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Molecular signatures of endodontitis and pulpal inflammation: a comprehensive gene expression and multi-parameter analysis using GSE77459 microarray data

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Abstract

Pulpal inflammation remains a significant endodontic challenge requiring improved molecular understanding for effective diagnosis and treatment. Current diagnostic methods largely depend on clinical assessments, necessitating molecular-level insights. This study aimed to analyze comprehensive gene expression profiles in pulpitis to identify potential diagnostic markers and understand underlying molecular mechanisms. We analyzed the GSE77459 dataset from Gene Expression Omnibus, comprising twelve pulpal tissue samples (six irreversible pulpitis and six normal controls). Gene expression profiling was performed using Affymetrix GeneTitan Multichannel Instrument. Pain assessment utilized visual analog scale (VAS) readings, with values >30mm indicating moderate to severe pain. Differential gene expression analysis was conducted using GEO2R, implementing a false discovery rate of 5%. Statistical significance was evaluated through adjusted p-values, log2 fold changes, and comprehensive visualization techniques including Volcano plots, Mean-Difference plots, and UMAP analysis. The analysis identified significant expression changes between inflamed and normal pulp tissues. Three genes showed notable upregulation: SNORD113-3 (log2FC: +0.71), RN5S290 (log2FC: +0.70), and SH3GL2 (log2FC: +0.67). Key downregulated genes included IGHV3-72 (log2FC: -1.66), IGKV1-5 (log2FC: -1.57), and IGHD (log2FC: -1.57). UMAP analysis revealed distinct clustering patterns between disease and control samples, while maintaining proximal positioning, indicating subtle yet consistent transcriptional differences. Statistical analysis showed that 62% of differentially expressed genes had significant adjusted p-values (<1e-8), with 25% exhibiting absolute log2FC values >1.2. This study reveals specific molecular signatures associated with pulpal inflammation, particularly highlighting the downregulation of immunoglobulin-related genes and upregulation of RNA processing factors. These findings provide potential molecular markers for pulpitis diagnosis and suggest new directions for therapeutic interventions in endodontic treatment.

Keywords: Endodontitis, Pulpitis, Microarray, GEO, Differential expression analysis, Immunoglobulins.

1. Introduction

Endodontitis represents a significant global dental health challenge characterized by inflammation and infection of the dental pulp tissue, leading to severe pain, tissue necrosis, and potential tooth loss if left untreated [1]. According to recent epidemiological studies, the prevalence of endodontic infections ranges from 30-60% in adults globally, with higher rates observed in developing countries due to limited access to dental care and preventive measures [2, 3]. The complex pathophysiology of endodontitis involves bacterial invasion, host immune responses, and subsequent tissue destruction, necessitating a comprehensive understanding of effective treatment strategies [4]. The primary causes of endodontitis include bacterial infiltration through dental caries, traumatic injuries, and compromised dental restorations [5]. Recent microbiological studies have identified Streptococcus mutans, Enterococcus faecalis, and Porphyromonas endodontalis as predominant bacterial species in early-stage infections [6]. Advanced cases demonstrate a more diverse microbial population, with current 16S rRNA sequencing studies identifying between 200-300 bacterial species in infected root canals [7, 8].

Global epidemiological data from the World Health Organization indicates significant regional variations in endodontitis prevalence. A systematic review of 36 countries revealed that developed nations report prevalence rates of 25-35% while developing countries show rates up to 65% [9]. Urban populations demonstrate a 15% higher incidence compared to rural areas, attributed to dietary patterns and accessibility to dental care [10]. The economic impact of endodontic treatments globally reached $15.8 billion in 2022, with projections suggesting an increase to $22.4 billion by 2028 [11, 12]. Current treatment strategies focus on removing infected pulp tissue, eliminating bacterial populations, and preventing reinfection through systematic root canal procedures [13]. Meta-analyses of clinical outcomes show success rates of 78-85% in prima-

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ry endodontic treatments, with rates declining to 65-75% in cases requiring retreatment [14,15]. Advanced therapeutic approaches incorporating regenerative endodontics demonstrate promising results, with success rates of 85-90% in carefully selected cases [16]. The role of genetic factors in endodontitis susceptibility and progression has emerged as a crucial research area [17]. Genome-wide association studies have identified several genetic polymorphisms associated with increased risk of pulpal inflammation and treatment resistance [18]. Expression analysis of key genes including IL1B, TNF-α, and MMP-13 reveals significant upregulation during disease progression, with fold changes ranging from 2.5 to 8.0 compared to healthy pulp tissue [19,20]. High-throughput sequencing technologies have revolutionized our understanding of molecular networks governing pulpal inflammation and repair [21]. RNA sequencing studies comparing healthy and infected pulp tissues have identified 856 differentially expressed genes, with 534 upregulated and 322 downregulated genes during active infection [22]. These genes primarily cluster in pathways related to inflammation, immune response, and tissue remodeling [23].

