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Exosome profile and composite indices reflect immune exhaustion in periodontitis

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Introduction Exosomes are small extracellular vesicles that regulate immune responses, inflammation, and tissue homeostasis. While several exosomal molecules have been investigated individually in periodontitis, a functional characterization based on composite indices has not been explored. This study aimed to profile salivary exosomes in periodontitis using four composite indices (Total Cargo, Purity, Biogenesis, and Immune Index) and correlate them with clinically relevant cytokines.
Materials and methods A cross-sectional analytic study was conducted on periodontital patients. All patients underwent a clinical periodontal examination. Gingival crevicular fluid was collected and analyzed using a 6-plex exosome characterization panel (CD9, CD63, CD81, VLA-4, Syntenin-1, Cytochrome C) and a 4-plex cytokine panel (IL-1β, IL-8, IL-10, IL-17 A). Four exosomal composite indices were calculated, and Spearman correlations were performed between clinical, biochemical, and exosomal variables.
Results A total of 33 patients with periodontitis were included, of whom 27% were classified as Stages I-II. Amongst other statistical significances ($p < 0.05$), Cytochrome C showed correlations with 4–6 mm pockets ($r = 0.50$) and PIRIM ($r = 0.42$), while 7–8 mm pockets were associated with CD63 ($r = 0.49$) and Purity Index ($r = 0.48$), and negatively with the Biogenesis ($r = -0.45$), and Immune Indices ($r = -0.41$). IL-1β showed correlations with the Total Cargo ($r = 0.49$) and Purity Indices ($r = 0.48$), and negative correlations with the Immune and Biogenesis Indices ($r = -0.47$ and -0.50, respectively). CD81 was negatively correlated with periodontal staging, and IL-10 showed an inverse correlation with periodontal grading.
Conclusion Composite exosomal indices reflect the balance between canonical biogenesis, immune cell-derived vesicles, and cell damage–associated vesicles in periodontitis. The negative association of CD81 and Immune Index with disease severity suggests immune exhaustion, whereas reduced IL-10 supports impaired regulatory control in advanced stages.

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Keywords Periodontitis, Exosomes, Lymphocyte exhaustion, Biomarkers

The diagnosis of periodontitis is primarily clinical, with the current case definition established as the presence of detectable interdental clinical attachment loss (≥ 3 mm) at two or more non-adjacent teeth, together with pocket probing depth (≥ 4 mm) and bleeding on probing[1]. Nevertheless, in recent years several biomarkers have been proposed as complementary diagnostic tools. A recent meta-analysis by Blanco-Pintos et al. evaluated combined biomarker panels in gingival crevicular fluid (GCF) and saliva, showing that the association of IL-6 with MMP-8 or IL-1β with IL-6 achieved sensitivities and specificities above 80%[2].

Exosomes – small, single-membrane extracellular vesicles released by host cells and microorganisms[3] – have emerged as key mediators in periodontitis. These vesicles carry proteins, nucleic acids, and microRNAs that modulate immune responses, inflammation, and tissue remodeling[4]. Exosome biogenesis occurs through distinct pathways – canonical (ESCRT-dependent) and non-canonical (often associated with cellular stress) – which critically influence their molecular cargo and function (Fig. 1)[5,6]. While host-derived exosomes orchestrate immune communication, periodontal pathogens such as *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* secrete outer membrane vesicles (OMVs) that mimic exosomal functions, modulating host responses and promoting alveolar bone destruction[7].

Beyond their functional role, exosomes have gained increasing attention as potential diagnostic biomarkers in periodontitis. Differentially expressed exosomal RNAs have been identified in patients compared with healthy

