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Impact of Smoking on Macrophage-Related Chemokines During Initial Peri-Implantitis: A Prospective Cohort Study

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ABSTRACT

Objectives: Smoking disrupts macrophage chemokine response and delays healing. This study aims to investigate the effect of smoking on peri-implant crevicular fluid (PICF) levels of macrophage-related chemokines, C-C motif chemokine ligand 2 (CCL-2), C-C motif chemokine ligand 8 (CCL-8), C-X-C motif chemokine ligand 9 (CXCL-9), and C-C motif ligand 3 (CCL-3), before and after non-surgical treatment of initial peri-implantitis.

Methods: Fifty-five implants (27 non-smoking [NSPI] and 28 smoking [SPI]) with initial peri-implantitis (bleeding on probing [BOP+], probing pocket depth [PPD] of 6–7 mm) were included in the study. Clinical parameters were recorded, and PICF samples were collected before and 4 months after non-surgical treatment. PICF concentrations of CCL-2, CCL-8, CCL-3, and CXCL-9 were measured with Luminex assay. The Mann–Whitney *U*-test, Wilcoxon signed-rank test, and repeated measures analysis of variance test were used to analyze differences between and within the groups.

Results: Baseline CCL-2 ($p < 0.001$) and CXCL-9 ($p = 0.026$) levels (pg/30 s) were significantly lower in smokers compared to non-smokers, while no difference was observed for CCL-3 between the two groups ($p = 0.320$). Only CCL-2 levels (pg/30 s) decreased in the NSPI group in response to non-surgical treatment ($p = 0.037$).

Conclusion: Smoking disturbs the expressions of macrophage-related chemokines in the early phase of peri-implantitis. These findings may indicate the impaired control of infection during initial peri-implantitis and explain the accelerated progression of the disease in smokers. This study was not registered prior to participant recruitment.

Trial Registration: https://clinicaltrials.gov/study/NCT06810401

1 | Introduction

Peri-implantitis is a pathological condition related to microbial dysbiosis, affecting the tissues surrounding dental implants, and is characterized by inflammation of the peri-implant mucosa and subsequent progressive loss of supporting bone [1]. The most common risk factors for peri-implant diseases are inadequate microbial plaque control and habitual

The last two authors contributed equally to this article.

tobacco use [2]. Recent evidence demonstrates that smoking not only elevates the risk of peri-implantitis development but also significantly increases treatment failure rates [3, 4]. Notably, smokers exhibit less favorable clinical outcomes following mechanical debridement and local antiseptic therapy compared to non-smokers [5].

The recruitment of phagocytes to the site of infection is crucial for the formation of a robust immune response. However, excessive or dysregulated macrophage activity can result in tissue damage and chronic inflammation, as observed in the pathogenesis of peri-implantitis [6]. The local and systemic effects of smoking compound the impairment of macrophage function [7]. Smoking reduces vascularity and oxygenation in peri-implant tissues, disrupting immune-inflammatory responses and impairing the healing process [8, 9]. A recent study revealed that cigarette smoke suppresses the chemokine response of macrophages, compromising their innate response to infection [10]. Macrophage activation-related chemokines, such as C-C motif chemokine ligand 2 (CCL-2/monocyte chemoattractant protein [MCP]-1), C-C motif chemokine ligand 8 (CCL-8/MCP-2), C-X-C motif chemokine ligand 9 [CXCL-9/monokine induced by interferon-γ (MIG)], and C-C motif ligand 3 [CCL-3/macrophage inflammatory protein (MIP)-1α], play critical roles in orchestrating the inflammatory response by directing macrophage chemotaxis to sites of infection and tissue injury. Macrophage-derived CCL-2 explicitly facilitates the recruitment of monocytes and macrophages to inflamed tissues, promotes the differentiation of monocytes into macrophages, and drives their polarization toward the M2 phenotype, which is associated with tissue repair and the resolution of inflammation [11, 12]. CCL-8 contributes to the recruitment of monocytes and neutrophils to the site of inflammation [13, 14]. CXCL-9 is primarily expressed in macrophages and is known to attract T cells that express the CXCR3 receptor. It is produced in response to pro-inflammatory cytokines such as interferon-γ and is linked to M1 macrophage polarization [15, 16]. CCL-3 recruits CD8 lymphocytes, promotes osteoclast and M1 macrophage activation, and enhances the production of pro-inflammatory cytokines such as tumor necrosis factor-α and interleukin-6 [17, 18].

