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ORIGINAL ARTICLE

Inflammasomes NLRP3 and AIM2 in peri-implantitis: A cross-sectional study

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Abstract

Background: Inflammasome components NLRP3 and AIM2 contribute to inflammation development by the activation of caspase-1 and IL-1β. They have not been yet evaluated in samples from patients with active peri-implantitis. Thus, the aim of the present study is to analyze the expression of inflammasomes NLRP3 and AIM2 and subsequent caspase 1 and IL-1β assessing the microenvironment of leukocyte subsets in samples from patients with active peri-implantitis.

Methods: Biopsies were collected from 33 implants in 21 patients being treated for peri-implantitis. Biopsies from gingival tissues from 15 patients with healthy periodontium were also collected for control. These tissues were evaluated through conventional histological stainings. Then, immunohistochemical detection was performed to analyze NLRP3, AIM2, caspase-1, and IL-1β and markers of different leukocyte subsets. PCR for inflammasomes and related genes was also done.

Results: This manuscript reveals a high immunohistochemical and mRNA expression of NLRP3 and AIM2 inflammasomes, caspase-1, and IL-1β in biopsies collected from human peri-implantitis. The expression of the tested markers was significantly correlated with the increase in inflammatory infiltrate, probing depth, presence of biofilm, and bleeding on probing. In these peri-implantitis lesions, the area of biopsy tissue occupied by inflammatory infiltrate was intense while the area occupied by collagen was significantly lower. In comparison with periodontal healthy tissues, the inflammatory infiltrate was statistically significantly higher in the peri-implantitis biopsies and was mainly composed of plasma cells, followed by T and B lymphocytes.

Conclusion: In human peri-implantitis, chronic inflammation can be explained in part by the action of IL-1β/caspase 1 induced through NLRP3 and AIM2 inflammasome activation.

KEYWORDS
AIM2, inflammasome, inflammation, NLRP3, peri-implant disease, peri-implantitis

1 | INTRODUCTION

Workgroup 4 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions defined peri-implantitis as 'a plaque-associated pathological condition occurring in tissues around dental implants, characterized by inflammation in the peri-implant mucosa and subsequent progressive loss of supporting bone' (Berglundh et al., 2018). Peri-implantitis shares many characteristics with periodontitis, such as a broad number of microorganisms as an etiological factor (Padial-Molina et al., 2016), bleeding upon probing, increased probing depth, and soft tissue attachment loss due to local inflammation as clinical findings (Dionigi et al., 2020) and alveolar bone resorption as a radiographic key finding (Schwarz et al., 2018). In addition, several of the biochemical intracellular pathways in both diseases are similar, including the activation of some pattern recognition receptors (PRRs), expression of DNA damage/repair mechanisms, and the presence of reactive oxygen/nitrogen species (Dionigi et al., 2020). Thus, there is a tendency to embrace the idea that many of the physio-pathological events underlying both diseases would be the same.

However, abundant current literature shows that peri-implantitis and periodontitis exhibit some important differences (Albrektsson, 2019). Prior studies show that, compared with periodontitis, biopsies of peri-implantitis have greater densities of neutrophils, macrophages, and plasma cells (Carcuac & Berglundh, 2014), higher macrophage polarization (Fretwurst et al., 2020), a quicker bone resorption pattern (Derks et al., 2016) with a non-linear trend (Galindo-Moreno et al., 2015). There is also limited evidence that other etiological factors are related to peri-implantitis (Schwarz et al., 2018) including post-restorative presence of submucosal cement (Linkevicius et al., 2013), the release of metal debris from implants (Fretwurst et al., 2016, 2018; Suárez-López Del Amo et al., 2017, 2018), the presence of ions in peri-implant soft tissue as a consequence of implant corrosion (Albrektsson et al., 2014), and modifications of the peri-implant microbiome structure and diversity as a consequence of titanium dissolution products (Daubert et al., 2018).

Inflammation and host immune dysfunction are gaining higher interest in explaining the pathogenesis of peri-implantitis. In this sense, one of the recently suggested inflammatory pathways in periodontal disease, inflammasomes (Marchesan et al., 2020), has not yet been analyzed in relation to the pathological processes occurring in peri-implantitis. Consequently, it would be interesting to analyze whether the expression of some inflammasomes is also present in this pathology, as it has been described in periodontitis.

Inflammasomes are intracellular supramolecular protein complexes, that consist of an inflammasome sensor molecule, an adaptor protein and a caspase-1 protein, orchestrating host response mechanisms against physiological aberrations, infectious agents (Man & Kanneganti, 2015), or external elements such as metallic debris or ions (Caicedo et al., 2008; Li et al., 2020). Inflammasomes are expressed by innate immune cells, but also in endothelial, epithelial, and many other cells of the adaptative immune system. Some pattern recognition receptors (PRRs) assemble inflammasomes as responses to pathogen-associated molecular patterns (PAMP) or damage-associated molecular patterns (DAMP). This promotes the release of pro-inflammatory cytokines, mainly through a caspase-induced production of interleukins (IL)-1β and -18 (Yilmaz & Lee, 2015). A complementary action of the inflammasome activation is the formation of caspase 1-mediated gasdermin D N-terminal form (GSDMD-N), that induces the formation of pores in cell membranes which triggers the mechanisms of pyroptosis, a lytic form of regulated cell death (He et al., 2015).