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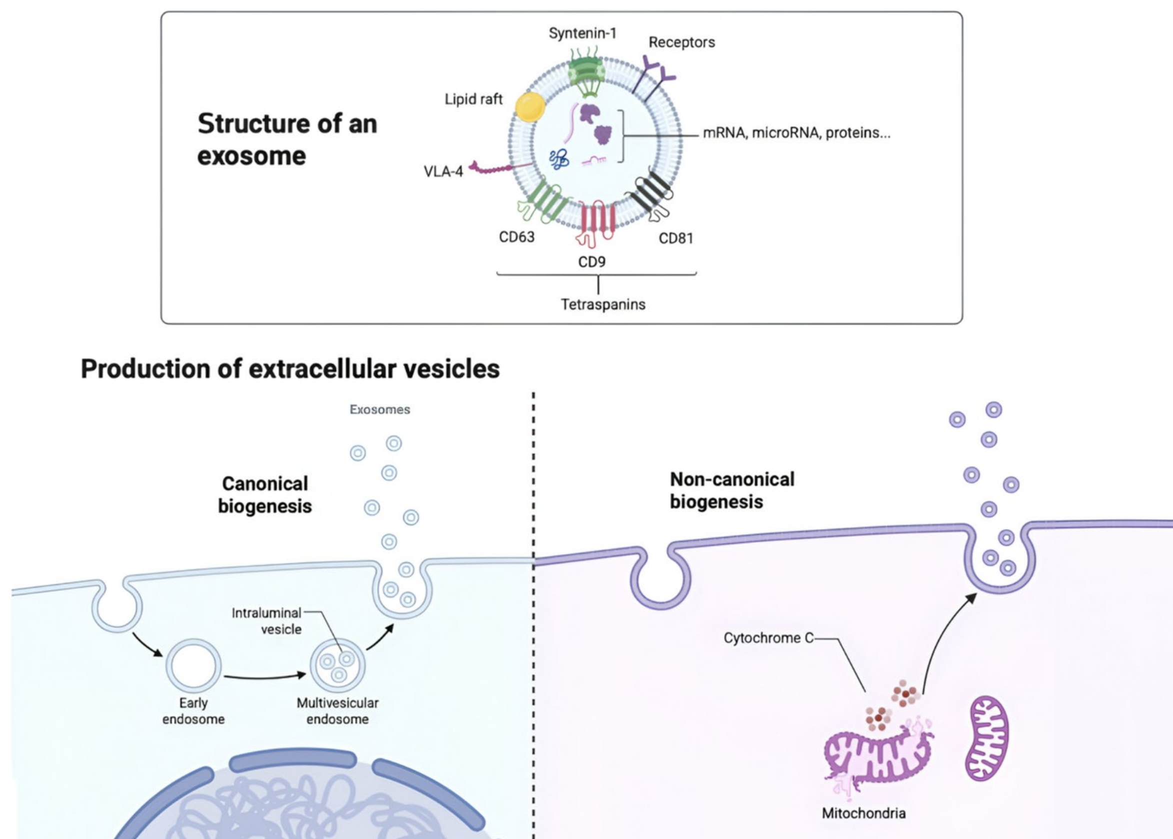

Fig. 1. Structure and biogenesis of exosomes. Exosomes are small extracellular vesicles enriched in tetraspanins (CD9, CD63, CD81), receptors, lipids, proteins, and nucleic acids. Their canonical biogenesis involves the formation of early endosomes, intraluminal vesicles, and multivesicular endosomes, which fuse with the plasma membrane to release exosomes. This process can be regulated by the ESCRT complex and accessory proteins. In contrast, non canonical pathways are frequently activated under stress or apoptosis conditions, and may involve the direct budding of vesicles from the plasma membrane or the incorporation of mitochondrial components (cytochrome C), contributing to mitochondrial quality control.

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controls, including microRNAs, small nucleolar RNAs, and mRNAs such as programmed death ligand-1, which correlate with disease severity and progression[8]. At the protein level, decreased expression of CD9 and CD81 in salivary exosomes has been negatively correlated with clinical parameters, supporting their potential relevance in the pathogenesis of periodontitis[9]. Together, these tetraspanins not only indicate exosome abundance but also provide functional insights into immune modulation and inflammatory activity in periodontitis.

Despite their potential, previous studies on periodontitis remain limited and have largely investigated single exosomal molecules (e.g., miR-146a, CD63) rather than integrated molecular profiles that capture the interplay between biogenesis pathways and immune.modulatory cargo[10,11]. To overcome this, we propose, for the first time, the use of composite indices for exosomal characterization. This innovative approach, inspired by integrative methodologies in other fields, enables a standardized functional assessment by normalizing key biomarkers, thereby providing crucial insights into the origin, integrity, and functional bias of the exosomal population.

To the best of our knowledge, a panel of six exosomal molecules transformed into four indices has not yet been investigated in patients with periodontitis. We therefore hypothesize that these novel composite indices could provide a superior and more nuanced functional characterization of the exosomal landscape in periodontitis compared to isolated markers.

This study has two objectives. First, to characterize in greater depth the exosomal profile in patients with periodontitis using composite indices (Purity, Biogenesis, Immune, and Total Cargo). Second, to correlate this profile with a panel of pro- and anti-inflammatory cytokines that are clinically highly relevant in periodontitis.

Materials and methods

Study design and participants

We conducted a cross-sectional, analytical study including patients diagnosed with periodontitis at the School of Dentistry, University of Granada, Granada, Spain. Patients were recruited between January and June 2025, and all eligible participants provided written informed consent. The study was approved by the Biomedical Research

Ethics Committee of the University of Granada (4566/CEIH/2024). The manuscript was prepared following the STROBE guidelines for observational epidemiological studies[12], which are included as Supplementary Material 1.

Inclusion criteria were patients aged over 18 years with a diagnosis of periodontitis according to the 2018 classification[1]. Exclusion criteria included patients who had taken antibiotics within the previous three months, received periodontal treatment in the last year, had active systemic inflammatory diseases that could affect the biochemical parameters analyzed, or were pregnant or breastfeeding.

Sociodemographic variables
Sociodemographic data were gathered from each patient: age, gender, and tobacco consumption (cigarettes per day).

