Effect of Periodontal Surgery on Tissue Plasminogen Activator and Plasminogen Activator Inhibitor Type-1 Gene Expression in Gingival Tissues of Periodontitis Patients: A Controlled Before-And-After Study

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Accepted for publication:
March 27, 2019

Abstract

Background: Regulation of the plasminogen activation system (PAS) is a vital component in governing proteolytic events within the extracellular matrix (ECM). PAS is believed to play a substantial role in the destruction and healing of periodontal tissues. Thus, the current work aimed to study the histopathological effect of open flap debridement (OFD) on periodontitis, as well as its effect on tissue plasminogen activator (t-PA) and plasminogen activator inhibitor type-1 (PAI-1) gene levels in gingival tissues. **Methods:** A total of 30 subjects were enrolled in the present study. They were divided into two groups: Group I (control group) included 10 periodontally healthy volunteers and group II (periodontitis group) comprised 20 patients suffering from stage III grade B periodontitis. Gingival tissue samples were collected from all periodontitis patients, before and after OFD, and from healthy controls. Hematoxylin and eosin (H&E) stained slides were subsequently examined and gene expression levels of t-PA and PAI-1 were assessed in the gingiva through quantitative reverse transcription polymerase chain reaction (RT-PCR). **Results:** Gingival tissue samples from periodontitis patients showed dilated blood vessels, diffuse hemorrhage, areas of edema, and disorganized collagen fibers together with large amounts of inflammatory cells in between. Following OFD, smaller sized blood vessels, a restored collagen fiber distribution, and an obvious decrease in the inflammatory infiltrate were noted. Gene expression levels of t-PA and PAI-1 were significantly higher in the periodontitis patients compared to the healthy controls. Although their levels showed a significant decrease following OFD in the periodontitis group, they were still significantly higher than the control group. **Conclusion:** OFD procedures resulted in down regulation of t-PA and PAI-1 expression levels in the gingiva of periodontitis patients, which could signify an important role of these proteins on periodontal disease progression.

Keywords: Tissue plasminogen activator; plasminogen activator inhibitor type-1; gingival tissues; periodontitis; open flap debridement.

Introduction

Periodontitis is one of the periodontal diseases that has been recognized to have a complex pathogenesis. It was suggested to be a host

response to a long-standing bacterial challenge leading to progression of this disease due to a combination of different factors. These factors encompass the existence of periodontopathic bacteria and increased levels of

proinflammatory cytokines, matrix metalloproteinases (MMPs), and prostaglandin E2 (PGE2).¹⁻⁵ Periodontitis is also marked by low levels of anti-inflammatory cytokines including interleukin (IL)-10, transforming growth factor (TGF), and tissue inhibitors of MMPs (TIMPs).⁵⁻⁷ Nevertheless, the exact molecular mechanisms of periodontal destruction are not well identified. Therefore, recent research has focused on systems that are implicated in connective tissue remodeling such as the plasminogen activating system (PAS).⁸

PAS is a complex enzymatic cascade whereby each component activates or deactivates the end product of this cascade – plasmin. The mechanism by which plasminogen is converted to plasmin is initiated by two activators, the urokinase plasminogen activator (PA) and the tissue-type PA (t-PA). These two activators are constrained by PA inhibitor type 1 (PAI-1) and type 2 (PAI-2).⁹ Plasmin acts as a proinflammatory agent by inducing neutrophil aggregation and platelet degranulation, as well as by synthesizing and secreting arachidonic acid derivatives. It also stimulates the release of proinflammatory cytokines including tumor necrosis factor (TNF), IL-1 α , and IL-1 β , which directly degrade the extracellular matrix (ECM) or contribute to its destruction via the activation of MMPs and blocking the effects of TIMPs.^{10,11} Therefore, the damaging potential of PAS might be vital for the commencement and progression of periodontal disease.¹²

