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Original Article

Periodontitis Through the Window of Bioinformatics: An Integrated Network Analysis Revealing Critical Genes, Regulators, and Pathways

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Abstract

Introduction: Periodontitis is a common chronic inflammatory condition that begins with an imbalance in the subgingival microbial community, triggering a damaging host immune response. Although the role of bacteria is well known, the complete picture of the molecular drivers, especially the central genes and regulatory networks, remains incomplete. Accordingly, an integrated bioinformatics analysis was conducted to identify principal regulator genes, associated pathways, and gene ontology terms involved in periodontitis.

Methods: The transcriptomic dataset GSE223924 was reanalyzed, including 10 healthy and 10 diseased gingival tissue samples. A differential expression analysis was performed with strict parameters (false discovery rate < 0.01, |Log2FC| > 2). Subsequent analyses involved constructing a protein-protein interaction (PPI) network, constructing a gene regulatory network via the STRING database and Cytoscape with iRegulon, and performing functional annotation using the g:Profiler database.

Results: The results revealed 1,281 genes that were differentially expressed in periodontitis tissues. From a PPI network, 41 high-priority hub genes were extracted, with interleukin (IL)-6 consistently emerging as the most prominent. The investigation of transcriptional control identified nuclear factor kappa B subunit 1 (NFKB1) as the primary regulator, controlling 15 out of 41 hub genes. Pathway analysis further confirmed a strong enrichment of immune system functions, including "cytokine-cytokine receptor interaction" and "immune response".

Conclusion: Our findings confirmed IL-6 and NFKB1 as central drivers within a dysregulated immune network in periodontitis. This molecular framework deepens our understanding of the disease's pathogenesis and provides a focused list of genes for further validation as potential therapeutic targets.

Keywords: Periodontitis, Bioinformatics, Genes, Immune system, Pathogenesis

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Introduction

Periodontitis is a highly prevalent inflammatory disorder with serious economic impacts, estimated to globally affect billions in its mild form and over one billion in its severe form (1, 2). Designated a social oral disease due to its high prevalence, it affected 42% of U.S. adults in studies performed from 2009 to 2014, with a lower percentage for severe cases (3). The 2017 age-standardized prevalence of severe periodontitis was 9.8% worldwide, with trends demonstrating a decline in middle-income populations but an increase in high-income countries over the past three decades (4, 5). The disease is characterized by the irreversible destruction of the tooth's supporting tissues, the alveolar bone and periodontal ligament, resulting in tooth loss. Furthermore, periodontitis is associated with several systemic conditions and may be a predisposing factor for oral cancer (4).

While the etiology of periodontitis is initiated by the accumulation of dental plaques and colonization by pathogenic bacteria, notably the red complex, the associated tissue destruction is primarily mediated by the host's inflammatory response (6). It is worth mentioning that the susceptibility, prevalence, and progression of the disease are substantially modified by different risk factors, including diabetes mellitus, tobacco cigarette smoking, and genetic predisposition (7, 8).

The high prevalence and serious complications of periodontitis, alongside its potential to exacerbate systemic diseases, make it crucial to understand its pathomechanisms; such research is key to identifying new drug targets and developing more effective treatments (4).

This study provides valuable insights into the molecular mechanisms of periodontitis through a comprehensive bioinformatic analysis of the GSE223924 dataset (9). More precisely, it aims to identify principal regulator genes, related pathways, and gene ontology (GO) terms involved in periodontitis by performing a comprehensive bioinformatic analysis.

Differentially expressed genes (DEGs) were identified via high-throughput sequencing (GPL24676, Illumina NovaSeq 6000) by comparing gingival tissues from periodontitis patients and healthy controls. Protein-protein interaction (PPI) network, functional enrichment, and gene regulatory analyses uncovered hub genes, key pathways, and upstream transcription factors. These findings suggest candidate biomarkers and provide insights into the molecular drivers of periodontitis.

Materials and Methods

Sample Collection

This study reanalyzed the publicly available ribonucleic acid sequencing (RNA-seq) dataset GSE223924, initially generated by Oh et al (9) in their cross-sectional investigation on periodontitis and peri-implantitis. In the original study, human gingival tissue samples were obtained from 20 participants with proper ethical approval and written informed consent. The cohort included 10 healthy subjects and 10 patients presenting with both periodontitis and peri-implantitis. Overall, 30 gingival tissue samples were collected, including 10 from healthy sites, 10 from periodontitis sites, and 10 from peri-implantitis sites, with the disease status confirmed through clinical examinations.

Statistical Analysis

Dataset GSE223924 (9) was reanalyzed from the Gene Expression Omnibus (GEO) (10). The dataset had been generated on the GPL24676 platform (Illumina NovaSeq 6000). Differential expression analysis between periodontitis and healthy gingival samples ($n = 10$ each) was performed using the GEO2R online tool, which implements the limma R package for microarray and RNA-seq data analysis. Moreover, default GEO2R settings were applied, except for the significance thresholds, which were set to a false discovery rate (FDR) < 0.01 and an absolute log2 fold change ($|\log2FC|$) > 2.0 to identify robust differentially expressed genes (DEGs). A corresponding volcano plot was generated using the Shiny server (11).

Protein-Protein Interaction Network and Gene Set Enrichment Analyses

A PPI network for the DEGs was constructed using the STRING database, version 12.0 (12). In addition, a medium confidence score threshold of > 0.4 was applied to ensure the inclusion of biologically relevant interactions while maintaining network connectivity. Subsequently, the initial network was refined by removing nodes that were completely disconnected from the main interactome (i.e., proteins with no interaction partners), as these isolated proteins do not contribute to network topology or hub gene identification. Next, the processed network was loaded into Cytoscape software (version 3.10.1) for visualization and topological examination (13). Within this environment, the protein interaction network was rendered, and key network centrality metrics were calculated for each node. Afterward, the hub genes were selected by applying a threshold requiring nodes to have degree and betweenness centrality values exceeding twice the network mean, and a closeness centrality above the network's average.