Periodontal examination
Clinical periodontal examinations included a periodontal chart carried out at the School of Dentistry, using the periodontal chart of the University of Bern, Switzerland. All measurements were obtained using a standardized periodontal probe (PCPUNC15, Hu-Friedy, Chicago, IL, USA) and a dental mirror (SE plus^{R} mouth mirror, Hahnenkratt E. GmbH, Königsbach-Stein, Germany). Probing pocket depth (PPD) and clinical attachment loss (CAL) were recorded in millimeters at six sites per tooth. The percentage of bleeding on probing (BOP)[13] and visible plaque (VPI)[14] was documented for each tooth. Periodontitis severity was assessed using a modified version of the Periodontal Inflammatory Severity Index, referred to as PIRIM[15]. The total number of present teeth was also recorded.

All measurements were carried out by a calibrated examiner (C.G.) Prior to data collection, this examiner underwent inter- and intra-examiner calibration with a reference researcher (F.M.) at different time points using patients with periodontitis at the University of Granada's School of Dentistry Clinic. The calibration process for PPD and CAL measurements yielded an intraclass correlation coefficient of 0.78 in PPD and 0.70 in CAL, accepting a measurement variability of ± 1 mm; these figures are considered Substantial (range from 0.61 to 0.80)[16]. Periodontitis was defined according to the 2018 classification by the American Academy of Periodontology and the European Federation of Periodontology, as the presence of detectable interdental CAL ≥ 3 mm at two or more non-adjacent teeth, along with PPD ≥ 4 mm and BOP[1].

Sample collection
GCF samples were collected following the technique described by Mombelli et al.[17]. Sterile #30 absorbent paper points (Bestdent©, 0.02 taper) were gently inserted into the two deepest bleeding pockets of each quadrant, yielding a total of eight paper points per patient. Each paper point was left in place for 20 s, then immediately transferred to Eppendorf tubes containing 100 µL of phosphate-buffered saline (PBS), and stored at -80 °C until cytometric analysis.

Flow cytometry analysis
Cytometric analysis was performed using the Luminex xMAP technology. Cytokine analysis was performed using the ProcartaPlex™ Human Essential Panel 2, 4-plex (EPX040-10008-901), which allows the quantification of IL-1β, IL-8, IL-10, and IL-17 A. Exosomal analysis was carried out with the ProcartaPlex™ Human Exosome Characterization Panel, 6-plex (EPX060-15845-901). In both protocols, samples were first thawed at room temperature (RT) and centrifuged at 400 g for 4 min at RT. Standard curves were then prepared by serial dilutions ($n = 16$). Subsequently, 50 µL of capture beads were added to each well, incubated for 10 min at RT, washed with 150 µL of Wash Buffer, and allowed to dry. Next, 25 µL of sample or standard curve was added to the corresponding wells and incubated overnight at 4 °C under agitation. After incubation, 25 µL of Detection Antibody Mix was added and incubated for 30 min at RT with agitation, followed by two washes. Then, 50 µL of Streptavidin-Phycoerythrin was added, incubated for 30 min at RT with agitation, washed twice, and finally resuspended in 120 µL of Reading Agent. The sensitivity of each kit is provided in Supplementary Material 2.

Exosomes and composite exosomal indices
To provide a standardized functional assessment of exosome populations, we introduced four composite indices derived from the relative expression of key exosomal markers. These indices were designed to interrogate distinct biological features: the Purity Index (CD63/Cytochrome C) estimates the proportion of canonical exosomes versus vesicles from damaged cells; the Biogenesis Index (Syntenin-1/Total Cargo) evaluates vesicle production via the syntenin-1-dependent pathway; the Immune Index (VLA-4/Total Cargo) indicates the relative abundance of exosomes derived from immune cells; and the Total Cargo index (mean of CD9, CD63, CD81) reflects the overall exosomal load.

The analysis included the three main tetraspanins (CD9, CD63, CD81) as well as VLA-4 (for immune origin), Syntenin-1 (for canonical biogenesis), and Cytochrome C (for non-canonical, damage-associated biogenesis) to provide the basis for these indices.

Statistical method
During the study period, it was possible to recruit 33 patients.

A post hoc calculation of statistical power with this sample size is as follows. The sample size of $n = 33$ allows the detection of a correlation coefficient (r) of 0.45, which lies between a medium ($r = 0.3$) and a large ($r = 0.5$) effect size, with 80% power and a two-sided alpha error of 0.05[18]. Statistical analysis was performed using IBM SPSS Statistics 22.0 (IBM Corp., Armonk, NY), with descriptive and analytical methods detailed in the footnotes of each results table. Spearman correlation was used because it is less sensitive to the presence of outliers in the

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Variable	n (%)
Sex, n (%)	
Male	14 (42.4)
Female	19 (57.6)
Age, range (years)	36–71
Age, mean ± sd	53.2 ± 7.8
Smokers No. cig/day, n (%)	
0	21 (63.6)
1–10	7 (21.2)
11–25	5 (15.2)
Smokers, n (%)	
No	21 (63.6)
Yes	12 (36.4)

Table 1. Participants (n = 33).