At present, the conceptual basis for the treatment of periodontitis is the elimination or adequate suppression of putative pathogens in the subgingival microbiota. The main viable and dependable strategy for eradication of these microorganisms is the mechanical debridement of subgingival locations. Hence, the traditional therapies for periodontal disease were dependent upon scaling and root planing (SRP), or upon surgical approaches to achieve this goal. Periodontal surgical therapy, including open flap debridement (OFD), constitutes a key aspect of the treatment of severe periodontitis patients. The objectives of OFD are to provide access for root debridement, to achieve pocket reduction, and to regenerate the lost periodontal tissues.¹³

Therefore, the objective of the current work was to study the effect of OFD surgical treatment on the progress of periodontitis. This effect was evaluated clinically and histopathologically through hematoxylin and

eosin (H&E) staining. Moreover, quantitative reverse transcription polymerase chain reaction (qRT-PCR) was used to analyze the expression levels of t-PA and PAI-1 mRNAs in the gingival tissues in an attempt to investigate whether or not the expression of these proteins could be involved in the pathogenesis of periodontitis. In addition, we aimed to assess the effect that applied surgical treatment would have on their expression.

Materials and Methods

The current clinical trial was registered and identified as NCT03803176 at ClinicalTrials.gov, which is a resource provided by the U.S. National Library of Medicine. Written consents approved by the research ethics committee were obtained from all participating subjects following an explanation of the study including the treatment and follow-up appointments needed.

I. Study Population

Thirty subjects in total were enrolled in this controlled before-and-after study and were divided into two main groups:

Group I (control group): 10 periodontally healthy volunteers who served as control subjects (6 males and 4 females; age range: 30-45 years, with a mean age of 41 years)

Group II (periodontitis group): 20 patients suffering from stage III grade B periodontitis (12 males and 8 females; age range: 35-50 years, with a mean age of 42 years)

II. Inclusion Criteria

All participating subjects were selected from the Outpatient Clinic at the Department of Oral Medicine and Periodontology of the Faculty of Dentistry, Cairo University, between February 2018 and June 2018. They were free of systemic diseases according to the modified Cornell Medical Index.¹⁴ Patients with a probing depth (PD) ≥ 5 mm and a clinical attachment level (CAL) ≥ 5 mm were diagnosed with stage III grade B periodontitis, according to the classification scheme established at the “2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions.”¹⁵ The control group included systemically healthy subjects who attended the same clinic and were scheduled for a crown lengthening procedure. They presented with a clinically healthy gingiva demonstrating a PD

≤3 mm, and a CAL, plaque index (PI), and gingival index (GI) of zero.

III. Exclusion Criteria

Pregnant females, smokers, and individuals who received periodontal therapy within the six months prior to recruitment or who have taken medication or antibiotics within the three months prior to recruitment, were excluded from the present study.

IV. Clinical Parameters

A thorough clinical examination was completed for all periodontitis patients and healthy control subjects. Clinical periodontal parameters including PD, CAL, PI,¹⁶ and GI,¹⁷ were recorded for all participating subjects. These parameters were assessed by a single periodontist (S.H.) at six sites per tooth for all teeth: mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual and distolingual. Probing depth was measured from the free-gingival margin to the base of the periodontal pocket and CAL was measured from the cemento-enamel junction to the base of the periodontal pocket. Measurements were approximated to the nearest millimeter utilizing the Michigan O probe with Williams' markings.

V. Periodontal Therapy

Following primary examination, all periodontitis patients were given comprehensive guidelines on self-accomplished plaque control methods including the use of a soft toothbrush and interdental cleansing devices. Full mouth supragingival and subgingival scaling and root planing were performed by two periodontists (S.H. and G.M). Under local anesthesia, subgingival debridement was performed using ultrasonic devices^a and universal periodontal curettes.^b Removal of all subgingival deposits was the end point of mechanical debridement achieving a smooth surface. After 4 weeks, each patient was re-evaluated and individuals exhibiting a PD ≥5 mm and a CAL ≥5 mm were given appointments for an OFD conventional surgical procedure.