The literature contains limited studies evaluating the CCL-2, CCL-8, CCL-3, and CXCL-9 profiles during peri-implantitis. Most of these studies employ a cross-sectional design, but their findings are inconsistent. For instance, while CCL-2 concentrations in the peri-implant crevicular fluid (PICF) were reported to be comparable between healthy and diseased implant groups [19], elevated CCL-2 levels were also demonstrated in peri-implantitis tissues, in comparison to healthy peri-implant tissues [20]. Increased [21] or similar [19, 22] CCL-3 levels were also observed in PICF samples of peri-implantitis patients compared to individuals with healthy implants. Moreover, the expression of CCL-8 and CXCL-9 in peri-implantitis has not been investigated. Only two studies [19, 23] described changes in macrophage chemokine levels in relation to peri-implantitis treatment. Besides, expression profiles of these chemokines during the early stages (probing pocket depth [PPD] of 6–7 mm) of peri-implantitis remain unknown.

Peri-implantitis has a multifactorial etiology. As the advanced forms of peri-implantitis suffer from various etiological factors simultaneously, they present a limited opportunity to evaluate the effect of smoking on macrophage chemokine response. Furthermore, to our knowledge, the effect of smoking on expression profiles of CCL-2, CCL-8, CXCL-9, and CCL-3 during the development and progression of peri-implantitis remains poorly understood. In the present study, we hypothesized that smoking plays a regulatory role on macrophage-related chemokine responses already at the early stages of peri-implantitis. The aim of the present study is to investigate the changes in CCL-2, CCL-8, CCL-3, and CXCL-9 concentrations in PICF before and after non-surgical treatment of initial peri-implantitis in smoking and non-smoking individuals.

2 | Materials and Methods

2.1 | Ethics Approval and Determination of Sample Size

The study was approved by the Clinical Research Ethics Committee of Başkent University, Ankara, Türkiye (Study protocol number: D-KD24/01) in accordance with the Helsinki Declaration of 1975, as revised in 2013. The study was registered and approved by www.clinicaltrials.gov (ID: NCT06810401). This study's report follows the STROBE guidelines.

The power analysis was conducted using GPower software. Based on previous research comparing pre- and post-treatment MIP-1α levels in PICF samples [19] the required minimum sample size was calculated to be 52 participants, with 26 participants in each group, in order to achieve a power of 80% (effect size $f = 0.2$; α error = 0.05). Estimating a drop-out rate of 20%, the present study aimed to recruit 63 patients.

2.2 | Study Design

Study planning, patient recruitment, treatments, and sample collection were performed at the clinics of the Department of Periodontology, Faculty of Dentistry, Gazi University, Türkiye, between January 2024 and October 2024. All individuals who were diagnosed with peri-implantitis (peri-implant bleeding on probing [BOP+] and/or suppuration, PPD of ≥ 6 mm, and radiographic crestal bone loss of ≥ 3 mm apical of the most coronal portion of the intraosseous part of the dental implant [1]) at their initial visit at the periodontology clinics of Gazi University Faculty of Dentistry were invited to take part in the study.

2.3 | Inclusion and Exclusion Criteria

Inclusion criteria were: (1) willingness to participate in the study, (2) having a dental implant that was loaded at least 1 year before the initiation of the study, that was diagnosed with peri-implantitis, and that has PD of 6–7 mm and radiographic bone loss not exceeding the middle third of the intraosseous implant body (bone loss of 3–4 mm), (3) the implants had been in function for at least 1 year. Exclusion criteria were: (1) having received

periodontal therapy or any oral decontamination treatment or received antibiotics or steroids in the last 6 months before the initiation of the study, (2) being pregnant or breastfeeding, (3) being diagnosed with rheumatoid arthritis, lupus erythematosus, poorly controlled systemic diseases (such as diabetes or hypertension), (4) having a medical history of radiation or cancer therapy.

2.4 | Study Population

Comprehensive periodontal and radiographic examinations of the teeth and dental implants were performed for all participants at baseline. Site-level clinical parameters were measured at four sites of each implant (mesial, buccal, distal, and lingual/palatal). The measurements included PPD (mm), BOP [24] visible plaque index (VPI) [25], and width of peri-implant keratinized tissue (≥ 2 mm or < 2 mm) [26] assessments. Periapical radiographs were obtained to measure peri-implant radiographic bone level. All clinical measurements were performed using a periodontal probe (UNC 12, UNC #12 [1–12 mm], DuraLite ColorRings, Nordent). All measurements were conducted by the same investigator (BNBA). Patients who had PPD ≥ 8 mm around their implants were excluded from the study.

