**, which showed a significant difference between ozone and the control group at seven days. They claimed that the effects of ozone on the gingival bleeding index include alteration in plaque composition, removal of inflammation-inducing factors, and physical changes in tissue integrity [19].

This study is in agreement with the study made by Shoukheba, which indicated the presence of a significant difference between ozone and control groups in 1st month, and found that because of the inhibitory action of ozone gel on the NF-kappa B system makes it a potent anti-inflammatory agent and can halt progression disease activity[14][20].

A different point of view of the recent study disagreement with several studies that used ozone water such as[21][22] they used water

irrigation in the pocket and showed no significant difference between test and control group in the first month this may be due to used water ozone, since once it dissolves in water; it becomes extremely unstable and quickly decomposes through a complex series of chain reactions, so it cannot be stored[23].on the other hand when it is dissolved in an oil base, it has a life span that could be measured in years.

At three months there was no significant between groups of the recent study in accordance with previous studies[24][25][22] Since the great majority of clinical trials linked to periodontitis treatment have been carried out using curettes and/or scalers, hand instrumentation is typically considered as the gold standard[26].

PAF levels decreased significantly in each group from baseline through one and three months of treatment. The study group changed more than the control group each time they were compared. The study group's value is continuously lower than the control group's after one and three months from baseline, but the results are only statistically different after one month.

After searching in the international studies on this subject, this study is considered the first one taking the examination the use of ozone gel for periodontal therapy concerning PAF levels in both control and test groups before and after treatment, so it is challenging to compare our findings to earlier data. The present investigation indicated that experiencing periodontitis displayed an increased level of this mediator factor in the gingival fluid. PAF levels in GCF revealed substantial positive relationships with all clinical periodontal parameters. Previous studies reported that "PAF levels in gingival fluid of patients having periodontal diseases are elevated compared with healthy controls" [26], which is in consistence with the recent results, suggested that "the higher levels of PAF in the inflamed gingival tissue might have been due to an increase in PAF production" [27].

The elevated levels of PAF in GCF in patients having periodontitis suggested an "important role of this lipid mediator in the pathogenesis of periodontitis and a potential involvement of this mediator in the development of systemic diseases [11]. PAF is synthesized by activated neutrophils and regulates the induction of chemotaxis, superoxide generation, and degranulation [28]. Upon activation, it can stimulate the release of a variety of lysosomal enzymes, arachidonic acid metabolites, and cytokines, which can further induce PAF synthesis [29][30]. The importance of this mediator in the course of periodontitis was underlined by the reduction of PAF levels in GCF after initial periodontal therapy [31].

This present study focused on the changes in gingival crevicular fluid PAF levels during non-surgical periodontal treatment with ozone intervention, and the results clearly showed a significant decrease in gingival crevicular fluid PAF levels after SRP plus ozone and SRP alone. Based on this knowledge, the recent study hypothesizes that the regeneration and repair of the gingival connective tissue can account for decreased gingival crevicular fluid PAF levels following periodontal therapy. Moreover, after these drugs, a significant increase in angiogenesis and a drop in GCF PAF levels were seen. The decrease in GCF and PAF levels might be due to vasodilatation and inflammation resolution. An angiogenesis increase may be associated with healing activity, as angiogenesis is considered a crucial healing process [32].

According to this study, it may be revealed PAF phospholipid proinflamtory mediator, According to a previous study Shoukheba 2014 showed ozone application, causes an increase in phospholipase activity,

Additionally the inhibitory effect of ozone on the NF-kappa B system makes it a potent anti-inflammatory agent and can stop disease activity [14] therefore accelerated healing activity at first-month lead to the significant difference between groups.

The decline in GCF volume from baseline to the third month demonstrates a significant change, with the study group seeing a more remarkable change than the control group. Each group's data demonstrate a significant difference when compared to one another. However, there is no difference between them across groups.

The literature discussing the gingival fluid volume rise in patients with periodontitis was higher than that in periodontally healthy participants. The GCF flow increases during inflammation, and the composition looks like an inflammatory exudate. By flushing bacterial colonies and their metabolites out of the sulcus, the greater GCF flow aids in host defense. The main route for GCF diffusion is through the basement membrane and then through the junctional epithelium into the sulcus [33].