Open flap debridement was carried out by two experienced periodontists (G.M. and S.H.). Following administration of local anesthesia, intrasulcular incisions were made and

conventional full thickness mucoperiosteal flaps were reflected to fully expose the defects.¹⁸ Thorough debridement of the defects was then accomplished, and the flaps were repositioned and sutured to their original position to attain primary closure. No dressing was used. Postoperative instructions were clarified to patients which included 0.12% chlorhexidine gluconate oral rinse^c twice daily as an adjunctive plaque control measure for two weeks. Three weeks following OFD, patients were instructed to use the roll technique to gently brush the operated area with a soft toothbrush.

VI. Histopathological Examination of Gingival Specimens

Ten healthy gingival tissue samples (2 mm x 2 mm) were obtained from the marginal gingiva of control subjects during crown lengthening procedures, and 20 gingival biopsies (1 mm x 1 mm) were collected from the marginal gingiva of periodontitis patients during the OFD procedure as baseline samples. Three months following OFD, another 20 biopsies were collected from the marginal gingiva of the defect. They were dissected away using the tip of a sharp curette without disturbing the healing phase.¹⁹ Thin (5 μ) paraffin sections of tissue specimens were stained with H&E and were studied under ordinary light microscope to monitor the histopathological changes within the epithelium and connective tissue throughout the study period.

VII. Quantitative Analysis of mRNA Expression for t-PA & PAI-1 Genes in Gingival Tissues Through Quantitative Real Time Polymerase Chain Reaction (qRT-PCR)

The relative expression of mRNA for t-PA & PAI-1 genes was assessed using the SYBR® Green method^d via qRT-PCR.^e Polymerase chain reaction primers were designed using the Gene Runner software^f from RNA sequences taken from a gene bank (Table 1). The primer set had a calculated annealing temperature of 60°C. Quantitative RT-PCR was performed in duplicate in a 50-μl reaction volume consisting of a 2x SYBR® Green PCR Master Mix, 2 μl of the primers, and 0.5 μl of cDNA. Amplification

^c Antiseptol, Kahira Pharmaceuticals Co., Cairo, Egypt

^d SYBR® Green PCR Master Mix, Applied Biosystems™, Foster City, CA

^e ABI Prism™ 7500 Sequence Detection System, Applied Biosystems™, Foster City, CA

^f Hasting Software, Inc., Hasting, NY

^a NSK Non-Optic Ultrasonic Scaler, Kanuma-shi, Japan

^b HuFriedy Universal Curette; HuFriedy, Chicago, USA

conditions were 2 minutes at 50°C, 10 minutes at 95°C, 40 cycles of denaturation for 15 seconds, and annealing/extension at 60°C for 10 minutes. The RT-PCR result was analyzed with the StepOne™ Applied Biosystems™ Software. Changes in the expression of each target gene were normalized relative to the mean critical threshold (CT) values of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) housekeeping gene using the $\Delta\Delta C_t$ method. The actual operation of these quantification methods was performed via qRT-PCR software.

Table 1. Primer sequence specific for each studied gene

Gene	Primer sequence: 5'- 3'
t-PA	F: GGCCTTGTCCTTCTATTCG R: AGCGGCTGGATGGGTACAG
PAI-1	F: GGCTGACTTCACGAGTCTTTCA R: TTCACTTTCTGCAGCGCCT
GAPDH	F: ACAGTCCATGCCATCACTGCC R: GCCTGCTTCACCACCTTCTTG

t-PA, tissue plasminogen activator; **PAI-1**, plasminogen activator inhibitor type-1; **GAPDH**, glyceraldehyde-3-phosphate dehydrogenase; **F**, forward; **R**, reverse; **T**, thymine; **A**, adenine; **C**, cytosine; and **G**, guanine.

VIII. Sample Size Calculation

The sample size was calculated using the G*Power software.²⁰ The calculation was based on the mean expression levels of t-PA and PAI-1

for both study groups. A total of 30 patients were divided into 2 groups: group I (control group) comprised 10 subjects and group II (periodontitis group) comprised 20 subjects, which were needed to achieve an 80% power test at a significant difference ($p\leq0.05$).