Few in vitro studies relate the activation of the inflammasome NLRP3 (NLR family pyrin domain containing 3) in response to the potential causes of peri-implantitis; this is by biofilms and/or metal ions and particles from implants (Li et al., 2020; Petterson et al., 2011). Other inflammasomes, such as those of the ALR family (mainly interferon-inducible protein AIM2, or absent in melanoma 2), are a family of DNA-binding proteins that cannot be activated by non-organic elements. Nevertheless, it is worth highlighting that, to our knowledge, there is no study in humans analyzing the role of the inflammasomes in clinical samples of peri-implantitis.

Because of this lack of knowledge, the aim of the present study is to analyze the expression of inflammasomes NLRP3 and AIM2 and subsequent caspase 1 and IL-1β assessing the microenvironment of leukocyte subsets in samples from patients with active peri-implantitis.

2 | MATERIALS AND METHODS

2.1 | Study design, primary locations, and participants

In this cross-sectional study, we included patients who were treated for peri-implantitis at a faculty clinic of the Department of Oral Surgery and Implant Dentistry, School of Dentistry of the University of Granada. The Ethics Committee for Human Research of the University of Granada approved the methodology followed in this study (587/CEIH/2020). All the procedures were explained to the participants, who signed informed consent forms. All patients were included from June 2020 through July 2021.

To participate in the study, the patients were required to be older than 18, with no significant systemic conditions and in a state of periodontal health. A complete clinical and radiographic examination was conducted including, specifically, the recording of probing depth (PD), bleeding or suppuration either spontaneous or upon probing and presence of biofilm in 4 sites around the implant, after removal of the prosthetic crown. The diagnosis of peri-implantitis followed the criteria of the 2017 World Workshop on the Classification of Periodontal and Peri-implant Diseases and Conditions (Renvert et al., 2018). Cases of peri-implantitis were included only if the implant had been in function for at least 1 year after prosthetic loading. The sites were not treated within 6 months of the study. Peri-implantitis tissue samples were collected during surgical therapy.

Briefly, during surgical therapy under local anesthesia, a band of soft tissue was gently removed by an internal bevel incision from the vestibule/lingual aspect of the lesion to the bottom of the peri-implant pocket.

Supracrestal soft tissues from around the teeth, that were healthy from the clinical point of view, were collected to be analyzed as control. In this case, the site was classified as healthy according to the 2017 World Workshop on the Classification of Periodontal and Peri-implant Diseases and Conditions (Chapple et al., 2018). The indication for tooth extraction had to be for orthodontic or caries reasons. Similarly, before the tooth was extracted, internal bevel and intrasulcular incisions were made to collect a representative band of gingival tissue.

2.2 | Outcomes measures

2.2.1 | Histopathological analysis

For conventional morphology and morphometrical analyses, the biopsies were fixed in buffered 10% formaldehyde at room temperature for 48 h. The samples were then paraffin-embedded in an Autotechnicon tissue processor (Leica TP1012), sectioned at 3 μm thickness, and stained with hematoxylin-eosin (H&E), Masson's trichromic (MT), picrosirius red (SR), and Movat's pentachromic (MP) modified by Russell (Suvarna et al., 2019).

Morphometrical analyses were conducted in the central, sulcular, and vestibular areas of the lamina propria (Figure 1a). ImageJ software (http://imagej.nih.gov/ij/) was used to assess the area occupied by inflammatory infiltrate, connective tissue, and fibrillar collagens in each zone. A total of 1440 10× images with a CD70 camera coupled to a BX51 microscope (Olympus Optical Company, Ltd.) were taken, 15 per each slide of the Movat's pentachromic, and picrosirius red staining. For each image, we calculated the relative area occupied by cells, the connective tissue, and the fibrillar collagens (measured with picrosirius red stain and visualized with polarized light).

2.2.2 | Immunohistochemical analysis

Immunohistochemical techniques were used to visualize the expression, location, and number of leukocyte subsets and other relevant cell types per mm^2. We used different prediluted monoclonal antibodies: anti-CD45 (all leukocytes), anti-CD20 (B lymphocytes), anti-CD3 (T lymphocytes), anti-CD15 (neutrophils leukocytes), anti-CD68 (monocytes/macrophages), anti-CD138 (plasma cells), and anti-CD34 (endothelial cells). Following the methodology of our group's previous studies, the slides were deparaffinized, rehydrated, and treated in a pre-treatment thermal PT module (Thermo Fisher Scientific Inc.) with a 1 mM EDTA buffer pH 8, for 20 min at 95°C. After peroxidase blocking for 15 min and washing with TBS + Tween20 buffer, the aforementioned primary antibodies were applied at the manufacturer recommended concentration for 30 min at room temperature. The staining was then developed in an automatic immunostainer (Autostainer480S, Thermo Fisher Scientific Inc.) using a peroxidase-conjugated micropolymer and diaminobenzidine as chromogen. Contrast was performed with Mayer's progressive hematoxylin stain. All prediluted antibodies were purchased from the same manufacturer (Vitro-Master Diagnóstica).