To detect dense modules, the network was analyzed with the MCODE application. Significant clusters that fulfilled predefined criteria, specifically, an MCODE score of three or more and comprising at least ten genes, were retained for further investigation into their functional roles.

Additionally, the functional annotation of the pooled proteins from all clusters was conducted using the g:Profiler web tool (14) in order to identify overrepresented biological processes and signaling pathways. Moreover, the entire list of DEGs was separately analyzed using g:Profiler to assess enrichment for molecular functions and cellular components. A rigorous threshold was also applied across all enrichment analyses, with only terms with an FDR below 0.05 and annotated by at least ten genes considered statistically significant.

Cluster-Specific Functional Enrichment Analysis

To gain deeper insight into the biological significance of the most densely connected modules, separate functional enrichment analyses were performed for the top two MCODE clusters. For each cluster, the constituent genes were extracted and submitted to the g:Profiler web tool for the gene ontology biological process and pathway enrichment analysis.

Identification of Upstream Transcription Factors

A key objective of this study was to identify the key transcriptional regulators of the hub genes associated with periodontitis. To this end, the iRegulon plugin (15) for Cytoscape was utilized to predict TFs with potential regulatory control over these essential genes. The prediction was based on the normalized enrichment score (NES) calculated by the iRegulon algorithm. Further, a stringent threshold of $NES > 5$ was applied to minimize false-positive predictions and ensure the identification of only high-confidence master regulators, prioritizing specificity over sensitivity to capture the most robust transcriptional control elements in periodontitis pathogenesis.

Cross-Validation Study

An additional microarray dataset (GSE106090) was obtained from the NCBI GEO database, generated using the GPL21827 platform (Agilent-079487 Arraystar Human LncRNA microarray V4, Probe Name version). This dataset comprised 18 periodontal tissue samples, including 12 from inflamed sites and six from healthy controls. Analysis was performed using the OralExplorer online tool (available at https://smuonco.shinyapps.io/OralExplorer/). Furthermore, thresholds for DEGs were set at FDR < 0.01 and $|\log_2 FC| > 2$, consistent with the criteria applied to the primary dataset (GSE223924). Ultimately, overlapping DEGs between the two datasets were identified, along with common genes between the hub genes from GSE223924 and the DEGs from GSE106090. All intersection analyses were conducted using the InteractiVenn server (https://www.interactivenn.net/).

Results

Differentially Expressed Genes in Periodontitis

The GSE223924 dataset was reanalyzed, which contained gingival samples from 10 healthy controls, 10 periodontitis patients, and 10 peri-implantitis patients. The investigation specifically compared 10 periodontitis samples with 10 healthy controls. To identify DEGs, an analysis was conducted via GEO2R, applying a stringent FDR of < 0.01 and an absolute log2 fold change threshold of > 2. Finally, this process identified 1,281 DEGs that robustly distinguished periodontitis from health, with 1,072 being upregulated and 209 downregulated (Supplementary Data 1). The distribution and significance of these DEGs are depicted in a volcano plot (Figure 1).

Hub Genes, Clusters, and the Main Gene Ontology Categories

A network analysis was performed to study the functional interactions among periodontitis-associated genes. A PPI network was generated from the STRING database, unconnected nodes were removed, and the graph was subsequently imported into Cytoscape for further examination. The final construct contained 872 nodes that were linked by 8,683 edges. Using topological parameters, 41 hub genes were identified as those with degree and betweenness centrality values exceeding twice the network mean and demonstrating above-average closeness centrality (Table 1). The average centrality values for the entire network were calculated as 19.915 (degree), 0.005 (betweenness), and 0.329 (closeness). The interactions and comparative ranking of these key hub genes are depicted in Figure 2.

Within the PPI network, 11 distinct clusters met the threshold of an MCODE score > 3 and contained at least 10 genes each. Figure 3 displays these modules (1, 2, 3, 4, 6, 7, 9, 10, 15, 16, 18), and Table 2 outlines their specifications. Among them, Cluster 1 was the largest, comprising 52 genes and 1,098 edges, with *CCRL2* as the seed gene.

The functional enrichment analysis of the 11 gene clusters was conducted to elucidate the dysregulated signaling pathways and biological processes in periodontitis. This analysis revealed a pronounced emphasis on immune system functionality. Key pathways, such as "Cytokine-cytokine receptor interaction," "Leukocyte transendothelial migration," "Viral protein interaction with cytokine and cytokine receptor," and "Neutrophil extracellular trap formation", were highly enriched. Concurrently, the most significant biological processes were related to immune activity, including "Immune system process," "Immune response," and "Leukocyte activation". The top ten entries for both pathways and biological processes are illustrated in Figures 4a and 4b, respectively.

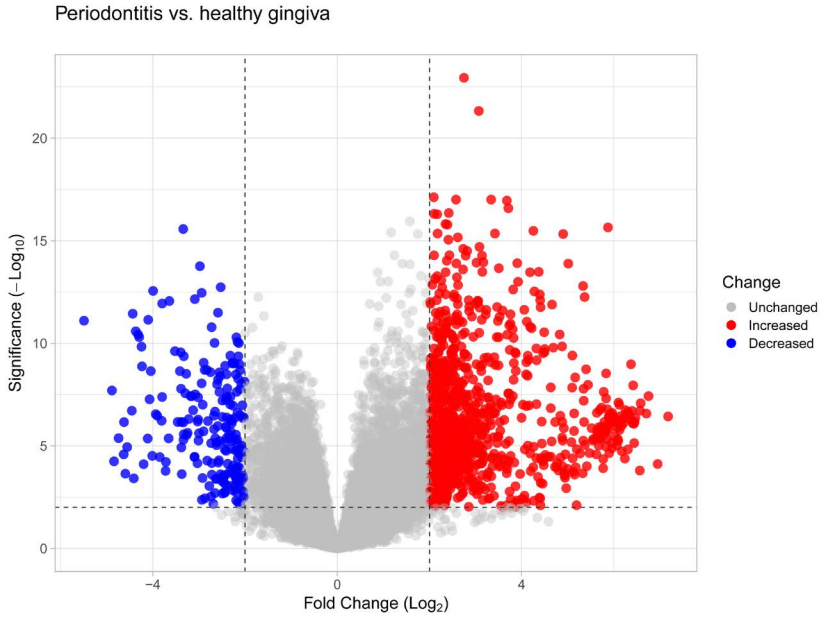

Figure 1. A Volcano Plot Representing the Transcriptomic Differences Between Periodontitis Gingiva and Healthy Subjects, Based on Differential Gene Expression Analysis