A total of 63 peri-implantitis patients who met the inclusion and exclusion criteria were recruited for the study. All participants were provided verbal and written information regarding the nature of the study, and written informed consent was obtained from each participant. The study population was later divided into two groups based on self-reported cigarette smoking [27]. Smokers were individuals who reported smoking at least one cigarette per day for at least 1 year (smoking peri-implantitis patients [SPI], $n = 30$, range of smoking was 3–20 cigarettes/day), while never-smokers were individuals who had never used tobacco in any form (non-smoking peri-implantitis patients [NSPI], $n = 33$).

2.5 | Peri-Implant and Periodontal Treatment

All patients in both groups received full-mouth non-surgical periodontal treatment using sterile ultrasonic scalers (Woodpecker Piezo Cavitron Ultrasonic Scaler Handpiece [HW-5L], EMS, Switzerland), dental curettes (Cgr1-2, Cgr11-12, Cgr13-14, Osung MND, Korea), and sterile saline irrigations. Non-surgical treatment of peri-implantitis involved the debridement of hard- and soft accumulations on implant surfaces using dental curettes with titanium tips (Titanium Implant Scaler 204SD, Hu-Friedy) for 15 min, followed by irrigation with sterile saline solution. Infiltration anesthesia was used if requested by the patient. Customized oral hygiene instructions were given to all participants after treatment. After 2 months (8 weeks), patients received a call for a control visit, including supragingival debridement and oral hygiene instructions. All clinical and radiographic measurements were repeated 4 months after the completion of non-surgical peri-implant treatment.

2.6 | Sample Collection

PICF samples were collected from all participants before (T0) and 4 months after (T1) peri-implant non-surgical treatment. A 30-s sampling protocol was used [28]. Before sampling, the sample area was isolated with cotton rolls and dried using a gentle air stream. A single PICF sample was collected from each implant pocket using PerioPaper strips (Oralflow Inc., New York, USA). These paper strips were placed 1–2 mm under the crevice of the pocket and kept for precisely 30 s. To minimize evaporation, volume quantification was performed immediately after sampling using a Periotron 8000 device (Oralflow Inc., New York, USA). The Periotron 8000 was calibrated before starting the study and recalibrated periodically. After quantifying the PICF volume, each paper strip was placed in a dry Eppendorf tube. The samples were stored at −80°C until being transferred in dry ice to the University of Turku, Institute of Dentistry, Finland, for biochemical analyses.

2.7 | Biomarker Analysis

Each PICF sample was eluted in 200 μL of phosphate-buffered saline containing 0.5% bovine serum albumin [29]. The tubes were then centrifuged at 10 000*g* at 4°C for 10 min. The CCL-2, CCL-8, CCL-3, and CXCL-9 concentrations were detected with Luminex 200 using multiplex immunoassay kits (Bio-Plex Pro Human Chemokine Assays, Bio-Rad Laboratories, California, USA) according to the manufacturer's recommendations. Concentrations (pg/ml) were converted to the mediator amount collected at 30 s (pg/30 s) using the equation described by Wassall and Preshaw [28]. The lower limits of quantification for each chemokine were as follows: 0.3 pg/mL for CCL-2, 0.03 pg/mL for CCL-8, 0.4 pg/mL for CCL-3, and 1.8 pg/mL for CXCL-9.

2.8 | Statistical Analysis

Data analysis was performed using IBM SPSS Statistics (Version 29.0 for Windows; IBM Corp). The normality of the outcomes was evaluated with the Shapiro–Wilk test. Group differences in categorical variables were assessed using the Pearson Chi-square test, while age distribution between groups was compared using the Independent Samples *t*-test. The Mann–Whitney *U*-test was applied to compare clinical and biochemical outcomes between groups. To compare baseline (T0) and 4-month post-operative (T1) outcomes of biochemical and clinical variables within each group, the Wilcoxon signed-rank test was used. Repeated measures analysis of variance test (controlled for age) was used to determine whether there is a difference in the effectiveness of treatment over time. Logarithmic conversions ($\log_{10}$) were applied to chemokine levels (pg/30 s) before the application of repeated measures analysis of the variance test. A p-value of < 0.05 was considered to be statistically significant for all the parameters. A four-parameter logistic regression model was used to calculate chemokine concentrations in PICF. As a result, CCL-2 was detected in all samples, while CCL-8 was below the limit of detection (LOD) in 77 samples (T0: 55%, T1: 85%), CCL-3 in