IX. Statistical Analysis

Data acquired from clinical periodontal assessment were statistically described as mean $\pm$ standard deviation (SD). Within the periodontitis group, the paired *t*-test was used to compare changes in periodontal parameters before and after OFD. Tissue plasminogen activator and PAI-1 gene expression levels obtained from qRT-PCR analysis were presented as median and range. Tissue plasminogen activator and PAI-1 gene expression within the study groups was compared using the Kruskal-Wallis test with post hoc multiple two-group comparisons. P-values ≤0.05 were considered statistically significant. All statistical calculations were performed using the computer software IBM Statistical Package for the Social Sciences® (SPSS) (IBM Corp, Armonk, NY, USA, Release 22 for Microsoft Windows).

Results

I. Clinical Parameters

Data for clinical periodontal parameters of all participating subjects are shown in Table 2. The present data shows a statistically significant decrease in all clinical parameters in periodontitis patients following OFD ($p\leq0.05$).

Table 2. Clinical periodontal parameters in the study groups (mean $\pm$ SD)

Periodontal Parameters	Group I (Control Group)	Group II (Periodontitis Group)	
		Before OFD	After OFD
PD (mm)	1.8 $\pm$ 0.32	5.2 $\pm$ 0.46	3.33 $\pm$ 0.6*
CAL (mm)	0.7 $\pm$ 0.21	6.27 $\pm$ 0.61	4.94 $\pm$ 0.56*
PI	0.3 $\pm$ 0.12	1.66 $\pm$ 0.32	0.63 $\pm$ 0.27*
GI	0.2 $\pm$ 0.11	1.92 $\pm$ 0.39	0.892 $\pm$ 0.45*

SD, standard deviation; **PD**, probing depth; **CAL**, clinical attachment level; **PI**, plaque index; **GI**, gingival index; and **OFD**, open flap debridement; *statistically significant difference after OFD ($p\leq0.05$).

II. Histopathological Results

Light microscopic examination of the gingivae of the control group revealed a normal keratinized stratified squamous epithelium separated from the underlying lamina propria by a heavily

undulated basement membrane. The superficial lamina propria was loose, with small sized blood vessels, while the deeper portion was denser with thick collagen fibers arranged in a parallel manner and contained fibroblasts and scattered inflammatory cells in between (Figure 1).

Figure 1. Photomicrographs of the human gingiva of a control patient

- a. Keratinized stratified squamous epithelium with numerous long, narrow epithelial ridges (arrows) (H&E x100)
- b. Basal cell layer (B), prickle cell layer (P), and lamina propria (LP) displaying normal collagen distribution and small sized blood vessels (arrow heads) (H&E x400)
- c. Granular cell layer (G) and keratin layer (K) containing a few nuclear remnants (arrows) (H&E x400)

Examinations of the gingival specimens of the periodontitis patients revealed an obvious decrease in keratin thickness, together with the presence of cytoplasmic vacuolizations in some superficial cell layers. The epithelial ridges lost their normal configuration as they were broader and shorter than those in the control group, with discrete areas of loss in the basement membrane continuity. Regarding the underlying lamina propria, widely dilated blood vessels and diffuse hemorrhage were observed. The ECM was severely damaged displaying areas of edema, especially within the perivascular area. Collagen fibers were highly disorganized with

large amounts of inflammatory cells throughout the lamina propria (Figure 2). Following OFD, gingival tissues obtained from surgically treated periodontitis patients showed an apparent increase in keratin thickness, fewer cytoplasmic vacuolizations within the surface epithelial cells, and the epithelial ridges were restored to their normal configuration to a great extent. Beneath the covering epithelium, the lamina propria displayed small sized blood vessels and restored collagen fiber distribution with rare areas of dissociation. There was an obvious decrease in the inflammatory infiltrate compared to the presurgical state (Figure 3).