Table 1. The 41 Hub Genes Identified in the Periodontitis PPI Network, Sorted in Descending Order of Degree Centrality

Name	Degree	Betweenness	Closeness
IL6	207	0.0702	0.483
IL1B	185	0.0385	0.472
PTPRC	184	0.0419	0.462
ITGAM	163	0.0182	0.451
IL10	159	0.0179	0.451
TLR4	151	0.0277	0.455
FCGR3A	138	0.0104	0.429
FCGR3B	137	0.0166	0.433
MMP9	136	0.0318	0.449
CXCL8	135	0.0179	0.438
ITGB2	135	0.0179	0.432
ICAM1	133	0.0138	0.439
CXCR4	127	0.0140	0.438
CD19	121	0.0150	0.421
PECAM1	120	0.0310	0.444
SELL	108	0.0136	0.428
BTK	106	0.0172	0.413
CTLA4	102	0.0100	0.417
CD34	101	0.0098	0.427
APOE	96	0.0469	0.438
MMP2	96	0.0144	0.423
CD38	88	0.0112	0.410
KDR	86	0.0244	0.421
PXDN	86	0.0220	0.436
CYBB	81	0.0101	0.418
FGR	76	0.0117	0.396
ITGA4	72	0.0116	0.410
CDH5	71	0.0183	0.413
NT5E	70	0.0215	0.415
PLEK	70	0.0184	0.401
HGF	70	0.0114	0.409
CALML5	69	0.0643	0.416
PDGFRB	67	0.0109	0.414
LILRB2	62	0.0101	0.391
ITGB3	61	0.0161	0.405
LUM	60	0.0183	0.389
DCN	59	0.0146	0.396
RUNX2	57	0.0115	0.407
BLK	46	0.0121	0.356
COL4A1	45	0.0100	0.375
GPX2	40	0.0111	0.388

Note. PPI: Protein-protein interaction.

The enrichment analysis of the 1,281 DEGs was performed to identify altered molecular functions and cellular components in periodontitis. The results confirmed a strong immune signature, with significant molecular functions, including "antigen binding," "immune receptor activity," "cytokine binding," and "immunoglobulin receptor binding". Among cellular components, the "immunoglobulin complex" was identified as one of the most prominently dysregulated elements. The ten most significantly affected molecular functions and cellular components are detailed in Figures 4c and 4d, respectively.

Cluster-Specific Functional Enrichment Revealing Distinct Biological Roles

To elucidate the specific functions of the most significant modules, separate enrichment analyses were performed for the top two MCODE clusters (Figure 5). Cluster 1 was predominantly enriched for immune-related biological processes, including "immune system process", "leukocyte migration", "leukocyte activation", "inflammatory response", and "regulation of immune system process". Pathway analysis revealed enrichment for "Rheumatoid arthritis", "Interleukin-10 signaling", and "Cytokine-cytokine receptor interaction". Moreover, the top-ranked biological processes enriched by cluster 2 included "Immune system process", "Immune response", and "Response to stimulus". Based on pathway analysis, significant enrichment was observed for "Innate immune system", "Immune system", and "Hemostasis".

Upstream Transcription Factors Involved in Periodontitis

A gene regulatory network was built with the iRegulon plugin to elucidate the transcriptional regulators of the periodontitis-associated hub genes (Figure 6). The network was constructed by mapping significant TFs to their target genes. Four potential master regulators were identified using a stringent cutoff (NES > 5; Table 3). The most significant regulator was NFKB1 (NES = 6.844), which directly modulated 15 hub genes, including *MMP9*, *ICAM1*, *ITGAM*, *CXCR4*, *FGR*, *BLK*, *ITGB2*, *IL10*, *CD19*, *IL1B*, *ITGA4*, *SELL*, *PDGFRB*, *APOE*, and *PECAM1*.

Validation Results

An external cross-validation analysis was conducted using an independent periodontitis transcriptomic dataset, GSE106090, which contains gingival tissue samples from periodontitis patients and healthy controls. Additionally, the differential expression analysis of GSE106090 was performed using the same threshold criteria (FDR < 0.01, $|\log_2 FC| > 2$), and 876 DEGs were identified accordingly (Supplementary Data 2). A comparative analysis was conducted between the primary RNA-seq dataset (GSE223924) and the validation microarray dataset, revealing a substantial overlap; overall, 246 genes were commonly dysregulated in both cohorts. More importantly, of the 41 hub genes previously identified through PPI network analysis in GSE223924, *PTPRC*, *FCGR3A*, *MMP9*, *CXCL8*, *CXCR4*, *CD19*, *SELL*, *BTK*, *CTLA4*, *CD38*, *ITGA4*, *PLEK*, *CALML5*, *LILRB2*, and *GPX2* (n = 15) were also found to be differentially expressed in the independent GSE106090 dataset, further confirming their central role in periodontitis pathogenesis (Figure 7).