30 samples (T0: 15%, T1: 40%), and CXCL-9 in 26 samples (T0: 15%, T1: 34%). In descriptive analyses, due to a high number of missing data, CCL-8 was excluded from statistical comparisons, while CCL-3 and CXCL-9 concentrations below the regression curve were substituted with a value equal to half of their lowest limit of quantification (LOD/2) [30].

3 | Results

Eight patients left the study during the 4-month follow-up period due to personal reasons. A total of 55 peri-implantitis patients (28 SPI and 27 NSPI) completed the study. Among them, five were diagnosed with gingivitis, 26 with Stage I periodontitis, 19 with Stage II periodontitis, and 5 with Stage III periodontitis. Baseline (T0) and post-operative (T1) full-mouth periodontal indexes are presented in the Table S1. The description of the study population by age, gender, and site-level clinical parameters at T0 and T1 is presented in Table 1. Following non-surgical peri-implant treatment, PPD reduction (PPD ≤ 5 mm at T1) was observed in 33.3% of NSPI and 21.4% of SPI groups. After the treatment, BOP% decreased to 74.1% for NSPI and 64.3% for SPI groups. The decrease in VPI% scores was more pronounced in smokers compared to non-smokers. Additionally, the volume of PICF decreased in both smokers and non-smokers after non-surgical peri-implant therapy (Table 1).

Table 2 presents the PICF CCL-2, CCL-3, and CXCL-9 levels in total chemokine amount collected at 30 s (pg/30 s) and in PICF concentrations (pg/μL). When the time-dependent PICF chemokine levels were compared, significantly lower CCL-2 ($p < 0.001$) and CXCL-9 ($p = 0.026$) levels were observed in smokers (SPI) at baseline compared to non-smokers (NSPI). No significant difference was found when CCL-3 levels were compared between groups ($p = 0.32$). Four months after non-surgical treatment, a significant reduction was found only in CCL-2 levels in the NSPI group ($p = 0.037$) (Table 2).

When comparing PICF chemokine concentrations between groups at baseline, no significant differences were observed (Table 2). However, after non-surgical treatment, SPI exhibited an increase in PICF CCL-2 ($p = 0.016$) and CXCL-9 ($p = 0.007$) concentrations.

According to the repeated measures analysis of the variance test, the effectiveness of non-surgical treatment on PICF CCL-2, CCL-3, and CXCL-9 levels over time did not differ between smokers and non-smokers (Figure 1).

4 | Discussion

To the best of the authors' knowledge, this study is the first to investigate smoking- and non-surgical treatment-related changes in PICF levels of macrophage chemokines (CCL-2, CCL-8, CCL-3, and CXCL-9). According to our findings, smoking suppresses CCL-2 and CXCL-9 levels already during the early phase of peri-implantitis. Furthermore, non-surgical therapy results in a limited reduction in chemokine levels.

Focusing on early peri-implantitis is the main strength of our research. Tissue degradation in peri-implantitis is rapid, extensive, and has a multifactorial character. For instance, apoptosis and oxidative stress, two critical pathways in the pathogenesis of advanced peri-implantitis, are regulated by smoking [31, 32]. By selecting implants with BOP+ and PPD of 6–7 mm, we were able

TABLE 1 | Description of the study population together with the site-level clinical parameters.

			Smoking status		
			Non-smoker n:27	**Smoker n:28**	***p***
Age (years), mean ± SD			51.4 ± 12.0	45.3 ± 10.6	**0.048**
Female (%)			62.3	64.3	0.919
Site-level measurements	KTW	< 2 mm	11	8	0.343
		> 2 mm	16	20	
	VPI+ (%)	T0	100	100	**0.028**
		T1	40.7	14.3	
	PPD = 6-7 mm (%)	T0	100	100	0.246
		T1	66.7	78.6	
	BOP+ (%)	T0	100	100	0.432
		T1	74.1	64.3	
	PICF (μL) (median, interquartile range [IQR])	T0	0.55 (0.31)	0.48 (0.36)	0.155
		T1	0.4 (0.22)	0.37 (0.2)	0.296
		P	**0.006**	**0.02**	

Note: PICF values (μL) are presented as median values with interquartile ranges (IQR). Significant differences ($p < 0.05$) are indicated in bold.
Abbreviations: BOP, Bleeding on probing; KTW, Keratinized tissue width; PICF, Peri-implant crevicular fluid; PPD, Probing pocket depth; T0, Baseline; T1, 4-month post-operative; VPI, Visible plaque index.