Variable	mean ± sd
Clinical attachment loss (mm)	-3.9 ± 1.0
Plaque index (%)	23.6 ± 20.7
Bleeding on probing (%)	33.4 ± 19.8
No. teeth	23.4 ± 5.3
No. pockets 4–6 mm.	34.8 ± 22.3
No. pockets 7–8 mm.	0.48 ± 1.20
PIRIM (periodontal severity index)	7.2 ± 5.2

Table 2. Periodontal variables.

exosome variables. Given that Pearson's correlation requires normality and the absence of outliers, and after visually diagnosing (figures not shown) the violation of these assumptions in several variables, we decided to use Spearman's non-parametric correlation in all cases for the sake of parsimony. It should also be noted that we did not apply Bonferroni correction despite performing multiple correlations, in order not to reduce statistical power and thereby increase Type II error[19], particularly because this is a study with a limited sample size.

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Results

The study included 33 periodontitis patients. Of these, 9 individuals (27%) were classified with mild to moderate periodontitis (Stages I-II), whereas 24 patients (73%) presented advanced to severe periodontitis (Stages III-IV). Regarding disease grading, 21 patients (64%) were categorized as Grade A, 6 patients (18%) as Grade B, and 6 patients (18%) as Grade C.

Table 1 summarizes the demographic characteristics of the study population, with a mean age of 53.2 years and a male/female ratio of approximately 1:1. Table 2 shows the main periodontal parameters, with a mean CAL of -3.9 mm, BOP of 33.4% and PIRIM of 7.2. Table 3 presents the mean levels of the biochemical variables, along with 4 exosomal indices.

Table 4 presents the Spearman correlation coefficients between clinical variables, cytokines, exosomes and exosomal indices, with key patterns visualized in Fig. 2 for enhanced interpretation. Notably, Cytochrome C exhibited a significant positive correlation with the number of 4–6 mm pockets ($r = 0.50$, $p < 0.05$) and PIRIM ($r = 0.42$, $p < 0.05$). 7–8 mm pockets showed significant correlations with CD63 ($r = 0.49$, $p < 0.05$), and the Biogenesis ($r = -0.45$, $p < 0.05$), Purity ($r = 0.48$, $p < 0.05$) and Immune Indices ($r = -0.41$, $p < 0.05$).

Table 5 shows the Spearman correlations between cytokines and exosomal indices, complemented by the visual overview in Fig. 3. Among other statistical significances, IL-1β demonstrated a significant positive correlation with Total Cargo ($r = 0.49$, $p < 0.05$) and Purity Index ($r = 0.48$, $p < 0.05$), and a significant negative correlation with Immune ($r = -0.47$, $p < 0.05$) and Biogenesis Indices ($r = -0.50$, $p < 0.05$). Cytochrome C exhibited a significant negative correlation with the Immune Index ($r = -0.42$, $p < 0.05$).

Table 6 presents the Spearman correlation coefficients between periodontal staging, grading, and biochemical variables. A significant negative correlation was observed between IL-10 and Periodontal Grading ($r = -0.40$, $p < 0.05$), as well as between CD81 and Periodontal Stages ($r = -0.34$, $p < 0.05$).

Discussion

This study revealed that proinflammatory cytokines (IL-1β, IL-8, IL-17 A) were closely associated with key exosomal markers (CD63, Cytochrome C, Syntenin-1) and composite indices (Purity, Biogenesis, Immune Index). Advanced disease stages exhibited reduced Biogenesis and Purity indices, along with lower levels of anti-inflammatory signals (IL-10 and CD81). To the best of our knowledge, no studies have employed

Variable	mean (pg/mL) ± sd
IL-1β	3772 ± 3139
IL-8	1506 ± 1068
IL-10	4.79 ± 4.33
IL-17 A	7.01 ± 3.06
CD9	10,412 ± 11,114
CD81	1937 ± 1370
VLA-4	65.7 ± 29.4
Syntenin-1	2783 ± 4822
Cytochrome C	6279 ± 4749
CD63	74,208 ± 89,705
Total Cargo[a]	28,852 ± 31,012
Immune index[b]	0.01 ± 0.01
Purity index[c]	12.6 ± 14.2
Biogenesis index[d]	0.56 ± 1.03

Table 3. Biochemical variables. [a]Total Cargo: (CD9 + CD63 + CD81)/3 [b]Immune Index: VLA-4/Total Cargo [c]Purity Index: CD63/Cytochrome C [d]Biogenesis Index: Syntetin-1/Total Cargo