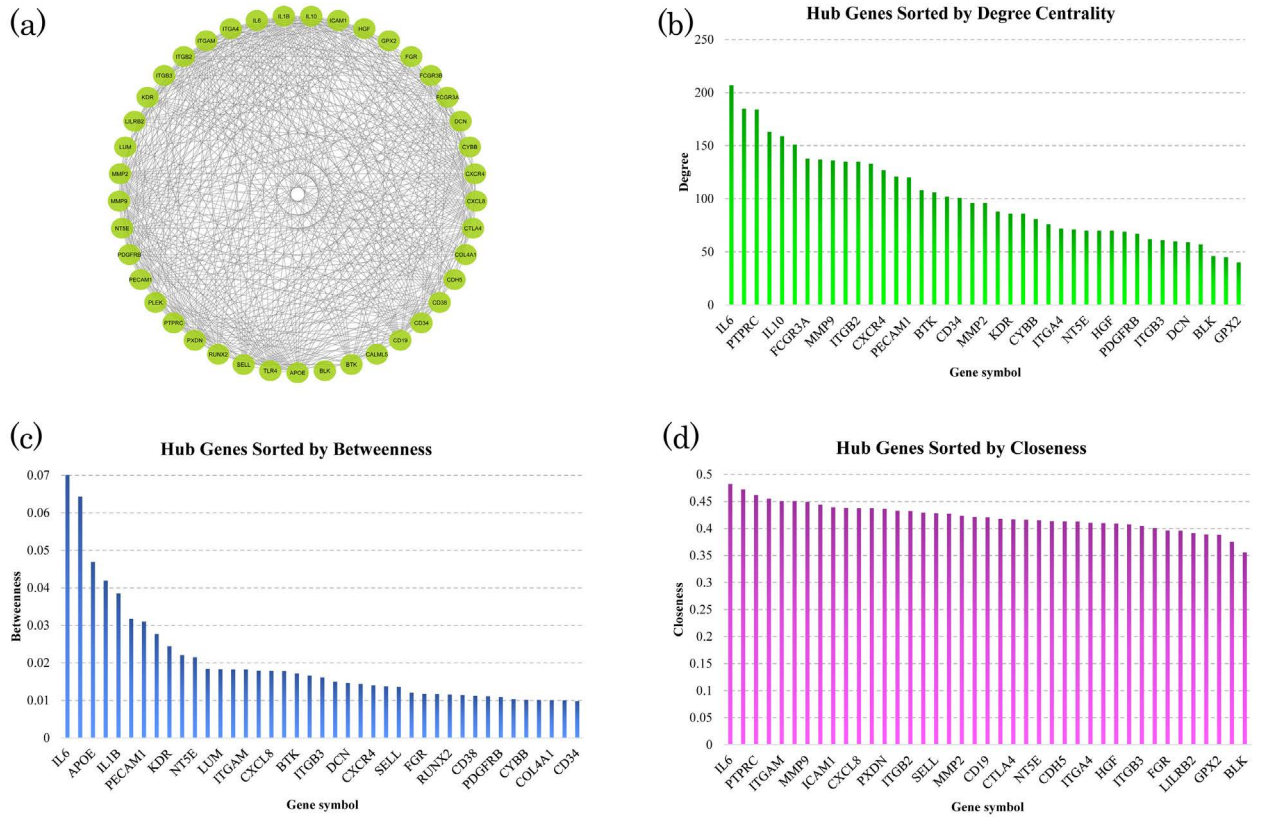

Figure 2. Visualization of Hub Gene Properties Within the Periodontitis PPI Network: **(a)** The Interaction Network and **(b-d)** The Rankings of These Genes Based on Degree (Green), Betweenness (Blue), and Closeness Centrality (Violet)
Note. The charts (b-d) plot gene names on the x-axis against their respective centrality values on the y-axis. PPI: Protein-protein interaction

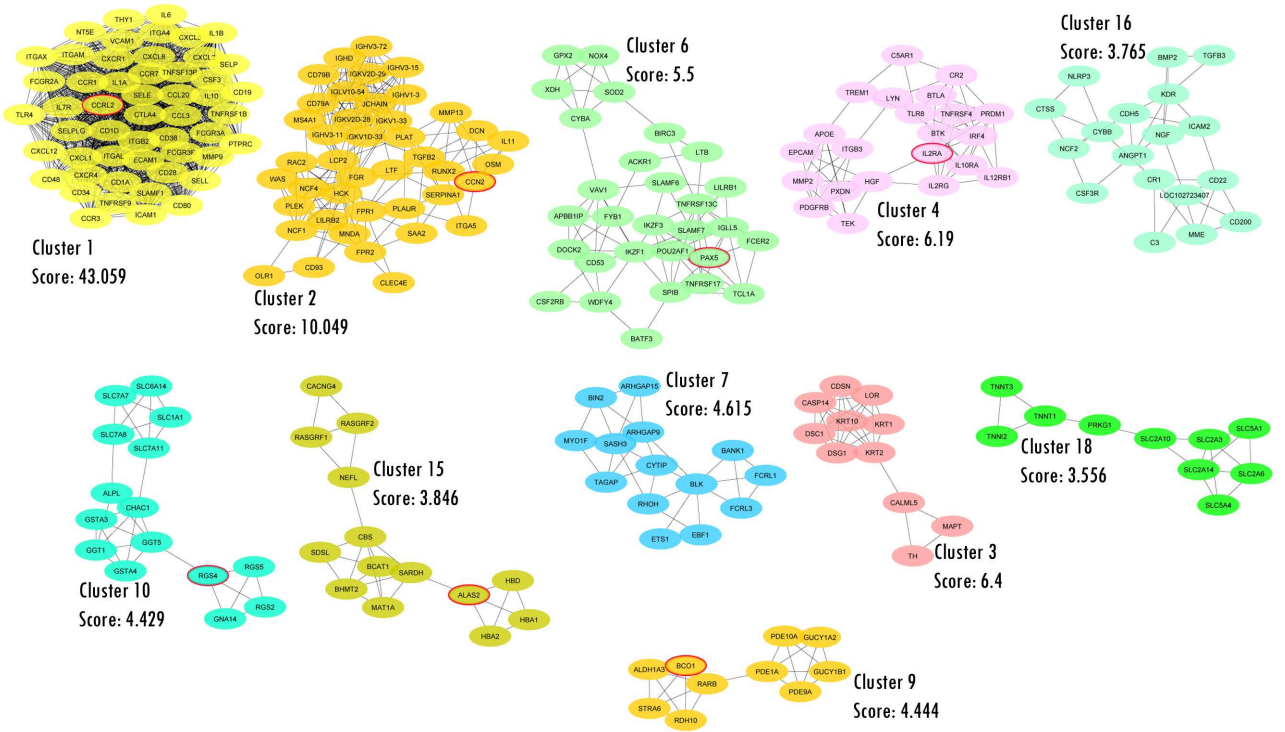

Figure 3. The 11 Significant Clusters Identified in the Periodontitis Protein-Protein Interaction Network by the MCODE Algorithm
Note. A red border visually distinguishes seed nodes. MCODE: Molecular complex detection complex