TABLE 2 | CCL-2, CXCL-9, and CCL-3 levels at T0 and T1 in NSPI and SPI groups are presented as concentrations (pg/μL) and as total chemokine amounts collected at 30 s (pg/30 s).

Variables (Median and IQR)			Smoking		
			Non-smoker	Smoker	*p*
Chemokine levels collected at 30 s (pg/30 s)	CCL-2	T0	0.37 (1.5)	0.24 (0.16)	**< 0.001**
		T1	0.21 (0.26)	0.27 (0.22)	0.270
		p	**0.037**	0.190	
	CXCL-9	T0	0.86 (1.47)	0.5 (0.89)	**0.026**
		T1	0.5 (1.47)	1.12 (2.28)	0.525
		p	0.234	0.114	
	CCL-3	T0	0.25 (0.83)	0.19 (0.62)	0.320
		T1	0.24 (0.37)	0.05 (0.33)	0.240
		p	0.100	0.101	
	CCL-8	T0	0.03 (0.02)	0.03 (0)	
		T1	0.03 (0)	0.03 (0)	
PICF chemokine concentration (pg/μL)	CCL-2	T0	0.79 (0.66)	0.51 (0.36)	0.062
		T1	0.7 (1)	0.7 (0.71)	0.285
		p	0.605	**0.016**	
	CXCL-9	T0	1.132 (2.32)	0.86 (1.98)	0.112
		T1	1.48 (4.19)	2.795 (5.15)	0.449
		p	0.782	**0.007**	
	CCL-3	T0	0.41 (1.8)	0.368 (1.04)	0.668
		T1	0.3 (1.37)	0.225 (1.02)	0.363
		p	0.337	0.168	
	CCL-8	T0	0.06 (0.11)	0.06 (0.06)	
		T1	0.08 (0.04)	0.1 (0.02)	

Note: Data are presented as median values with interquartile ranges (in parentheses). Significant differences ($p < 0.05$) are indicated in bold.
Abbreviations: T0, baseline; T1, 4-month post-operative.

to examine the effect of smoking on macrophage chemokines during the initial phases of peri-implantitis. To standardize the effect of treatment on peri-implant tissues, all peri-implantitis lesions were treated with non-surgical treatment by a single practitioner. In this study, chemokine levels were presented as both concentrations in PICF (pg/μL) and the total chemokine amount collected over 30 s (pg/30 s). To account for variations in crevicular fluid volumes before and after treatment, the total chemokine amount collected at 30 s was presented as the primary outcome. The present study focuses on the expression profiles of CCL-2, CCL-8, CCL-3, and CXCL-9 to investigate the macrophage chemotactic response in the context of early peri-implantitis and smoking. Earlier studies have demonstrated that in peri-implantitis, macrophages predominantly exhibit the M1 pro-inflammatory phenotype, producing elevated levels of inflammatory mediators that foster an environment conducive to bone resorption and impair effective inflammation resolution and tissue regeneration [33–35]. M2 macrophages, which typically secrete anti-inflammatory cytokines such as interleukin-10 and transforming growth factor-β, are less prevalent, creating a higher M1/M2 ratio, particularly at advanced peri-implantitis sites [34]. A more comprehensive evaluation of macrophage polarization could provide valuable insights into the pathogenesis of initial peri-implantitis. Although the present study was limited to a 4-month follow-up, the overall timeline for complete wound healing following non-surgical periodontal treatment can vary [36]. Therefore, extended follow-up periods, for example, up to 12 months, could offer additional insights into the regulation of macrophage chemotaxis [37]. Finally, the authors acknowledge that the difference in mean age between the NSPI and SPI groups may create a confounding effect on pre- and post-treatment levels of chemokines. It is known that macrophages display dysregulated chemokine production with aging [38, 39]. These changes in signaling can lead to either an overproduction or an insufficient response to pro-inflammatory chemokine signals, ultimately affecting the effectiveness of immune surveillance and tissue repair in older individuals [38, 40]. In order to minimize the impact of age on chemokine levels, ANOVA analysis was used, and age was implemented into the model as a covariate. Nevertheless, future studies may benefit