Figure 2. Photomicrographs of the human gingiva of a periodontitis patient before open flap debridement

- a. Broader and more flattened epithelial ridges (arrows) (H&E x100)
- b. Discrete areas of basement membrane discontinuity (arrows), widely dilated blood vessels (arrow heads), hemorrhage and extravasated red blood cells (stars), and lamina propria (LP) displaying a massive chronic inflammatory cell infiltrate (H&E x200)
- c. Areas of collagen degeneration (stars) and clusters of numerous chronic inflammatory cells between collagen fibers (I) (H&E x200)
- d. Apparent decrease in keratin thickness (K) and a granular cell layer (G) showing numerous cytoplasmic vacuolizations within its cells (arrows) (H&E x400)

Figure 3. Photomicrographs of the human gingiva of the treated group

- a. Greatly restored configuration of the epithelial ridges (arrows) (H&E x100)
- b. Lamina propria (LP) with mild chronic inflammatory cell infiltrate, rare dissociation of collagen fibers, and small sized blood vessels (arrow heads) (H&E x200)
- c. Obvious increase in keratin thickness with nuclear remnants (K) and fewer cytoplasmic vacuolizations within the granular cell layer (G) (arrows) (H&E x400)

III. Quantitative RT-PCR Results

Quantitative RT-PCR results revealed a statistically significant difference between the two study groups, for both t-PA and PAI-1 mRNAs expression ($p\text{-value} \leq 0.05$). The expression of t-PA and PAI-1 mRNAs was significantly higher in group II (periodontitis group) (median=2.442 and 1.495 respectively)

compared to group I (control group) (median=0.638 and 0.621 respectively). Within the periodontitis group, gene expression of both proteins was significantly lower after OFD (median=1.443 and 0.884 respectively) compared to before OFD (median=2.442 and 1.495 respectively) but was significantly higher compared to the control group (Table 3).

Table 3. t-PA and PAI-1 gene expression within the study groups

	Group I (Control Group)	Group II (Periodontitis Group)	
		Before OFD	After OFD
t-PA Median (Range)	0.638 (0.632-0.651)	2.442* (2.314-2.521)	1.443*# (1.421-1.766)
PAI-1 Median (Range)	0.621 (0.563-0.653)	1.495* (1.434-1.535)	0.884*# (0.815-0.952)

*Statistically significant difference compared to the control group ($p \leq 0.05$); #statistically significant difference after OFD ($p \leq 0.05$).

Discussion

Periodontitis is a microbial and inflammatory disease that is mediated by the interaction between pathogens and the host immuno-inflammatory responses. It is characterized by an intense inflammatory infiltrate, impaired host immune response and destruction of the connective tissue attachment. This tissue breakdown is thought to be the result of activation of host cells by inflammatory mediators.²¹ Due to its high prevalence and consequences in dentistry, periodontal disease has been implicated as a global public health

problem.²² In fact, it is well recognized that the complex pathogenic mechanism of periodontitis involves both the presence of microbial plaque and the immune-inflammatory response of the host.²³ This mechanism is characterized by the production of cytokines, free radicals, and enzymes in the periodontal tissues which leads to advanced gingival inflammation, periodontal ligament injury, alveolar bone resorption, and ultimately leads to tooth loss.²⁴ Thus, the aim of the present study was to investigate the effect of OFD surgical treatment on the progress of periodontitis. This effect was evaluated clinically,

histopathologically through H&E staining, and by measuring t-PA and PAI-1 gene expression through qRT-PCR.