Discussion

Periodont itis, a widespread oral inflammatory condition, arises from an ecological imbalance in the subgingival biofilm. This dysbiosis triggers a persistent and damaging host immune response, ultimately leading to the degradation of periodontal tissues and the alveolar bone (16). Recent breakthroughs in microbial identification technologies have refined preventive and therapeutic

strategies that primarily target the oral microbiome (17). Despite these advances, the field has made less headway in deciphering the precise immunological drivers of the disease, and this limited understanding has stalled the effective clinical implementation of host-modulating treatments (18).

The present study employed a comprehensive bioinformatics approach to delineate core molecular mechanisms and pivotal genes involved in periodontitis. The methodology involved reanalyzing a public RNA-seq dataset (GSE223924), which identified a substantial number of genes with altered expression in diseased gingival tissues. Network analysis distilled this list to 41 hub genes, with interleukin-6 (IL-6) emerging as the top-ranked gene across all three centrality metrics. Receptor-type tyrosine-protein phosphatase C (PTPRC) ranked second in terms of degree and closeness centrality, whereas Apolipoprotein E (APOE) ranked second with regard to betweenness centrality. Moreover, the construction of a regulatory network identified nuclear factor kappa B subunit 1 (NFKB1) as a key transcriptional regulator, directly influencing 15 hub genes, including *APOE*. Concurrently, enrichment analysis demonstrated a significant overrepresentation of immune-related pathways, biological processes, molecular functions, and cellular components, strongly underscoring the central role of immune system dysregulation in the pathogenesis of periodontitis.

IL-6 is a potent pleiotropic cytokine that is not only responsible for the intense activation of B-cell antibody production (19) but also functions as a key transcriptional regulator and growth factor (20, 21). Additionally, its established link to tissue injury and chronic inflammatory diseases (22) provides a plausible mechanism for the tissue destruction observed in periodontitis, positioning our bioinformatic discovery within a well-defined pathological framework. Relvas et al (23) investigated the potential of specific cytokines as biomarkers for the severity and progression of periodontitis. They quantified a panel of immune mediators in the saliva of patients and healthy controls and reported significantly elevated levels of pro-inflammatory cytokines IL-6 and IL-1β in periodontitis,

Table 2. Key Features of the Modules Detected in the Periodontitis Protein-Protein Interaction Network

Cluster Number	Number of Nodes	Number of Edges	MCODE Score	Seed Node
1	52	1098	43.059	CCRL2
2	42	206	10.049	CCN2
3	11	32	6.4	Na
4	22	65	6.19	IL2RA
6	29	77	5.5	PAX5
7	14	30	4.615	Na
9	10	20	4.444	BCO1
10	15	31	4.429	RGS4
15	14	25	3.846	ALAS2
16	18	32	3.765	Na
18	10	16	3.556	Na

Note. Na: Not available.