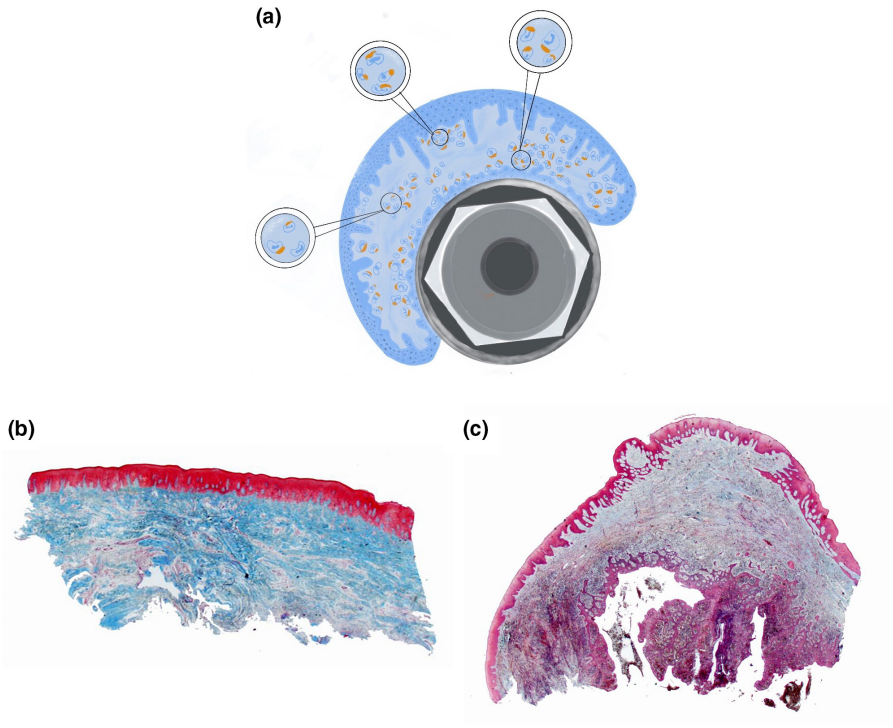

FIGURE 1 (a) Explanatory diagram showing the location where the analyses were conducted within the lamina propria and panoramic microphotography of representative biopsies of free gingival mucosa from (b) control group and (c) peri-implantitis group. Note the intense inflammatory infiltrate in lamina propria near the sulcular epithelium. Masson's trichromic stain, original magnification 1.25×.

For the analysis of the immunohistochemical expression of inflammasome multiprotein complexes, the samples were treated as mentioned above and the incubated with polyclonal primary antibodies anti-NLRP3 (1:400 dilution), anti-AIM2 (1:200 dilution), anti-Caspase-1 (1:4000 dilution), and anti-Interleukin 1β (1:400 dilution). All antibodies were purchased from Thermo Fisher Scientific Inc., except AIM2, that was purchased from Abcam.

Immunohistochemical expression was semi-quantitatively evaluated by assessing the intensity of immunostaining in the lamina propria and epithelium with a semi-quantitative scale (0–3): 0: absent, 1: mild, 2: moderate, and 3: intense. The extent of the infiltrate was assessed according to the area occupied in the lamina propria by the leukocyte subset (CD45 positive) with a semi-quantitative scale (0–3): 0: <5%; 1: 6%–25%; 2: 26%–50%; and 3: >50%. In addition, the number of positive cells per mm^2 was quantitatively evaluated in 10 fields of each section selected randomly. The sections were visualized with a BH2 microscope (Olympus Optical Company, Ltd.) with a 40× objective and a millimeter scale in the eyepiece.

2.2.3 | mRNA analysis

The mRNA was evaluated by keeping the biopsies in TriZol™ reagent at −80°C immediately after collection. To process the sample, the tissue was homogenized in a tissue blender and total RNA extracted following the conventional protocol recommended by the manufacturer (TriZol™ Plus RNA Purification kit, Invitrogen). The total amount of RNA was quantified in a Nanodrop instrument. Thirty μL of cDNA with 1 μg of RNA were generated with the PrimeScript RT Master Mix (Takara Bio Europe) and a conventional cycle. Subsequently, a quantitative real-time PCR was done using the TB Green Premix Ex Taq (Tli RNase H Plus, Takara Bio Europe) on a Real-Time PCR Thermal Cycler qTOWER3 system. Two μL of each sample were used per each of the 2 replicates. Finally, to calculate the gene expression levels relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) we used the $2^{-\Delta\Delta C_t}$ method. As a control, data were normalized to the expression of each gene in the control samples. The primers of each gene used for RT-PCR are shown in Table S1.

2.3 | Statistical analysis

Statistical analysis was performed using the SPSS 28.0 software for macOS (IBM Inc.). Given the sample size, for the comparison of each independent sample, the Mann–Whitney *U*-test was used. Correlations between variables were evaluated by Spearman's rho correlation coefficient. Quantitative data are reported as the mean (standard deviation) while categorical variables are reported as percentages. A $p < .05$ was considered to represent statistically significant differences between control and peri-implantitis groups.

3 | RESULTS

3.1 | Clinical variables

Demographic data are summarized in Table 1. A total of 33 peri-implantitis biopsies and 15 clinical healthy soft tissue samples were evaluated. The peri-implantitis samples were obtained from 14 women and 7 men with a mean age of 58 years; healthy soft tissue samples were obtained from 9 women and 6 men with a mean age of 51 years. Most patients in both groups neither smoked nor consumed more than 10 g of alcohol per day. None of these clinical variables were statistically different between groups. Only the number of sites with biofilm and bleeding on probing as well as the magnitude of probing depth were significantly higher in the peri-implantitis group.

3.2 | Histopathological results

Representative images of each histological staining are presented in Figure 2. The relative area of tissue occupied by the inflammatory infiltrate was significantly higher in peri-implantitis lesions both in the lamina propria and vestibular areas compared with the control group. In the sulcular area, although the area of tissue occupied by cells was also higher in peri-implantitis lesions, the comparison with the control group did not reach statistical significance. Consequently, the area occupied by connective tissue was lower in peri-implantitis samples (Figure 2a and Table S2). The analysis of picrosirius red staining images with and without polarized light showed that relative areas of connective tissue were significantly lower in peri-implantitis lesions; within those areas of connective tissue, relative areas of fibrillar collagen were also significantly lower in peri-implantitis (Figure 2b and Table S3).