Variable	Age	Sex	No.Cigs.	CAL	VPI	BOP	No.Teeth	Pockets 4–6 mm	Pockets 7–8 mm.	PIRIMX
IL-1β	−0.37* **−0.68 to −0.05**	0.07	0.09	−0.10	−0.09	0.29	0.24	0.27	0.15	0.18
IL-8	−0.32	−0.24	0.20	0.19	−0.01	−0.11	−0.02	0.21	−0.01	0.15
IL-10	0.06	0.12	−0.38* **−0.67 to −0.1**	0.14	0.05	−0.00	0.11	−0.21	−0.15	−0.29
IL-17 A	0.27	0.12	0.01	0.33	0.05	−0.19	−0.04	−0.01	−0.48* **−0.7 to −0.26**	−0.02
CD9	0.01	0.17	−0.18	−0.03	−0.28	0.04	0.22	0.24	0.30	0.11
CD81	−0.01	0.01	−0.19	−0.04	−0.27	0.06	0.27	0.27	0.18	0.08
VLA-4	−0.04	0.05	−0.15	0.03	−0.35* **−0.66 to -0.04**	0.01	0.34* **0.08 to 0.61**	0.04	0.24	−0.04
Syntetin-1	0.33	−0.05	0.01	0.12	−0.04	−0.08	0.02	0.05	−0.24	0.04
Cytochrome C	−0.18	0.32	0.14	0.08	0.21	0.15	−0.09	0.50* **0.24 to 0.75**	0.20	0.42* **0.16 to 0.67**
CD63	−0.26	0.21	−0.10	−0.12	−0.04	0.30	0.23	0.20	0.49* **0.2 to 0.77**	0.07
Total Cargo	−0.22	0.26	−0.12	−0.12	−0.08	0.28	0.18	0.21	0.48* **0.2 to 0.76**	0.09
Immune Index	0.26	−0.17	0.03	0.17	−0.05	−0.29	−0.15	−0.18	−0.41* **−0.7 to −0.13**	−0.07
Purity Index	−0.17	0.15	−0.23	−0.11	−0.21	0.19	0.31	−0.02	0.48* **0.21 to 0.76**	−0.12
Biogenesis Index	0.28	−0.13	0.15	0.16	0.01	−0.20	−0.12	−0.07	−0.45* **−0.74 to −0.17**	0.00

Table 4. Spearman correlation coefficients (with 95% confidence intervals shown below if significant) between biochemical, sociodemographic, habits, and periodontal variables ($n = 33$). Inside the table, Spearman correlation coefficients (rs) are presented. *: $p < 0.05$ Significant values are in bold

composite indices such as Purity, Biogenesis, or Immune to assess the functional profile of extracellular vesicles in periodontitis. Our findings suggest that these indices provide a more integrative evaluation than isolated markers, capturing not only the presence of vesicles but also their origin, functional integrity, and potential role in immune modulation and tissue homeostasis. These parameters could help standardize exosomal characterization in future research and better differentiate vesicles derived from active inflammation, cellular stress, or tissue damage across different stages of the disease.

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CD63, CD9, and CD81 are essential components of extracellular vesicles[20]. CD9 is broadly expressed in endothelial cells, keratinocytes, and leukocytes, including B cells, $CD4^+$ and $CD8^+$ T cells, NK cells, granulocytes, and macrophages, as well as in endothelial cells[21]. Additionally, CD9 participates in the regulation of adhesion molecules such as VLA-4 and activated leukocyte cell adhesion molecule (ALCAM), influencing immune cell adhesion, migration, and activation[22]. It also contributes to the formation of adhesive platforms in the endothelium, facilitating leukocyte extravasation[21]. Similarly, CD63 is involved in cholesterol sorting into intraluminal vesicles,

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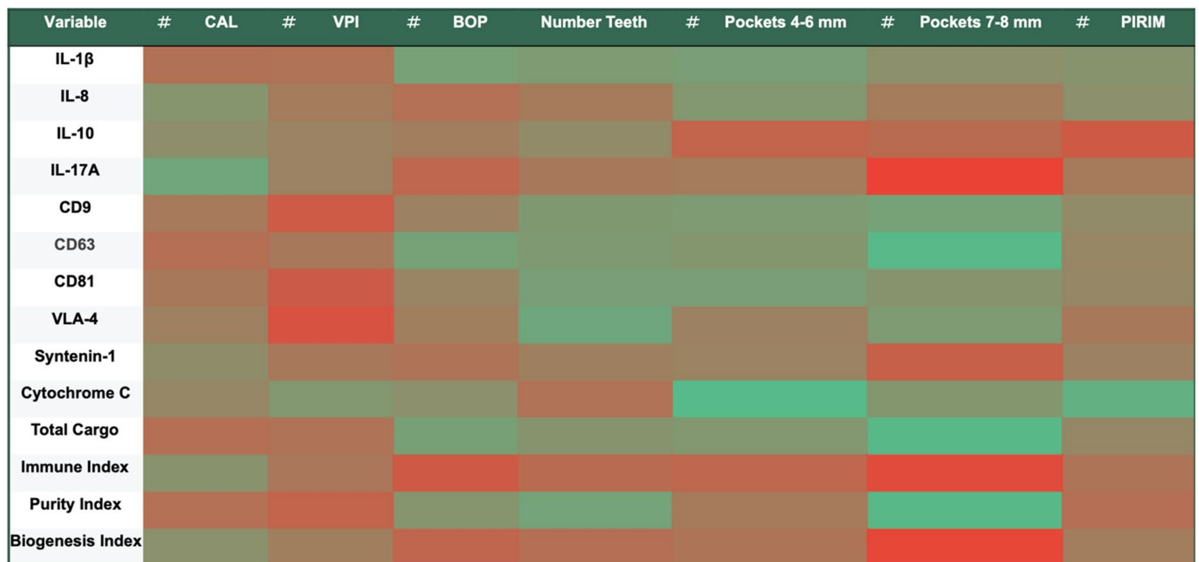