The osmotic gradient increases when the dental biofilm is present, followed by more significant protein leakage. This increase will result in a rise in hydrostatic pressure and vascular permeability, thereby exceeding the capacity of lymphatics to drain fluids and leading to the upregulation of GCF flow[34]. These results are consistent with earlier results. Moreover, patients with periodontitis had a greater increase in gingival fluid volume [6]. Following treatment with non-surgical periodontal therapy, many researchers have reported reduced GCF levels in their patients[35]. It is well known that scaling and root planning can reduce periodontal tissue inflammation within two weeks, leading to decreased GCF volume after treatment. A further decrease in GCF volume was more evident after four weeks of treatment, mainly attributed to a reduced bacterial load that elicited a host response[36,37,38]

Studies also revealed that adjunct ozone in periodontal therapy was beneficial for reducing inflammation in periodontal tissues. This reduction, in turn, led to a decrease in GCF volumes, which was consistent with this study's conclusion that there was no significant difference between groups[36,39]. This GCF decrease was attributed to the antiseptic action of ozone gel on perio pathogens, which caused a disturbance in tissue homeostasis and host response modulation. On the other hand, SRP benefits the periodontal status of patients with periodontitis, so it was concluded that SRP is the treatment of choice. In addition, there are limitations to the biochemical efficacy achieved with ozone administration in clinical settings. Despite the similarity in results with most studies, there was a previous study disagreement[40]. It used gas ozone to evaluate the efficiency of ozone gas on GCF volume and showed a significant difference between groups. Nonetheless, subgingival ozone was applied once every two days for one full week, following the guidelines provided by the product maker. Repeated delivery may be the explanation for the additional improvement that was found following ozone therapy in the treatment of periodontal disease.

Except for the study group at the one-month mark, which exhibited a moderately significant positive association, all other groups and periods demonstrated a weak positive significant correlation between PAF and GCF.

The current study's findings, in accordance with a previous study Chen (2010) show a positive correlation between PAF and GCF volume [11]. These findings demonstrated that the inflammatory process of the periodontal tissues was the cause of the higher PAF levels in GCF and that this

mediator is essential to the inflammatory process of the periodontal tissue. Elevated PAF levels in the GCF sample of periodontal disease support the destructive role of this lipid mediator in human periodontal tissues. PAF plays an essential role in the pathophysiology of periodontal disease in concert with other mediators to regulate inflammatory and immune responses [41].

Ozone gel's ability to accelerate the repair of the periodontal disease led to a somewhat positive-significant correlation after just one month in the study group [42]. According to our knowledge, no study exists about the correlation between the PAF levels in the GCF with ozone application. Thus, further research is needed to understand their potential relationships and clinical significance in managing periodontal disease.

It is unsurprising to observe comparable amounts in areas at the sampling sites with similar symptoms of periodontal disease, given the damaging impact of excessive PAF production in sites with periodontal tissue deterioration. On the other hand, this literature disagrees with the previous study [41], where the study group showed no correlation between PAF levels and PLI in the patients with periodontitis in the test group. Also, there was no correlation between PLI and GCF in the control group, while there was a positive-weak not significant correlation in the study group.

To the best of our knowledge, there is no study about the correlation between PAF levels and PLI with ozone application. As the previous study [11]**.** Suggested, PAF and BOP exhibit a positive-weak correlation in each group.

The current investigation results show that the correlation between the PPD with the PAF and GCF is mostly weak-positive not significant at three months.

At baseline, when correlating PPD with PAF in both groups, the correlation is moderately positive-significant, and after 1 month, the correlation between PPD and PAF is positive-weakly significant. At the same time of treatment (1 month), the correlation between PPD and GCF in the study group was moderately positive-significant.

According to earlier research [11], there is a favorable link between the PPD and the PAF and GCF, which helps all periodontal metrics. The PAF levels in the GCF of all patients had significant positive relationships, and the PAF levels increased as the severity of the periodontal inflammation and damage increased. These findings demonstrated that the periodontal tissues' inflammatory process was the cause of the elevated PAF levels in GCF and that this mediator is essential to the inflammatory process of the periodontal tissues. The outcomes supported newly released information for patients with periodontitis.

The correlations between CAL with PAF and GCF are positive-weak not significant. As suggested in a previous study [41], no significant correlation exists between CAL and PAF levels in GCF. However, a disagreement with **(Zheng et al., 2006)** shows a significant correlation between the CAL with the PAF and GCF. This mismatch could be explained by the disparity in patient numbers and unique characteristics between the ethnic groups represented in the two studies.