In the present study, assessment of the clinical parameters (PD, CAL, PI, and GI) revealed a significant decrease following a surgical OFD procedure. These results support those formerly achieved by Hassan et al. who reported that performing OFD as a treatment modality for chronic periodontitis patients resulted in a 38.5% PD reduction, 16.7% CAL gain and a significant decrease in all clinical parameters compared to untreated chronic periodontitis patients.²⁵

Concerning the histopathological results in the present work, gingival tissues of periodontitis patients showed widely dilated blood vessels and diffuse hemorrhage in the lamina propria. The ECM was severely damaged displaying intense edema, and collagen fibers were highly disorganized. Additionally, large amounts of inflammatory cells were noted between the collagen fibers. Collagen is the major ECM component of the gingiva and its degradation is considered the main marker of periodontal disease progression.²⁶ Heidari et al. suggested that decreased collagen fiber density might be related to disease severity. Several recent studies have demonstrated alterations in the ECM and collagen of gingival tissues of chronic periodontitis patients.²⁷ For instance, Almeida et al. reported ECM disorganization, with altered collagen fiber distribution, and large amounts of inflammatory cells dispersed throughout the gingival tissues of chronic periodontitis patients, indicating a chronic inflammation.²⁸ Golijanin et al. found a gradual loss of the gingival fibroblastic population which in turn, was related to a significant loss of collagen fibers in severe chronic periodontitis patients.²⁹

Regarding the vasodilatation and hemorrhage observed in the present study, this could be explained through the findings of Boşca et al. who induced experimental periodontitis in rats and monitored its progression.³⁰ Their results demonstrated lysis of the gingival ECM and its replacement by inflammatory cells, edema, and diffuse hemorrhage. The authors clarified that gingival edema was the result of vasodilatation and enhanced vascular permeability, while hemorrhage was the consequence of capillary fragility. Recently, Sheibak et al. found that the increased number and diameter of blood vessels is possibly beneficial to allow for the infiltration

of immune agents from the plasma to the inflammatory sites, where they can control tissue damage.³¹

The surgical approach to the treatment of periodontitis is a modality used in an attempt to increase the efficacy of root debridement, especially at sites with deep probing depths or furcation involvement; this provides better access for the removal of etiologic factors, reduces deep probing depths, and regenerates or reconstructs periodontal tissues.³² To achieve these goals, OFD was performed in the present study on patients with severe periodontitis and the results revealed an obvious improvement in the histological features of the gingival tissues represented by the absence of edema, the appearance of highly organized collagen fibers, the regression of inflammatory cell infiltration to a great extent, and the presence of small sized blood vessels.

Plasmin as a final cascade product for PAS was first regarded as a key enzyme in removing fibrin clots and preventing pathological blood clot formation due to its high affinity to fibrin but, it may also play a dual role in infectious diseases as plasminogen is used by some bacteria to invade the host by degrading the ECM. On the other hand, plasmin may be important for host defense against infection.³³ Thus, the main point of interest of the present work was to investigate, through qRT-PCR, the gene expression of t-PA and PAI-1 mRNAs – two important elements of PAS – in the gingival tissues of periodontitis patients before and after being subjected to OFD procedures in order to explore their involvement in disease progression and tissue homeostasis.

In the present investigation, qRT-PCR results revealed a highly significant increase in the expression levels of both t-PA and PAI-1 in the gingival tissues of periodontitis patients compared to those of healthy control samples. Tissue plasminogen activators and its inhibitors are the principal components of PAS in periodontal tissues. Plasmin, as a pro-inflammatory mediator, could contribute to tissue destruction indirectly by converting latent MMPs into their active forms. In addition to the high proteolytic activity of MMPs, they can degrade non collagenous proteins of the ECM. Thus, it was hypothesized that increased t-PA levels in chronic periodontitis could contribute significantly to the connective tissue destruction associated with periodontitis, and consequently – together with other mediators – it plays an important role

in the pathogenesis of periodontitis.³⁴ Plasminogen activator inhibitor type-1 inhibits t-PA and has been identified to inhibit the activity of MMPs that are involved in tissue remodeling. However, it causes destruction of connective tissue during chronic inflammation.³⁵ Thus, the presented results of higher levels of t-PA and its inhibitor in diseased periodontal tissues compared to healthy controls could be attributed to these postulations.