Fig. 2. Heatmap of Spearman correlations between clinical periodontal parameters and exosomal markers/indices. Color intensity represents correlation strength from – 1.0 (red, negative correlation) to + 1.0 (green, positive correlation). CAL: Clinical Attachment Loss; VPI: Visible Plaque Index; BOP: Bleeding on Probing; PIRIM: Periodontal Inflammatory Severity Index.

modulating the lipid composition and functional properties of exosomes[23]. Immunologically, CD63 is associated with neutrophil activation and has been found elevated in the saliva of patients with periodontitis, correlating with CAL[24]. Therefore, in periodontitis, CD63 not only reflects exosome abundance but also suggests their functional involvement in sustaining inflammation and dysregulated lipid trafficking. CD81 is also expressed in B cells, T cells ($CD4^+$, $CD8^+$), and NK cells, acting as a co-stimulatory protein for T cell activation and proliferation[25]. Together, these tetraspanins not only indicate exosome abundance but also provide functional insights into immune modulation and inflammatory activity in periodontitis.

VLA-4 (very late antigen-4) is a key integrin expressed on monocytes and lymphocytes, whose primary function is to mediate adhesion of these cells to periodontal fibroblasts. This interaction has been hypothetized to be critical in periodontitis, as it could facilitate monocyte infiltration and the release of inflammatory mediators and proteases that contribute to tissue destruction[26]. Syntenin-1 is an essential adaptor protein involved in the biogenesis and release of exosomes. Reduced expression of syntenin-1 decreases exosome release and their characteristic markers, while overexpression increases both vesicle number and content[27]. In the context of periodontitis, elevated syntenin-1 levels may reflect hyperactive secretion by immune or epithelial cells, contributing to vesicle overproduction during chronic inflammation. Lastly, cytochrome C is a mitochondrial protein primarily released by eukaryotic cells in response to cellular damage. Its presence in exosomes reflects activation of intrinsic apoptotic pathways, particularly under conditions of mitochondrial stress or inflammation[28]. Recent studies have shown that OMVs from pathogens such as *P. gingivalis* can directly induce mitochondrial fragmentation and cytochrome C release in host immune cells, linking bacterial virulence to host cell apoptosis and chronic inflammation[29]. Therefore, exosomal cytochrome C may serve not only as a biomarker of mitochondrial distress but also as a mediator amplifying the local inflammatory milieu.

Our findings suggest a functional axis between inflammation, exosomes, and tissue damage in periodontitis. Elevated levels of IL-1β and IL-8 reflect an intense and patient-specific inflammatory profile, whereas low IL-10 indicates insufficient anti-inflammatory control, contributing to disease progression[30]. This cytokine pattern correlates with exosomal load and the presence of cytochrome C, suggesting that extracellular vesicle release could respond not only to immune activation – via canonical exosome secretion – but also to local cellular stress and damage – non-canonical exosome secretion –, signaling neighboring cells of tissue injury[31]. The heterogeneous exosomal abundance, as reflected by CD63 (74,208 ± 89,705) and CD9 (10,412 ± 11,114), indicates that vesicle release is a dynamic and individualized process in periodontitis. This variability suggests that patients may present distinct vesicular profiles, potentially linked to differences in immune activation, tissue remodeling, or disease activity. In addition, our results showed an association between cytochrome C and 4–6 mm periodontal pockets, supporting a tissue stress profile predominantly regulated by apoptotic pathways. This finding is consistent with Salehe et al.[32], who reported a link between cytochrome C and apoptotic phenomena, further indicating that apoptosis-driven mechanisms prevail in mild-to-moderate stages of periodontitis. In contrast, in our patients with deep periodontal pockets, no association was found between cytochrome C and the purity index. We hypothesize that, as suggested by Vringer et al.[33], this may be explained by a shift from regulated apoptosis to uncontrolled necrotic processes, where mitochondrial collapse impairs exosome biogenesis and leads to the uncoordinated release of intracellular contents as damage-associated molecular patterns.