3.3 | Immunohistochemical results

The semi-quantitative analysis indicated that the extension of the inflammatory infiltrate in samples from peri-implantitis was higher than in the control group, with a high percentage of samples presenting more than 50% of the area occupied by inflammatory cells. In contrast, most samples from the control group showed less than 25% of the area occupied by the inflammatory infiltrate (Table S4).

The immunophenotype of the inflammatory infiltrate in the peri-implantitis samples was mainly composed of plasma cells (CD138 positives), B lymphocytes (CD20 positives), and T lymphocytes (CD3 positives) with a notable presence of neutrophils (CD15 positives), and monocytes/macrophages (CD68 positives). The vascular structures (CD34 positives) were numerous due to the presence of granulomatous tissue below the ulcerated epithelium of the sulcular area. In summary, the total inflammatory infiltrate

TABLE 1 Clinical data of included patients and samples and comparison between cases of peri-implantitis and no periodontal disease controls.

Patient-level variable	Peri-implantitis group ($n = 21$)	Control group ($n = 15$)	p-Values[a]
Age [mean (min-max) in years]	59.19 (48–75)	50.87 (25–83)	.382
Gender [*n* (%)]			
Female	14 (66.67)	9 (60.00)	.681
Male	7 (33.33)	6 (40.00)	
Alcohol [*n* (%)]			
No	19 (90.48)	13 (86.67)	.720
Yes	2 (9.52)	2 (13.33)	
Smokers [*n* (%)]			
No	11 (52.38)	9 (60.00)	.650
<10 cigarettes per day	10 (47.62)	6 (40.00)	
Implant-level variable	**Peri-implantitis group ($n = 33$)**	**Control group ($n = 15$)**	**p-Values[a]**
Biofilm [*n* (%)]			
No	0 (0.00)	12 (80.0)	<.001
Yes	33 (100.00)	3 (20.0)	
Probing Depth [mean (SD) in mm]	6.80 (2.45)	2.21 (0.34)	<.001
Bleeding on probing [*n* (%)]			
No	0 (0.00)	13 (86.70)	<.001
Yes	100 (100.00)	2 (13.30)	

[a]Mann–Whitney *U*-test for continuous variables; chi-squared for categorical data.

(CD45 positives), the different leukocyte subsets, and the number of vessels per mm^2 were more abundant in the peri-implantitis samples. The difference was statistically significant when compared with the control group in all areas evaluated as well as when considering the sample as a whole (Figure 3 and Table S5).

Immunohistochemical determination of NLRP3 and AIM2 inflammasomes as well as caspase-1 and interleukin-1β in cases of peri-implantitis showed a statistically significant higher expression of all of them in the lamina propria and epithelium compared to control samples (Figure 4 and Table 2).

The expression of NLRP3 and AIM2 inflammasomes as well as caspase-1 and interleukin-1β were correlated with statistical significance with the expression of inflammatory infiltrate phenotype markers. In addition, all of them were correlated with statistical significance with probing depth, presence of biofilm, and bleeding on probing but not correlated with alcohol or tobacco consumption (Table S6). NLRP3, AIM2, caspase-1, and interleukin-1β were all significantly correlated between them.

3.4 | mRNA results

Evaluation of mRNA showed higher expression of all the genes under study in samples of peri-implantitis. Statistical significance was reached for the comparison of expression of AIM2, NLRP3, CASP1, and TLR4 (Table 3).

4 | DISCUSSION

The aim of the present study was to analyze for the first time the presence of inflammasomes NLRP3 and AIM2 in tissue samples obtained from humans around implants with active peri-implantitis. To the best of our knowledge, this is the first clinical study on this topic. Increased expression of interleukin-1β mediated by caspase-1 was also observed in these lesions in comparison with samples obtained from clinically healthy supracrestal soft tissue. We also find evidence, not only of augmented TLR4 expression in our human samples of peri-implantitis but also increased Gasdermin (GSDMD) expression. This finding could indicate an increased tissue inflammation due to cell death mediated by pyroptosis, since inflammasome-activated Gasdermin is known to induce such process by generating pores in the cell membrane (Liu et al., 2016).

In the present study, we found a more intense inflammatory infiltrate in the connective tissue correlated with the probing depth. In agreement with Carcuac and Berglundh (2014), and Dionigi et al. (2020), we also found a more severe inflammatory infiltrate in areas close to the pocket epithelium. The inflammatory cells found in our samples follow the same trends described in previous studies with a predominant role of the $CD138^+$ plasma cells (Bullon et al., 2004; Carcuac & Berglundh, 2014; Dionigi et al., 2020; Galindo-Moreno et al., 2017; Ghighi et al., 2018; Sanz et al., 1991). In our study, T subsets were more common than mature B subsets without taking B effector subsets into account