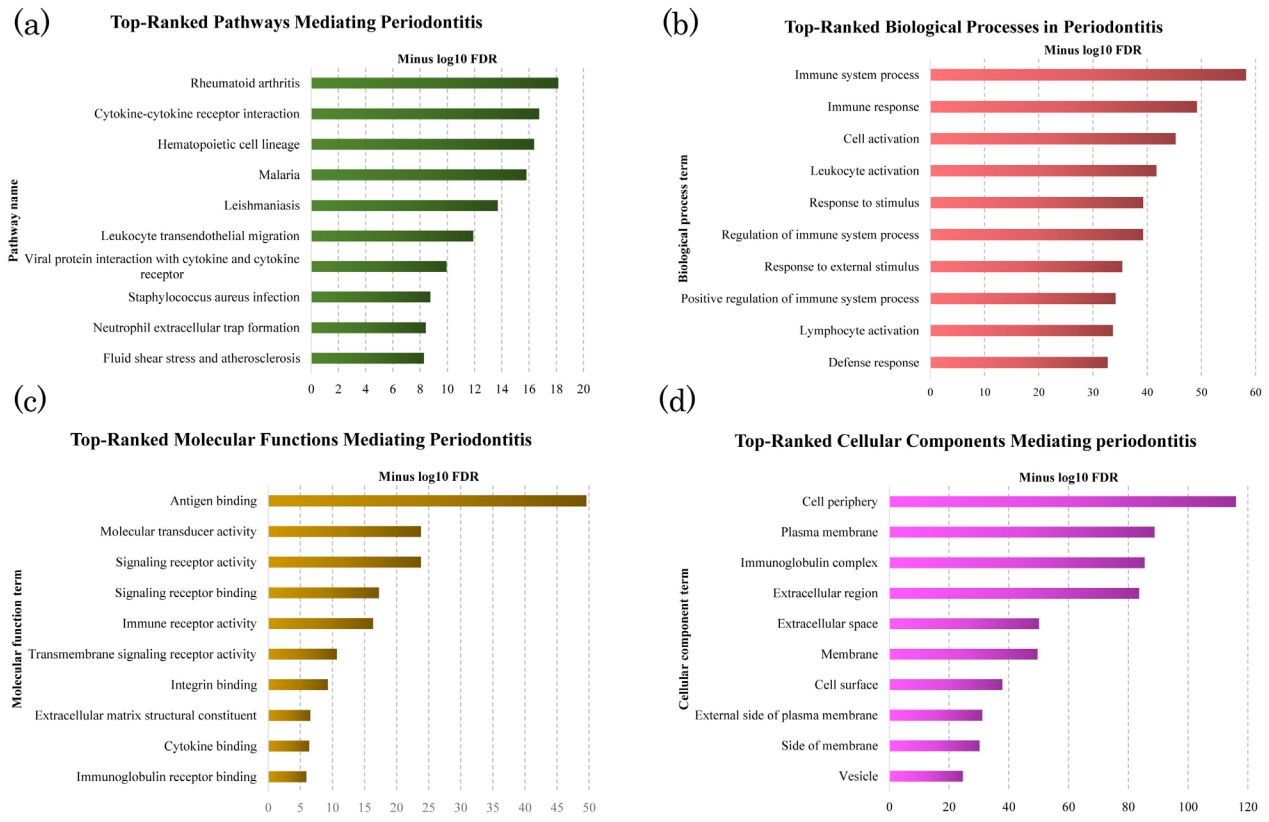

Figure 4. Functional Enrichment Analysis Results for Periodontitis-Associated Genes, Demonstrating Significant **(a)** Pathways, **(b)** Gene Ontology Biological Processes, **(c)** Gene Ontology Molecular Functions, and **(d)** Gene Ontology Cellular Components

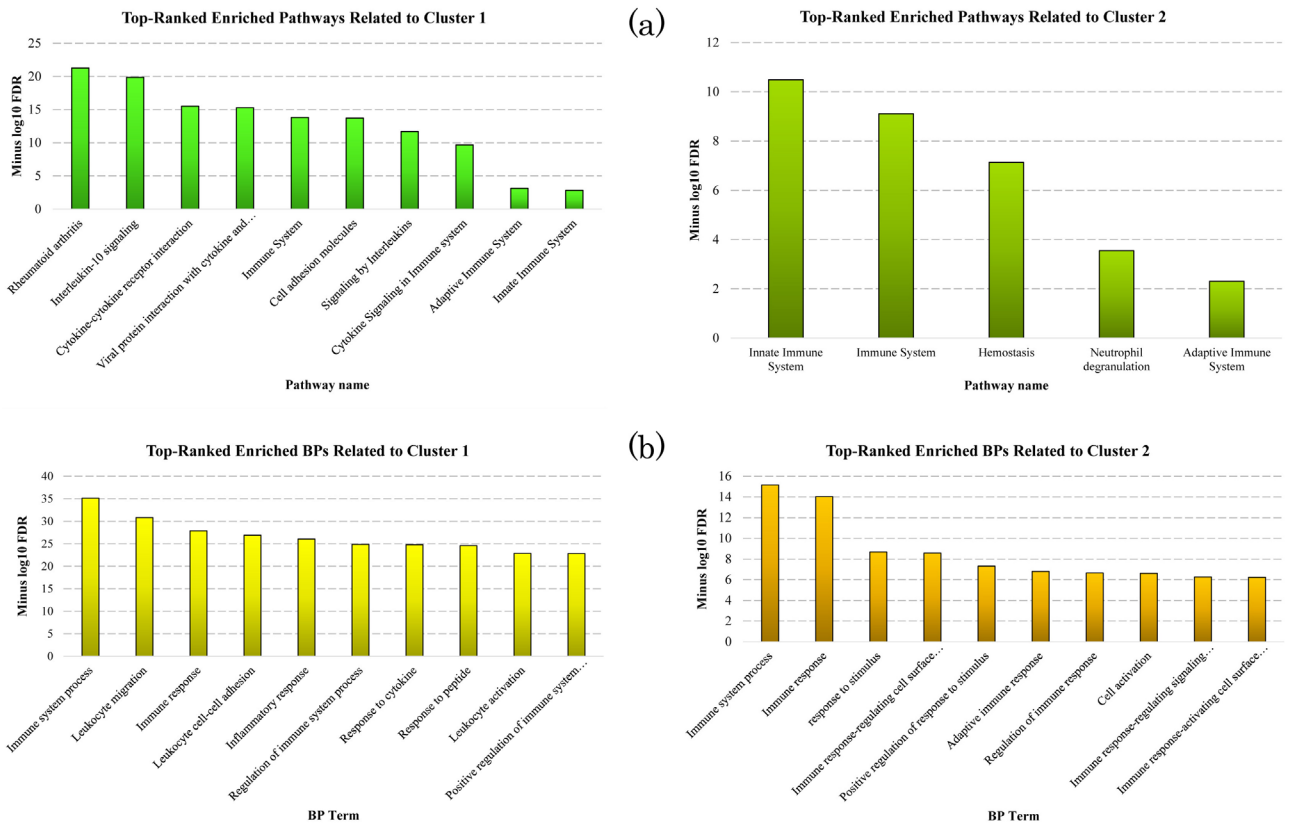

Figure 5. Top-Ranked Enriched Pathways **(a)** and Biological Processes **(b)** in Cluster 1 (Left) and Cluster 2 (Right)
Note. BP: Biological Process