Composite indices provide a more functional perspective than individual markers. In deep pockets (7–8 mm), the decrease in IL-17 A is accompanied by a higher Purity Index, suggesting that in advanced stages,

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Variable	IL-8	IL-10	IL-17 A	CD9	CD81	VLA-4	Syntetin-1	Cytochrome C	CD63	Total Cargo	Immune Index	Purity Index	Biogenesis Index
IL-1β	0.53* **0.28 to 0.77**	0.14	−0.25	0.28	0.12	0.22	−0.42* **−0.73 to −0.12**	0.37* **0.08 to 0.66**	0.51* **0.23 to 0.79**	0.49* **0.19 to 0.79**	−0.47* **−0.75 to −0.19**	0.48* **0.2 to 0.76**	−0.50* **−0.76 to −0.24**
IL-8	–	0.06	−0.09	0.37* **0.06 to 0.68**	0.13	0.14	−0.34	0.48* **0.21 to 0.74**	0.37* **0.07 to 0.68**	0.37* **0.08 to 0.67**	−0.38* **−0.66 to −0.1**	0.24	−0.39* **−0.73 to −0.05**
IL-10	–	–	0.25	0.35* **0.02 to 0.68**	0.34	0.33	−0.05	−0.17	0.21	0.27	−0.16	0.32	−0.21
IL-17 A	–	–	–	−0.21	−0.19	−0.15	0.56* **0.28 to 0.84**	0.02	−0.43*	−0.39* **−0.71 to −0.08**	0.38* **0.02 to 0.73**	−0.47* **−0.80 to −0.15**	0.56* **0.31 to 0.82**
CD9	–	–	–	–	0.75* **0.55 to 0.94**	0.58* **0.41 to 0.75**	−0.13	0.29	0.55* **0.28 to 0.83**	0.63* **0.39 to 0.88**	−0.45* **−0.78 to −0.13**	0.54* **0.25 to 0.83**	−0.44* **−0.72 to −0.16**
CD81	–	–	–	–	–	0.52* **0.26 to 0.78**	0.02	0.19	0.47* **0.20 to 0.74**	0.52* **0.26 to 0.77**	−0.38* **−0.69 to −0.08**	0.41* **0.13 to 0.70**	−0.28
VLA-4	–	–	–	–	–	–	−0.08	0.06	0.34	0.39* **0.09 to 0.69**	−0.05	0.42* **0.11 to 0.74**	−0.29
Syntetin-1	–	–	–	–	–	–	–	−0.14	−0.57* **−0.83 to −0.31**	−0.56* **−0.83 to −0.29**	0.51* **0.21 to 0.80**	−0.60* **−0.84 to −0.37**	0.87* **0.77 to 0.97**
Cytochrome C	–	–	–	–	–	–	–	–	0.45* **0.14 to 0.76**	0.45* **0.15 to 0.75**	−0.42* **−0.76 to −0.08**	0.14	−0.31
CD63	–	–	–	–	–	–	–	–	–	0.99* **0.97 to 1.00**	−0.92* **−1.00 to −0.83**	0.91* **0.83 to 1.00**	−0.88* **−0.98 to −0.77**
Total Cargo	–	–	––	–	–	–	–	–	–	–	−0.90* **−1.00 to −0.81**	0.92* **0.85 to 0.98**	−0.87* **−0.97 to −0.77**
Immune Index	–	–	–	–	–	–	–	–	–	–	–	−0.81* **−0.98 to −0.64**	0.79* **0.63 to 0.96**
Purity Index	–	–	–	–	–	–	–	–	–	–	–	–	−0.86* **−0.98 to −0.75**
Variable	IL-8	IL-10	IL-17 A	CD9	CD81	VLA-4	Syntetin-1	Cytochrome C	CD63	Total Cargo	Immune Index	Purity Index	Biogenesis Index

Table 5. Spearman correlation coefficients (with 95% confidence intervals shown below if significant) between biochemical variables ($n = 33$). Inside the table, Spearman correlation coefficients (rs) are presented. *: $p < 0.05$ Significant values are in bold

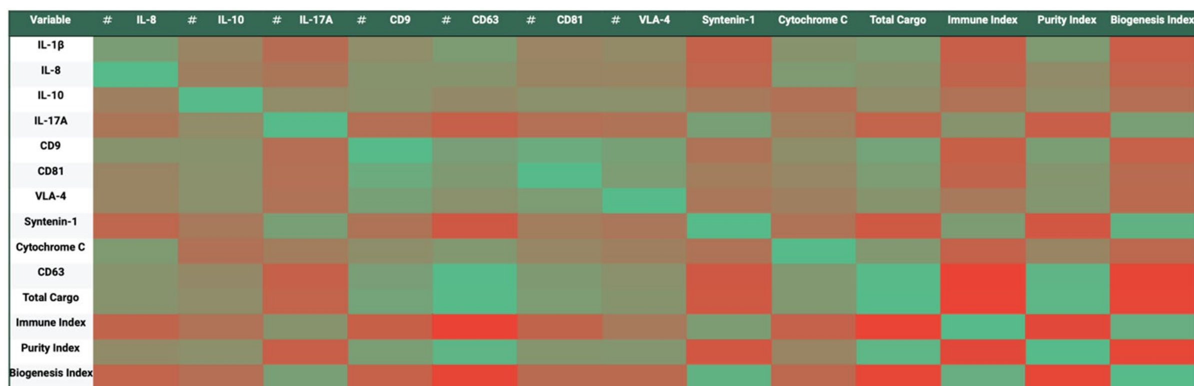

Fig. 3. Heatmap of Spearman correlations among cytokines and exosomal markers. Color intensity represents correlation coefficients ranging from − 1.0 (red, negative correlation) to + 1.0 (green, positive correlation).