Conclusion:

Scaling and root planning treatment has a positive effect on the periodontal health in patients with periodontitis. SRP followed by ozone therapy dose contribute to further improvement in clinical periodontal parameter (bleeding on probing) and platelet

activating factor level in gingival cervicular fluid in the first month only in patients with periodontitis. Re-evaluation after 3 month, the use of ozone gel as an adjunctive therapy to SRP produces no statistically significant benefit compared with SRP for all parameters.

Limitations of the study:

- There is a shortage of evidence on the various doses of ozone that applied only two times.
- Short time period for the study and loss of some patients because they did not attend follow-up appointments or did not comply with the study's instructions led to a small sample size.
- ELISA is a technically complex and demanding method that needs specialized laboratory equipment and a skilled technician to ensure that there are no technical errors.
- Sample collection was challenging because multi-center had been visited.

Suggestions:

1. Additional studies with larger sample sizes and extended durations, such as six months, are required to confirm the effects of ozone gel in periodontal therapy.
2. Studying the benefits of ozone gel and laser photodynamic therapy can be used to treat periodontitis.
3. Studying the effect of ozone gel on periodontal pathogens like *A.a, P.gingival and T. denticola* in vitro.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Figure(1) Ozone gel

Figure (2) Ozone application

Figure 3 (SARTORIUS scientific balance)

Table (1) Normality test of studied variables.

Variables	Shapiro-Wilk			
	Groups			
	Control		Study	
	Statistic	P value	Statistic	P value
PAF0	0.935	0.092	0.940	0.090
PAF1	0.944	0.150	0.960	0.310
PAF2	0.930	0.069	0.956	0.246
GCF0	0.956	0.297	0.936	0.071
GCF1	0.938	0.109	0.945	0.124
GCF2	0.929	0.060	0.951	0.180

Table (2) Distribution of BOP among groups and time
*Cohen's H=1.911, **=Cohen's H=1.019

Time		Groups			
		Control*		Study**	
		N	%	N	%
Baseline	With BOP	21	100.00	21	100.00
1M (Fisher exact=**0.010**)	With	14	66.67	5	23.81
	Free BOP	7	33.33	16	76.19
3M	Free	21	100.00	21	100.00

Table (3) Descriptive and statistical test of platelet activating factor at first and third month

Groups		Baseline	1M	3M	F	P value	MD	P value	Effect size
Control	Range	5.370	6.030	6.430	520.827	**0.000 Sig.**			0.951
	Mean	27.923	24.141	19.293		B X 1M=**0.000**	3.783	**0.000**	
						B X 3M=**0.000**	8.630	**0.000**	
	±SD	1.678	1.646	1.737		1M X 3M=**0.000**	4.848	**0.000**	
Study	Range	6.900	6.280	6.950	786.630	**0.000 Sig.**			0.967
	Mean	27.673	22.210	18.408		B X 1M=**0.000**	5.463	**0.000**	
						B X 3M=**0.000**	9.265	**0.000**	
	±SD	1.607	1.695	1.872		1M X 3M=**0.000**	3.802	**0.000**	
F		0.331	18.962	3.401					
P value		0.568	**0.000**	0.071					
ES		0.006	0.256	0.058					

1M=first month, 3M=third month, ES=effect size

Table (4) Descriptive and statistical test of GCF at first and third month.

Groups		Baseline	1M	3M	F	P value	Effect size
Control	Range	1.000	0.700	0.700	103.583	**0.000 Sig.** B X 1M=**0.000** B X 3M=**0.000** 1M X 3M=**0.000**	0.793
	Mean	0.822	0.470	0.300			
	±SD	0.247	0.188	0.159			
Study	Range	1.500	0.600	0.400	139.865	**0.000 Sig.** B X 1M=**0.000** B X 3M=**0.000** 1M X 3M=**0.000**	0.838
	Mean	0.833	0.417	0.240			
	±SD	0.348	0.162	0.122			
F		0.019	1.343	2.577			
P value		0.891	0.251	0.114			

Table (5) Correlation between PAF and GCF

Groups	Periods	R	p
Control	Baseline	0.061	0.762
	1 month	0.371	0.057
	3 month	0.049	0.809
Study	Baseline	0.181	0.388
	1 month	0.566	**0.001**
	3 month	0.033	0.866

Table (6) Correlation between BOP, PAF and GCF.

Groups		BOP1	
		Rsp	P
Control	PAF1	0.442	**0.049**
	GCF1	0.000	1.000
Study	PAF1	0.157	0.496
	GCF1	-0.094	0.684