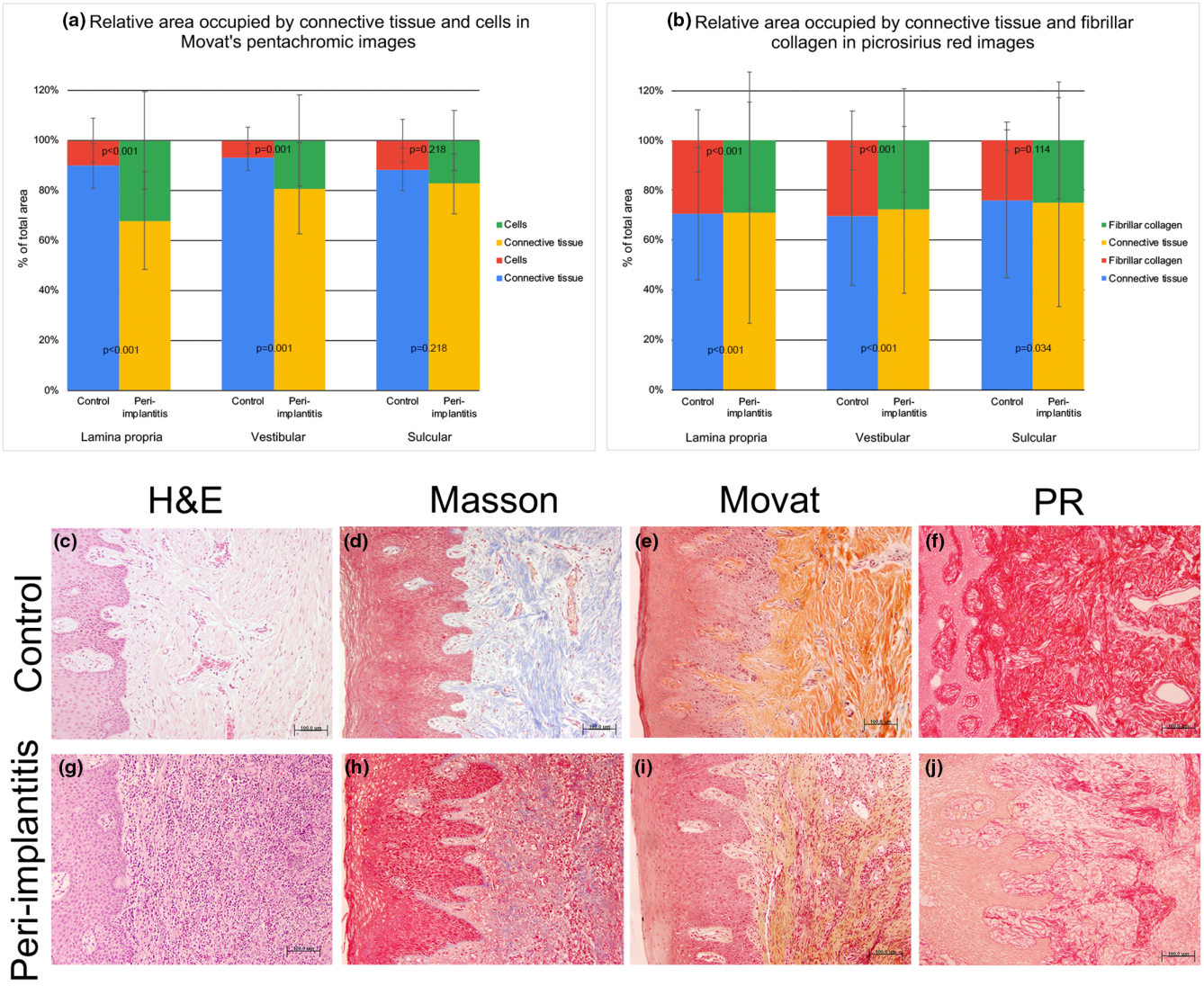

FIGURE 2 Graphical representation of the comparison between groups of (a) relative area occupied by connective tissue and cells visualized in Movat's pentachromic images and (b) relative area occupied by connective tissue and fibrillar collagen visualized in picrosirius red images, under regular and polarized light, respectively. Note the *p* values of the Mann–Whitney *U*-test. All values are expressed as mean percentage (standard deviation). Representative panoramic microphotograph from (c–f) gingival control, and (g–j) peri-implantitis biopsies stained with (c, g) H&E, (d, h) Masson's trichromic, (e, i) Movat's pentachromic and (f, j) picrosirius red. Note the decrease of connective tissue and increase of inflammatory infiltrate in cases of peri-implantitis. Original magnification 10×, Bar scale:100 μm.

(plasma cells). These findings are similar in other publications related to histopathological analyses of peri-implantitis (Carcuac & Berglundh, 2014; Cornelini et al., 2001). In contrast, Ghighi et al. (2018) found an inverse relation. This difference could be due to the fact that in Ghighi's study the tissue samples were obtained after mechanical therapy, which could have affected the local peri-implant environment.

The presence of AIM2 inflammasome in relation with human peri-implantitis is a very remarkable finding of the present study. In fact, to our knowledge, this is the first time such finding is described, both in vivo and in vitro. Studies in vitro have evidenced NLRP3 inflammasome activation after exposure of immune cells to titanium particles or other metals (Li et al., 2020; Pettersson et al., 2017). Moreover, a study in vivo has concluded that suppression of NLRP3 inflammasome could improve alveolar defect healing in diabetic rats (Li et al., 2019). In fact, these studies described intracellular pathways of NLRP3 inflammasome activation in presence of foreign body particles. However, this has not been described yet in clinical lesions of peri-implantitis. Interestingly, no study has described the relationship of AIM2 inflammasome activation, neither in the presence of metal particles, nor in peri-implantitis lesions. An explanation for this could be that the AIM2 inflammasome, in theory, only binds to double-stranded DNA; thus, it could only be activated in the presence of biological triggers (Marchesan et al., 2020). It is possible that the AIM2 inflammasome detection in our clinical samples was attributable to the microorganisms present in the biofilms around the implants. In addition, we detected AIM2 immunohistochemical