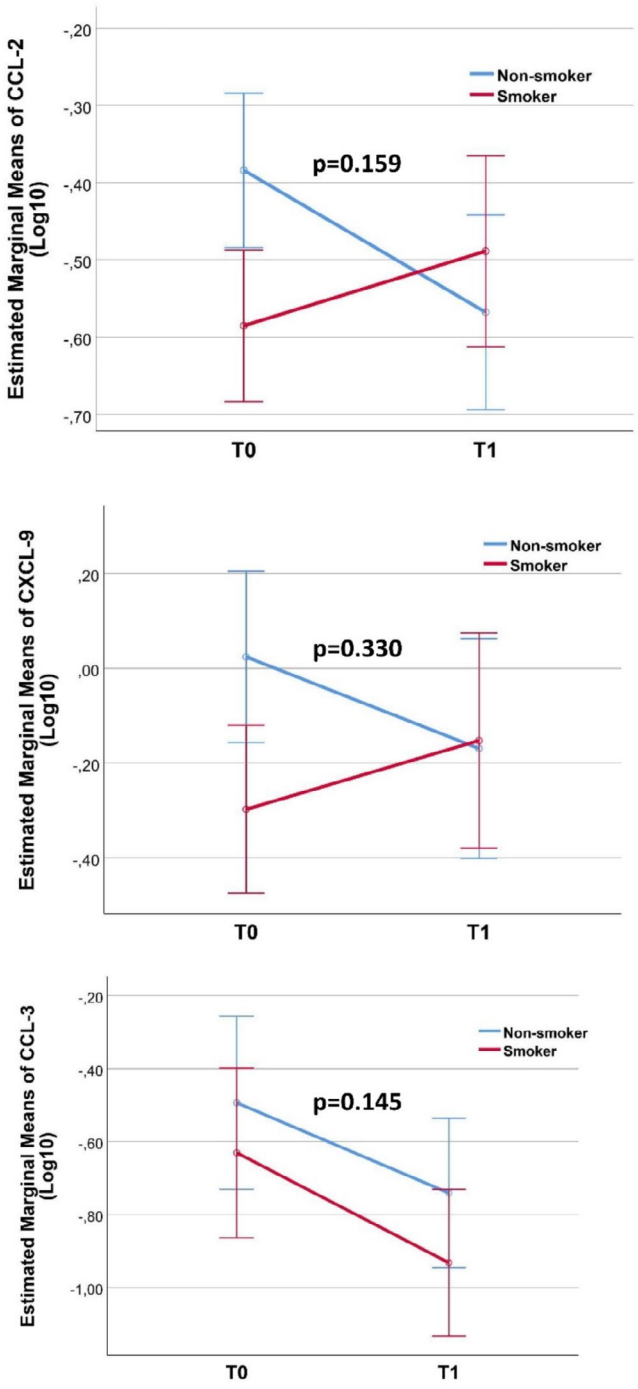

FIGURE 1 | Comparison of the effectiveness of non-surgical treatment on CCL-2, CCL-3, and CXCL-9 levels over time between smokers and non-smokers. *p* values were calculated using repeated measures analysis of variance after controlling for age. Data is presented as an estimated marginal means (with a 95% confidence interval) (T0: Baseline, T1: 4-month post-operative).

from stratifying age groups to investigate age-specific chemokine dynamics during the development and resolution of peri-implant diseases. Moreover, potential influences of other factors on study outcomes, i.e., implant surface characteristics, implant location, and variabilities in individual immune responses, cannot be entirely ruled out.