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Variable	Periodontal grading (A, B, C)	Periodontal staging (I, II, III, IV)
IL-1β	0.06	−0.03
IL-8	0.21	−0.09
IL-10	−0.40* **−0.65 to −0.15**	−0.24
IL-17 A	−0.02	−0.08
CD9	−0.17	−0.17
CD81	−0.16	−0.34* **−0.64 to −0.05**
VLA-4	−0.14	−0.06
Syntetin-1	0.00	0.00
Cytochrome C	0.15	0.01
CD63	−0.08	−0.14
Total Cargo	−0.10	−0.11
Immune Index	0.01	0.14
Purity Index	−0.22	−0.15
Biogenesis Index	0.12	0.09

Table 6. Spearman correlation coefficients (with 95% confidence intervals shown below if significant), between biochemical variables and periodontal grading/staging ($n = 33$). Inside the table, Spearman correlation coefficients (rs) are presented. *: $p < 0.05$ Significant values are in bold

the predominant exosomes may originate from epithelial cells rather than activated leukocytes. In contrast, the Biogenesis Index (Syntenin/Total Cargo) decreases in subjects with greater disease severity and elevated IL-1β/IL-8, indicating a shift toward a higher proportion of vesicles originating from non-canonical pathways (Syntenin-1-independent), capable of amplifying inflammation and tissue remodeling[34]. The Immune Index (VLA-4/Total Cargo) also reflects functional alterations; its low value suggests a reduced proportion of exosomes derived from leukocytes and declines further in patients with deeper pockets. This phenomenon may be related to macrophage death processes reported in periodontitis (apoptosis, pyroptosis, necroptosis, ferroptosis, and ETosis)[35]. This observation, together with reduced IL-17 A secretion, aligns with previous findings on T cell dysfunction in periodontitis, where an exhausted state limits immune activity and, potentially, extracellular vesicle production[36].

In line with this hypothesis, our results show that CD81 is negatively associated with periodontitis stage, reinforcing the notion of an exhausted phenotype – characterized by cells with reduced effector and proliferative capacity and diminished cytokine production – typically observed following chronic inflammation[37]. Furthermore, periodontitis severity was negatively correlated with IL-10, indicating a loss of compensatory anti-inflammatory mechanisms. These findings may suggest that exosomes from epithelial sources predominate in advanced stages, reflecting pronounced immune exhaustion. This regulatory failure may partly explain the progression of periodontitis and the local inflammatory imbalance.

Although direct comparisons should be cautious, exosomal immunoprofiling in systemic inflammatory and autoimmune diseases has revealed discrete vesicular signatures associated with immune dysregulation[38]. By introducing composite indices that summarize biogenesis, immune origin and cargo load, our approach may facilitate cross-disease comparisons and the identification of shared or disease-specific vesicular patterns.

Future studies could test whether the indices here described recapitulate immunopathological axes observed in conditions such as rheumatoid arthritis or metabolic inflammation, thereby expanding their clinical applicability.

As limitations, we note that although statistical significance was achieved, a larger sample size could allow for a more detailed exosomal profiling across all stages and grades of periodontitis. Additionally, the cross-sectional design without a control group limits the ability to infer causal relationships between immune exhaustion and disease progression. Furthermore, no multivariable analysis was performed. Finally, the absence of previous studies exploring a comprehensive exosomal profile and the use of composite exosomal indices in periodontitis patients restricts the possibility of comparing and contextualizing our findings within the existing literature.

Conclusion

Composite exosomal indices reflect the balance between canonical biogenesis, immune cell-derived exosomes, and cell damage-associated vesicles in periodontitis. The negative association of CD81 and Immune Index with disease severity suggests immune exhaustion, whereas reduced IL-10 supports impaired regulatory control in advanced stages. These observations highlight the potential of exosomal markers and composite indices as tools for monitoring disease progression and guiding future research into immunomodulatory mechanisms in periodontitis.

Data availability

The data are available from the corresponding author upon reasonable request.

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Author contributions

Conceptualization : MB (corresponding), FM; Methodology : MB (corresponding), IP; Formal analysis and investigation: MB; Writing - review and editing: FM, MB (corresponding), MB, IP; Funding acquisition : FM; Resources : FM; Supervision : FM.

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Declarations

Competing interests

The authors declare no competing interests.

Ethical approval

The study was approved by the Biomedical Research Ethics Committee of the University of Granada, with the code 4566/CEIH/2024. Informed consent was obtained from all participants involved in this study, ensuring they understood the study's purpose and their right to withdraw at any time without consequence.

Consent for publication

Not applicable.

Additional information

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