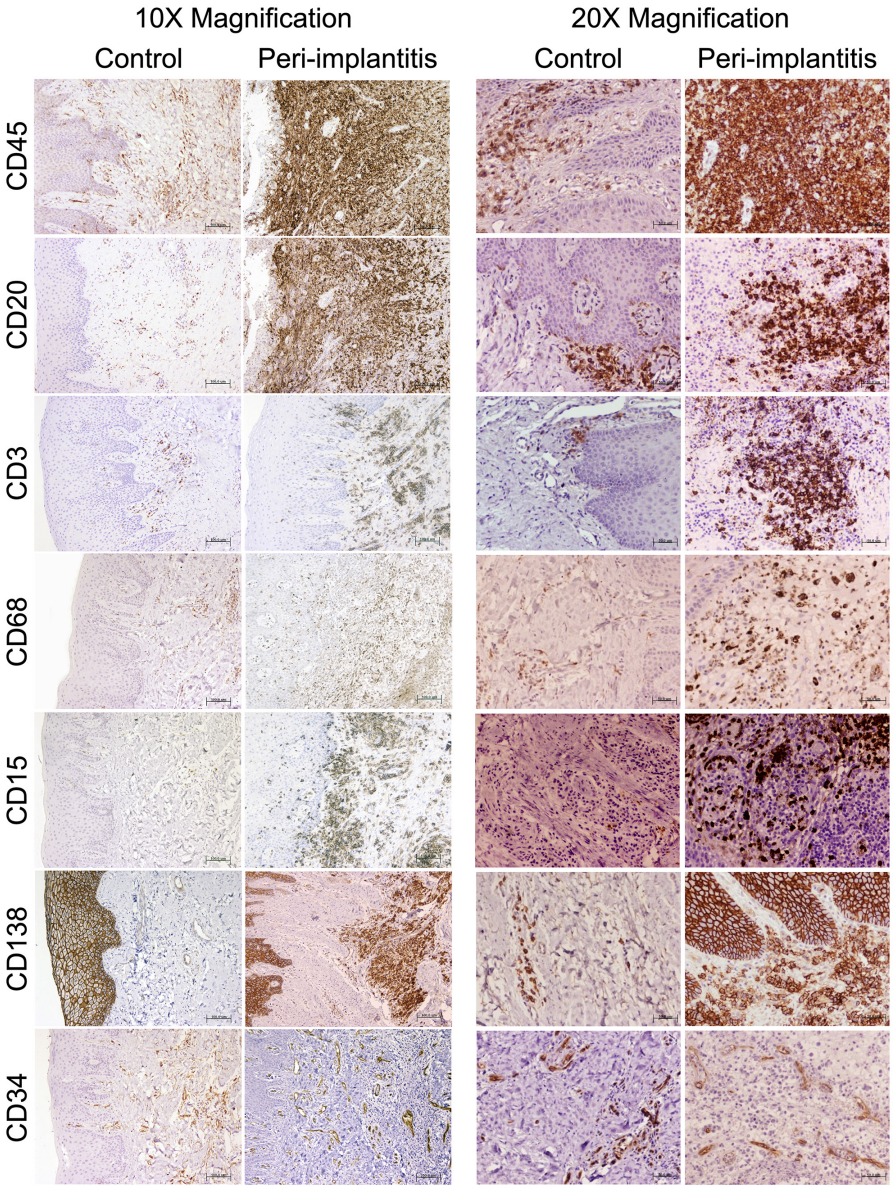

FIGURE 3 Representative microphotography of different leukocyte subsets (CD45, CD20, CD3, CD68, CD15, and CD138) and vessels (CD34) in the lamina propria of biopsies from peri-implantitis and control groups visualized at both 10× (scale bar: 100 μm) and 20× (scale bar: 50 μm) original magnifications: CD45 (leukocytes), CD20 (B lymphocytes), CD3 (T lymphocytes), CD68 (monocytes/macrophages), CD15 (polymorphonuclear neutrophils), CD138 (plasma cells), and CD34 (endothelial cells). Note the severe inflammatory infiltrate composed of plasma cells and macrophages in cases of peri-implantitis. Peroxidase-conjugated micropolymer method visualized with diaminobenzidine.

expression in correlation with the severity of the inflammatory infiltrate and the probing depth.

In a recent study, Rakic et al., using X-ray spectrometry, evidenced that titanium particles were a common finding in peri-implantitis lesions. However, they claimed that these particles did not evoke a marked biological response. In their study, the intensity of the inflammation measured by the Nf-kB expression did not demonstrate any significant difference when comparing peri-implantitis versus periodontitis granulomatous tissues. However, they reported an increased number of macrophages and higher neovascularization patterns in the peri-implantitis granulomatous tissue. This later finding was related with a more severe pattern of destruction (Rakic et al., 2022). This is a really interesting point, because obviously the increased inflammation in peri-implantitis could be mediated by a different inflammation pathway and not the classically described Nf-kB pathway toll-like receptor-mediated and promoted by microorganisms. In our study, we were able to evidence increased expression of TLR4. TLR4 can be activated through interaction with microorganisms, and also, as DAMP receptors. Thus, TLR4 receptors are capable of recognizing histones, hyaluronate fragments, or some thermal shock proteins, among others. In summary, these receptors can also activate the non-canonical pathway of the NLPR3 inflammasome (Suárez & Buelvas, 2015).