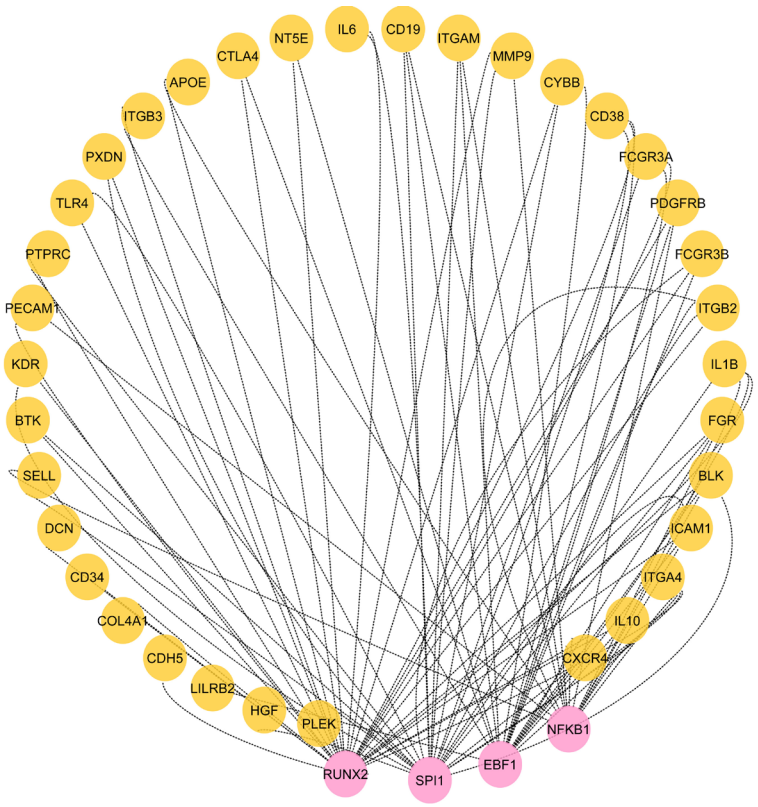

Figure 6. The Gene Regulatory Network Illustrating the Hub Genes Implicated in Periodontitis (Light Brown Nodes) and Their Corresponding Upstream Transcription Factors (Purple Nodes)

which correlate with the clinical parameters of disease severity (stage) but not with the progression rate (grade). The study also noted an increased IL-1β/IL-1RA ratio and higher levels of the anti-inflammatory cytokine IL-10 in patients, highlighting a complex immune response. Based on these findings, the researchers proposed that

Table 3. Key Transcription Factors Identified as Significant Regulators of Hub Genes in the Periodontitis Network

TF	NES	Targets
NFKB1	6.844	MMP9, ICAM1, ITGAM, CXCR4, FGR, BLK, ITGB2, IL10, CD19, IL1B, ITGA4, SELL, PDGFRB, APOE, PECAM1
SPI1	5.932	ITGA4, FGR, CYBB, PLEK, CD38, PTPRC, IL1B, MMP9, IL10, TLR4, BTK, ITGB2, BLK, CD19, CXCR4, HGF, KDR, IL6, ITGAM, ICAM1, FCGR3B, FCGR3A, SELL, PXDN
EBF1	5.618	CD19, IL10, FGR, ITGB2, ITGAM, BLK, ITGB3, CD38, PDGFRB, LILRB2, IL1B, ITGA4, CTLA4, CXCR4, NT5E, ICAM1, CYBB, FCGR3B, FCGR3A
RUNX2	5.354	RUNX2, MMP9, ITGB2, BTK, CYBB, CDH5, IL1B, KDR, CD38, FCGR3A, PDGFRB, FGR, FCGR3B, PECAM1, BLK, PTPRC, TLR4, CXCR4, COL4A1, PXDN, ITGB3, APOE, CTLA4, ICAM1, NT5E, CD34, ITGA4, IL10, IL6, DCN

Note. TF: Transcription factor; NES: Normalized enrichment score; NFKB1: Nuclear factor kappa B 1; SPI1: Spi-1 proto-oncogene; EBF1: Early B-cell factor 1; RUNX2: Runt-related transcription factor 2.

Figure 7. External Validation of Hub Genes Using an Independent Microarray Dataset: **(a)** Overlap Between DEGs From GSE223924 (n = 1281) and GSE106090 (n = 876), With 246 Genes Common to Both and **(b)** Intersection of 41 Hub Genes From GSE223924 With 876 DEGs From GSE106090, Identifying 15 Validated Hub Genes Central to Periodontitis Pathogenesis
Note. DEG: Differentially expressed gene

these salivary cytokines, particularly IL-6 and IL-1β, hold promise as biomarkers for refining diagnostic strategies.

Encoded by a gene on chromosome 19, APOE is a 34 kDa lipoprotein consisting of 299 amino acids (24). Genetic variation in the *APOE* gene is primarily characterized by three common alleles, ε2, ε3, and ε4. From these, six distinct genotypes can be formed, comprising three homozygous (ε2/ε2, ε3/ε3, and ε4/ε4) and three heterozygous (ε2/ε3, ε2/ε4, and ε3/ε4) combinations (25). Krishnamurthy et al (25) provided compelling evidence linking APOE polymorphisms to systemic inflammation, highlighting its relevance beyond neurology and cardiology. Their large-scale clinical analysis demonstrated that specific APOE genotypes correlate with distinct inflammatory and lipid profiles. Crucially, they found that individuals carrying the ε4 allele (particularly the E3/E4 and E4/E4 genotypes) exhibited remarkably elevated levels of key inflammatory markers, including high-sensitivity C-reactive protein and myeloperoxidase, compared to the common E3/E3 genotype. This established a direct genetic link between the APOE4 isoform and a pro-inflammatory state. The scholars concluded that the ε4 allele confers a heightened risk for inflammatory and metabolic disorders, positioning APOE as a critical modulator of the immune response.

Likewise, Salamone et al (26) evaluated the relationship between *APOE* gene polymorphism and bone loss in 392 healthy premenopausal, perimenopausal, and postmenopausal women with a mean follow-up of 2.5 years. They reported that perimenopausal and postmenopausal women carrying the APOE*4 allele exhibited considerably greater annualized spine bone loss (-1.75% ± 1.5%) compared to non-carriers (-0.98% ± 1.4%; $P = 0.018$). Notably, this effect was modified by hormone replacement therapy (HRT): APOE*4 carriers not on HRT showed nearly twofold higher bone loss (-2.31% ± 1.5% vs. -1.27% ± 1.3%; $P = 0.033$), whereas no significant difference was observed among HRT users. The researchers proposed several mechanisms to explain this association, including estrogen-APOE interactions and the role of APOE in vitamin K transport, which is a key nutrient for osteocalcin carboxylation and bone mineralization. These findings are particularly relevant to periodontitis, given that alveolar bone resorption is a hallmark of disease progression. Generally, the identification of *APOE* as a hub gene in the present results, coupled with its established role in systemic bone loss, suggests that APOE polymorphism may influence susceptibility to periodontal bone destruction, potentially through similar pathways involving vitamin K metabolism and estrogen-mediated regulation. This connection warrants further investigation as a possible link between periodontitis and systemic osteoporosis.