A recent systematic review indicated that there is moderate evidence to suggest that peri-implantitis is associated with elevated cytokine levels. On the other hand, the same study stresses that the confounding effect of smoking was not adequately addressed in the literature, as smokers are usually excluded from the study population [41]. Another systematic review also states that smoking does not influence the cytokine (IL-1β) and chemokine (IL-8) levels during osseointegration of implants. However, the evidence is derived from only two studies [42]. Therefore, there is a need to demonstrate the effect of smoking on cytokine and chemokine expression profiles around implants, both in health and in disease. In the literature, only a limited number of studies have analyzed CCL-2 and CCL-3 levels in peri-implantitis. Their findings are conflicting, and the impact of smoking has not been assessed [19–21, 23]. Our findings reveal that smoking suppresses CCL-2 and CXCL-9 levels in early peri-implantitis, whereas no measurable difference was detected for CCL-3. Smoking has a long-term chronic impact on immune response, with its detrimental effects including impaired wound healing, compromised fibroblast function, decreased collagen synthesis, reduced peripheral circulation, and diminished functionality of neutrophils and macrophages [43]. Nicotine, an essential component of cigarette smoke, is a potent immunomodulatory molecule for macrophages, and nicotine exposure suppresses the bacteria-induced CCL-2 and CXCL-9 expressions of macrophages, leading to impaired macrophage response during infection [44]. A disturbed macrophage response during the early stages of infection may facilitate increased replication of intracellular bacteria and accelerated disease progression [45]. As a matter of fact, patients at low risk for developing peri-implantitis exhibit elevated M1/M2-like macrophage ratios since M1 macrophages control the virulence of pathogens by promoting Th1 responses [46]. Taken together, the present observation of suppressed macrophage chemokine response in smokers during the early stages of peri-implantitis may partially explain the common finding of accelerated progression of peri-implantitis.

Non-surgical treatment serves as a critical initial intervention, laying the foundation for potential further treatments if required [47]. This approach typically involves mechanical debridement, which aims to control biofilm, suppress inflammation, and induce soft tissue healing [48]. According to our findings, non-surgical treatment of early peri-implantitis (PPD = 6–7 mm) provides limited clinical improvement after 4 months, both in smokers and non-smokers. A systematic review highlights that while non-surgical treatments can lead to some clinical improvements, the resolution of peri-implant mucosal inflammation, indicated by the absence of BOP, is not consistently achieved across studies [49]. The review noted that the mean difference in PD reduction between treatment groups did not reach statistical significance, suggesting that while some patients may benefit, the overall effectiveness remains limited. Further supporting this notion, a study reported that only 14%–47% of cases achieved disease resolution, defined as the absence of BOP and further bone loss, within 6–12 months post-treatment [50]. These results align with the present study's findings, where the resolution of inflammation occurs in only 25.9% of non-smokers and 35.7% of smokers. Indeed, non-surgical therapy for moderate to severe peri-implantitis often fails to halt disease progression, reinforcing the idea that

non-surgical methods may be insufficient for more advanced cases [51]. Few studies have investigated the effect of non-surgical peri-implantitis treatment on PICF biomarkers, and none have assessed the impact of smoking on the chemokine response [19, 52, 53]. In the present study, the only change in chemokine levels after non-surgical treatment was the reduction in CCL-2 levels of the NSPI group. This finding aligns with previous research that reported no significant differences in most of the studied cytokines [19, 53]. While the evidence is limited, it can be proposed that the insufficient improvement in clinical parameters after non-surgical treatment of early peri-implantitis may be associated with the stability of macrophage chemokine levels in response to therapy. Our current findings do not indicate any difference between smokers and non-smokers in terms of their overall chemokine responses to therapy. Nevertheless, the changes in CCL-2 and CXCL-9 levels after therapy showed opposite patterns in smokers and non-smokers. Considering the suppressed chemokine responses in smokers during the initiation of peri-implantitis, it can be hypothesized that peri-implant therapy normalizes the chemokine levels by elevating the suppressed chemokine expressions in smokers and decreasing the elevated chemokine levels in non-smokers. Yet, further studies with longer follow-up periods are needed to prove this hypothesis.

In conclusion, smoking exerts a suppressive effect on macrophage-related chemokine levels, which may lead to the deterioration of macrophage chemotaxis and limited elimination of infection already at the early phases of peri-implantitis. It is also possible that smoking impairs macrophage functions already during peri-implant health and peri-implant mucositis. Therefore, further studies are necessary to describe the interplay between smoking and chemokine expression patterns in both peri-implant health and diseases. Responsiveness to non-surgical treatment is a common phenomenon that is seen in both smokers and non-smokers and is likely linked to a prolonged inflammatory response in peri-implantitis.

Author Contributions

B.N.B.A.: participant recruitment, sample collection, laboratory and data analysis, and writing – original draft preparation. **Z.T.C.:** writing – review and editing. **M.Y.:** data analysis, and writing – review and editing. **M.G.:** laboratory analysis and writing – review and editing. **A.B.:** supervision and writing – review and editing. **U.K.G.:** conceptualization, resources, supervision, and review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.