It is quite interesting that despite so many previously described similarities between periodontitis and peri-implantitis, as Rakic et al. described, both lesions exhibit different patterns of bone destruction and progression, both in shape and time. Literature has described the intimal differences in the pathogenesis of both entities. In this sense, and in relationship with the inflammasome-mediated inflammation from the 10 different inflammasomes described in the literature (Guo et al., 2015), a recent revision has presented clinical and preclinical data supporting a potential role of the inflammasomes NLRP1, NLRP3, AIM2, IFI16, and pyrin in the

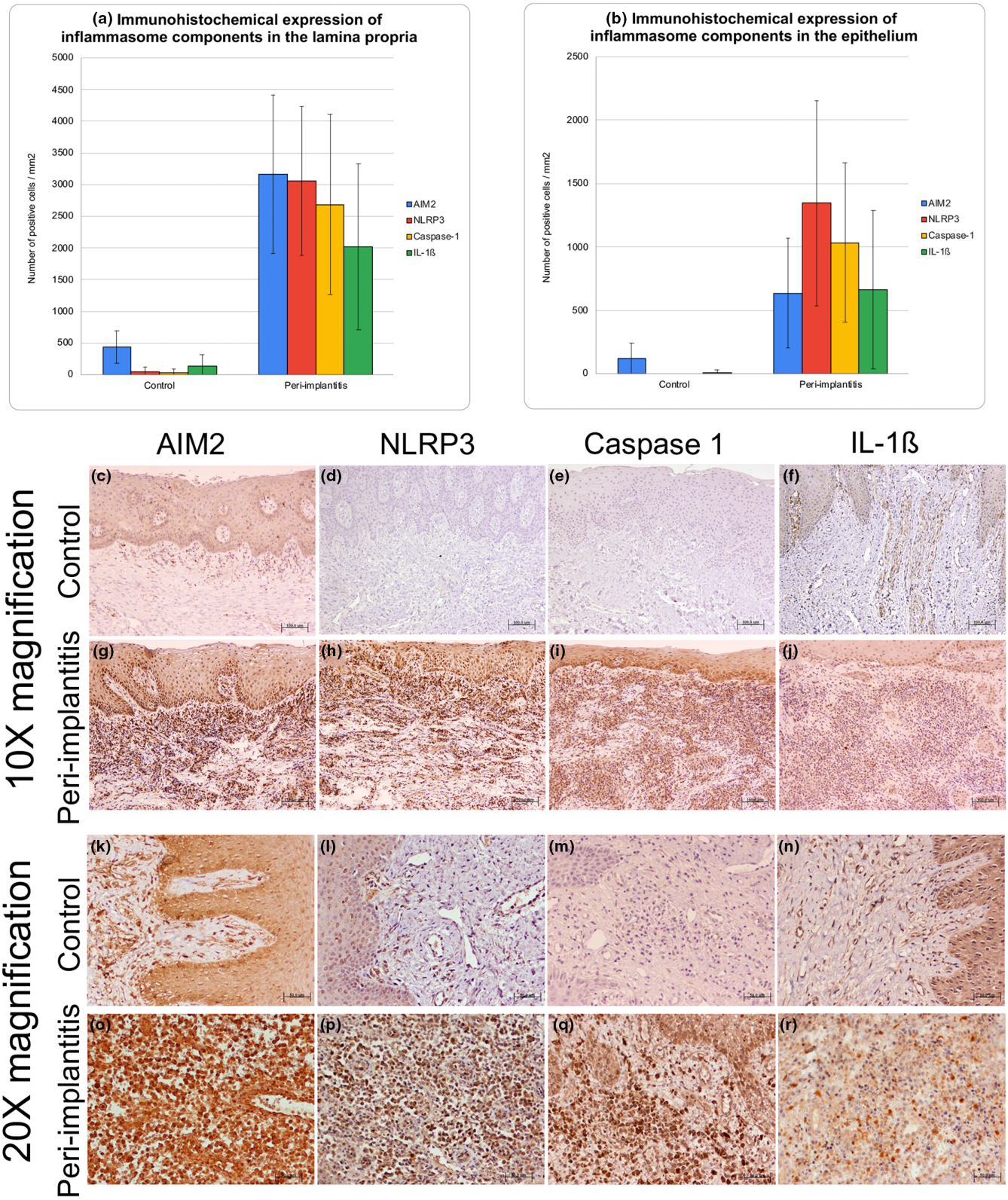

FIGURE 4 Graphical representation of the number of positive cells/mm^2 of each of the inflammasome components under study, both in (a) the lamina propria and (b) the epithelium. The comparisons between groups of all markers were statistically significant ($p < .001$, Mann–Whitney *U*-test). All values are expressed as mean of positive cells per mm^2 (standard deviation). In the lower panel (c–r), differential immunohistochemical expression of inflammasome components NLRP3, AIM2, Caspase-1, and IL-1β in control and peri-implantitis groups visualized at both 10× (scale bar: 100 μm) and 20× (scale bar: 50 μm) original magnifications. Peroxidase-conjugated micropolymer method visualized with diaminobenzidine.

TABLE 2 Immunohistochemical expression of inflammasome components by group and statistical comparisons.

Marker	Zone	Peri-implantitis group ($n = 33$)	Control group ($n = 15$)	p-Values (Mann–Whitney U-test)
AIM2	Lamina propria	3160.28 (1251.36)	434.41 (260.54)	<.001
	Epithelium	635.08 (435.93)	119.35 (123.10)	<.001
NLRP3	Lamina propria	3057.84 (1177.27)	44.93 (70.50)	<.001
	Epithelium	1345.62 (811.41)	0.00 (0.00)	<.001
Caspase-1	Lamina propria	2685.21 (1421.50)	33.33 (48.57)	<.001
	Epithelium	1034.56 (625.90)	0.00 (0.00)	<.001
Interleukin-1β	Lamina propria	2016.24 (1312.15)	140.86 (170.53)	<.001
	Epithelium	660.05 (625.22)	8.60 (19.15)	<.001

Note: All values are expressed as mean of positive cells per mm^2 (standard deviation).

TABLE 3 Relative expression of mRNA.