The present results are consistent with previous studies on t-PA and PAI-1 in periodontally affected patients. Precisely, Kardesler et al. evaluated gingival crevicular fluid (GCF) levels of IL-1 β , PGE2, t-PA, and PAI-2, in a diabetic periodontitis group, a systemically healthy periodontitis group, and a systemically and periodontally healthy group. The authors demonstrated an increased t-PA concentration in both periodontitis patients and diabetic periodontitis patients compared to healthy controls.³⁶ Moreover, Pamuk et al. demonstrated significantly higher t-PA levels in the GCF of chronic and aggressive periodontitis samples compared to controls.³⁷ They also reported higher PAI-1 levels in gingival biopsies of patients with periodontal disease when compared to healthy individuals. These relatively high concentrations of those activators and inhibitors in GCF indicate that these proteins are produced locally in gingival tissues.³⁴ The demonstrated higher gene expression levels of PAI-1 in the present investigation were in accordance with a former study where Akman et al. suggested that serum PAI-1 could be an important marker in periodontal disease where increased PAI-1 level was accompanied with increased inflammation.³⁸ These findings could explain the present histological signs of tissue destruction in periodontitis tissue samples including lysis of the ECM and collagen destruction, edema, and intense inflammatory cell infiltrate.

On the other hand, the present qRT-PCR results revealed a statistically significant decrease in the expression levels of both t-PA and PAI-1 in the gingival tissues of periodontitis patients following OFD surgical treatment. Consistent with the current findings, previous studies evaluating changes in t-PA in relation to periodontal treatment, including the study performed by Kurgan et al., reported that a significant reduction in t-PA levels was found in chronic periodontitis patients following mechanical nonsurgical periodontal therapy which was associated with reduced local

inflammation.³⁹ More recently, Pamuk et al. found a significantly reduced GCF t-PA and PAI-1 levels following low-level laser therapy together with scaling and root planing in chronic periodontitis subjects.³⁷ Again, these findings indicate a possible role that t-PA and PAI-1 may play in periodontal tissue destruction.

In contrast to our findings, Tuter et al. demonstrated no significant reduction in GCF t-PA levels following periodontal treatment.⁴⁰ This may be attributed to the different treatment modality used where the authors performed non-surgical periodontal treatment only, while in the current study, OFD was performed.

Based on the present observations, the significant reduction in the expression of t-PA following an OFD procedure could reflect the decreased tissue inflammation achieved through this treatment modality as the strict control of t-PA is important for maintaining the integrity of periodontal tissues. In addition, it could be suggested that PAI-1 controls t-PA and consequently tissue destruction in periodontal disease. This regulatory effect may, in turn, be supported by the decreased t-PA and PAI-1 expression following surgical OFD procedures.

Conclusively, the significantly higher t-PA and PAI-1 levels in the gingival tissues of periodontitis patients compared to periodontally healthy controls provide further evidence for the implication of these biomolecules in the pathophysiology of periodontal disease. Moreover, following surgical therapy, the overall expression profile of the studied genes was down regulated. This may implicate that PAS is largely involved in the deterioration of the periodontal status and that periodontal therapy resulted in a strong reduction of the bacterial component, hence regulating the inflammatory immune responses and hindering the progression of periodontal destruction. However, comparing the treated patients with the healthy controls, a significantly higher expression of the studied genes was found. This forces us to focus our attention on the possible presence of potential sites for disease activity. Thus, the surgically treated sites need further supportive periodontal therapy for proper maintenance in order to prevent tissue deterioration for a longer time.

Interestingly, the results of the present study revealed a strong association between the evaluated clinical, histopathological, and protein expression parameters during the progression of periodontitis and following surgical intervention.

Within the limitations of the present study, we can conclude that OFD results in a downregulation of t-PA and PAI-1 expression in the gingival tissues of periodontitis patients, highlighting the crucial role of these two biomarkers in the pathophysiology of the disease. Hence, we recommend that further studies be performed to explore new modalities for treating periodontitis through the biologic inhibition of t-PA and PAI-1.

Acknowledgments:

The authors thank all laboratory workers that were involved in the practical work of this study.

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Conflicts of interest: The authors declared no conflicts of interest related to this work.

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