Similarly, Bank et al (27) identified genetic variation in NFKB1 as a significant risk factor for Crohn's disease and/or ulcerative colitis. In their large-scale genetic association study, a specific polymorphism in the *NFKB1* gene (rs28362491) was substantially associated with an increased risk of disorders. This finding genetically positions NFKB1, a master regulator of inflammation, as a key susceptibility factor in a major chronic inflammatory

condition. They found that a genetically determined high inflammatory response contributes to disease risk, a paradigm in which NFKB1 is centrally implicated.

Periodontitis develops when the balance between the oral microbial community and the host's local immune response is disrupted (28). This immune overactivation triggers osteoclasts, which are cells that break down the alveolar bone that supports the teeth (28). Research has shown that this process involves the dysregulation of inflammatory signaling molecules, or cytokines (29). Furthermore, immune dysregulation in periodontitis is not confined to the mouth; it can fuel systemic inflammation (30). Consequently, a significant focus of periodontitis research involves understanding these critical alterations in host immunity (31).

Moreover, He et al (31) provided evidence that a significant disruption of the local immune landscape characterizes periodontitis. Their analysis revealed that periodontitis tissues exhibit a distinct immune cell infiltration profile, which is characterized by the broad suppression of multiple immune cell types. This finding indicates that an immunosuppressive microenvironment, rather than solely hyper-inflammation, is a key feature of the disease. Further, their network analysis identified key TFs, such as early growth response 1 and ETS proto-oncogene 1, which appear to regulate the expression of immune-related genes contributing to this suppressed state. The findings of the study confirmed that the pathogenesis of periodontitis involves a significant immune imbalance, providing a new perspective that moves beyond a purely clinical diagnosis toward understanding the underlying molecular and cellular dysregulation.

PPI network analysis further identified *IL1B*, *TLR4*, *PTPRC*, and *MMP9* as the key genes involved in periodontitis pathogenesis. This finding aligns with and is further supported by existing evidence from genetic and biomarker studies. For instance, de Alencar et al (32) presented compelling evidence strengthening the association between *IL1B* and periodontitis susceptibility while expanding the genetic landscape to include the broader inflammasome pathway. In their well-powered case-control study, the IL1B -511 T/T genotype was significantly more frequent in patients with periodontitis compared to controls. Moreover, Jin et al (33) performed a meta-analysis of *TLR4* polymorphisms, finding that the *TLR4* C > G (rs7873784) allele was remarkably associated with chronic periodontitis in Asian populations. Beyond genetic susceptibility, Kim et al (34) demonstrated that salivary MMP-9 and S100A8 proteins are substantially associated with periodontitis and display high diagnostic power when combined, achieving an excellent screening accuracy of 0.86. Notably, while both markers decreased following non-surgical treatment, S100A8 showed a greater reduction rate (83.7%) than MMP-9 (23.5%), suggesting that S100A8 may be a more sensitive indicator of treatment response. Additionally, Zhao et al (35) offered a novel perspective by uncovering shared molecular mechanisms between periodontitis and rheumatoid arthritis using weighted gene co-expression network analysis. Among 154 common DEGs, *PTPRC* emerged as a central hub, with significantly elevated expression in periodontitis patients compared to controls. Functional validation further revealed that the overexpression of *PTPRC* promotes fibroblast proliferation, migration, and invasion.

This study is subject to several limitations. The analysis relied on a single, publicly available RNA-seq dataset (GSE223924). While this provided a robust foundation for our bioinformatic exploration, the findings require validation in independent, larger patient cohorts to ensure generalizability. Finally, the *in silico* nature of this work, while powerful for generating hypotheses, necessitates subsequent *in vitro* and *in vivo* functional studies to experimentally confirm the roles of the identified hub genes (e.g., *IL6* and *APOE*) and the regulatory influence of NFKB1 in periodontitis pathogenesis.

Conclusion

This bioinformatics study successfully uncovered the molecular foundation of periodontitis, identifying 1,281 DEGs. From this set, a PPI network analysis pinpointed 41 high-confidence hub genes. In addition, the prominence of top-ranked genes, such as *IL6*, *PTPRC*, and *APOE*, underscores a central theme of aberrant immune and inflammatory signaling. The finding that NFKB1 is a key transcriptional regulator, supported by enrichment analyses that highlight immune pathways, confirmed that immunoinflammatory disruption is fundamental to the disease. Overall, these results offer a valuable set of potential therapeutic targets and highlight the promise of future host-directed treatments for periodontitis.

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Authors' Contribution

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Competing Interests

The authors declare that they have no competing interests.

Consent for Publication

Not applicable.

Data Availability Statement

The datasets used and/or analyzed during the current study are available upon reasonable request from the corresponding author.

Ethical Approval

The present study was approved by the Ethics Committee of Hamadan University of Medical Sciences, Hamadan, Iran (ethical No. IR.UMSHA.REC.1403.880).

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Supplementary Files

Supplementary Data 1

In general, 1,281 genes were identified as differentially expressed in periodontitis gingival tissues relative to healthy controls, based on an analysis threshold of FDR < 0.01 and |log2FC| > 2.

Supplementary Data 2

List of 876 Differentially Expressed Genes Identified in the External Validation Dataset GSE106090

The analysis was performed using the OralExplorer server with threshold criteria of FDR < 0.01 and $|\log_2 FC| > 2$.

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