Gene	Peri-implantitis group ($n = 33$)	Control group ($n = 15$)	p-Values (Mann–Whitney U-test)
AIM2	142.49 (516.78)	1.65 (1.68)	<.001
NLRP3	67.19 (87.16)	1.12 (0.65)	<.001
CASP1	5.45 (6.21)	1.01 (0.16)	.024
IL-1β	22.66 (65.34)	1.10 (0.52)	.084
GSDMD	4.62 (8.69)	1.05 (0.36)	.475
TLR4	30.04 (33.56)	1.02 (0.22)	<.001

Note: All values are expressed as relative mean (standard deviation).

pathogenesis of periodontal disease (Marchesan et al., 2020). In peri-implantitis, Ganesan et al. (2022) have reported the increased in NLRP2, NLRP8, and NLRP12. The current study has also identified NLRP3 inflammasome in peri-implantitis. Nowzari et al. (2012) reported no differences in the microbiota in healthy implants versus healthy teeth in an intra-individual study, but reported significantly higher concentrations of cytokines and chemokines in the healthy implants. In this sense, other authors demonstrate higher levels of IL1β in peri-implant crevicular fluid of healthy implants in comparison with gingival crevicular fluid obtained from healthy teeth (Gürlek et al., 2017; Yaghobee et al., 2013). Similarly, Jansson et al. (2021) have found similar differences in chemokines and cytokines expression from healthy implant sites in comparison with healthy tooth sites; in addition, they found that the cytokine profile did not differ between sites diagnosed with peri-implantitis in relation to those diagnosed with periodontitis. Taking together all this information, it can be suggested that other local factors beyond the microbiome alone could play a role in the homeostasis of the peri-implant tissues. Upregulation of IL-1β is a capital event in the development of peri-implantitis (Li & Wang, 2014). In our peri-implantitis samples, IL-1β is clearly overexpressed in conjunction with AIM2 and NLRP3 sensors and caspase 1 in comparison with the control samples. It is important to keep in mind that at least two stimuli are needed to promote the processing of pro-IL-1β and later releasing of mature IL-1β. If not released, pro-IL-1β would be either retained or degraded (Burns et al., 2003). Accordingly, several signaling pathways can be implicated in the transcriptional upregulation of pro-IL-1β, including other cytokines such as TNF and various Toll-like receptor (TLR) ligands, such as some lipopolysaccharide (LPS) (Dinarello, 1998). Additionally, inflammasome sensors activating caspase 1 or some metals present in the microenvironment, such as cobalt or titanium (Pettersson et al., 2018), can also induce IL-1β release.

In the present study, we have evidenced not only the activation of the inflammasome sensor NLRP3 but also the activation of the AIM2 sensor, in samples of peri-implantitis retrieved from humans. These expressions were significantly correlated with the severity of the peri-implantitis lesion. Likewise, it has been evidenced the overexpression of TLR4 in our samples, as expected in relation to a greater presence of biofilm, or in relation to the non-canonical pathway of NLRP3 inflammasome activation. We have also found an overexpression of Gasdermin, related to pyroptotic phenomena, which is also related to the activation of the inflammasome. This is important to keep in mind because inflammasome activation regulates the activities of many types of cells, including bone cells (osteocytes, osteoblasts, and osteoclasts) and others, such as periodontal ligament cells, monocytes, macrophages, neutrophils, and Th17 cells. This regulatory activity results in a reduction in bone mass and quality. Therefore, inflammasome activation may be not only a promoter but also a consequence of inflammatory bone loss, enhancing a positive feedback on the mechanisms of bone destruction through inflammation (Li et al., 2021).

This manuscript describes, for the first time, the role of some inflammasomes in human peri-implantitis, but it also has an

important limitation. We used healthy gingiva taken from tooth extraction for caries or orthodontic reasons as control. Obviously, some structural differences can be claimed between both environments, healthy gingiva versus diseased peri-implant mucosa. Additionally, the results presented in the current manuscript regarding the higher expression of NLRP3 and AIM2 in peri-implantitis compared to healthy gingiva could be expected because we are comparing a non-inflamed tissue with a highly inflamed one. Thus, future studies comparing tissues from all conditions of health and inflammation around teeth and implants should be conducted. Many questions remain about this pathway of inflammation activation and its regulation, which should be evaluated in future clinical trials.

5 | CONCLUSIONS

Peri-implantitis is associated with several inflammatory mechanisms among which inflammasome activation is included. In human peri-implantitis, chronic inflammation can also be explained due to the action of interleukin-1β/caspase 1 promoted induced through NLRP3 and AIM2 inflammasomes activation.

AUTHOR CONTRIBUTIONS

Pablo Galindo-Moreno: Conceptualization; methodology; investigation; writing—original draft; resources; writing—review & editing; supervision; funding acquisition; project administration. **Saray Montalvo-Acosta**: Methodology; investigation; writing—review & editing. **Natividad Martín-Morales**: Methodology; investigation; writing—review & editing. **Ana Belén Carrillo-Gálvez**: Investigation; data curation; writing—review & editing. **Elena González-Rey**: Conceptualization; resources; writing—review & editing; funding acquisition. **Francisco O'Valle**: Conceptualization; methodology; formal analysis; resources; data curation; writing—review & editing; visualization; funding acquisition. **Miguel Padial-Molina**: Conceptualization; methodology; validation; formal analysis; resources; writing—original draft; writing—review & editing; visualization; project administration; funding acquisition.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest, either directly or indirectly, in any of the products listed in the manuscript.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

All procedures performed in studies involving data from human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This clinical study was reviewed and approved by the Ethics Committee for Human Research of the University of Granada, Spain (587/CEIH/2020).

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SUPPORTING INFORMATION

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