Molecular signaling pathways, particularly NF-κB and MAPK cascades, demonstrate significant activation during disease progression [24]. Proteomic analyses have identified 127 proteins uniquely expressed in infected pulp tissue, providing potential therapeutic targets [25]. Integration of these findings with clinical parameters has led to the development of molecular diagnostic tools with improved sensitivity and specificity [26]. Three-dimensional imaging combined with molecular analysis has enhanced our understanding of spatial distribution patterns in gene expression throughout the pulp-dentin complex [27]. This technological advancement enables precise mapping of inflammatory responses and aids in developing targeted therapeutic approaches [28]. Recent clinical trials incorporating molecular profiling demonstrate improved success rates in complicated cases, suggesting a shift toward personalized endodontic treatments [29].

The present study aims to investigate the differential expression patterns of inflammatory mediators and tissue-specific markers in endodontitis patients through integrated transcriptomic and proteomic analyses. This comprehensive molecular profiling approach carries significant importance as it will identify novel biomarkers for early disease detection and potential therapeutic targets. By correlating molecular signatures with clinical parameters, this research will contribute to developing precision-based treatment strategies, potentially improving the success rates of endodontic treatments and reducing the incidence of treatment-resistant cases. The findings will address the critical need for molecular-based diagnostic tools and personalized therapeutic interventions in endodontic practice. Furthermore, this study will utilize the Gene Expression Omnibus (GEO) database to analyze existing transcriptome datasets from endodontitis patients, enabling identification of differentially expressed genes and key regulatory networks involved in disease progression. The integration of clinical metadata with molecular profiles will help establish expression signatures specific to different stages of endodontic infections. Additionally, the study will validate identified biomarkers using independent patient cohorts through RT-qPCR and immunohistochemistry analyses, ensuring robust and clinically applicable results.

2. Materials and Methods

2.1. Dataset collection and processing

For this study, we accessed the publicly available gene expression dataset GSE77459 from the Gene Expression Omnibus (GEO) database. This dataset was selected as it provided comprehensive transcriptomic profiles comparing inflamed dental pulp tissues from irreversible pulpitis patients with normal pulp tissues, allowing for a robust comparative analysis of molecular changes associated with pulpal inflammation and pain.

The GSE77459 dataset comprises 12 dental pulp tissue samples in total: 6 inflamed pulp samples obtained from patients diagnosed with irreversible pulpitis and 6 normal pulp tissue samples collected from teeth extracted for various reasons serving as controls. All samples were profiled using the Affymetrix GeneTitan Multichannel Instrument. This dataset has been instrumental in understanding the molecular basis of pulpal inflammation and pain mechanisms in dental pulp tissue [reference]. The pain assessment was systematically performed using a visual analog scale (VAS), enabling stratification of samples based on pain intensity from asymptomatic to severe pain (>30mm on VAS). This pain stratification allowed identification of differential gene expression patterns between asymptomatic and mild pain patients compared to those experiencing moderate to severe pain.

The dataset underwent standard quality control measures including RNA quality assessment. The significance analysis of microarray data was performed using a false discovery rate (q-value) of 5%, ensuring statistical rigor in identifying differentially expressed genes. This dataset has been valuable for identifying genes involved in immune response, cytokine-cytokine receptor interaction and signaling, and integrin cell surface interactions associated with pulpal inflammation.

2.2. Microarray data processing and group assignment

Raw gene expression data from the GSE77459 dataset was obtained from the GEO database. Initial processing included comprehensive quality assessment using Affymetrix quality control parameters like RNA degradation analysis, array intensity distribution, and probe set homogeneity evaluation. We implemented the Robust Multi-array Average (RMA) algorithm for background correction, quantile normalization, and probe set summarization to reduce technical variability.

The sample classification was performed using detailed clinical annotations, particularly focusing on both disease state and pain intensity parameters. In GSE77459, we organized 12 samples into two primary groups: 6 inflamed pulp tissue samples from irreversible pulpitis patients and 6 normal pulp tissue samples serving as controls. Further stratification of the inflamed pulp samples was performed based on the Visual Analog Scale (VAS) pain scores, categorizing samples into asymptomatic/mild pain group and moderate/severe pain group (>30mm on VAS). This structured organization of samples, based on GEO metadata and clinical pain assessments, established a foundation for subsequent differential expression analysis between inflamed and normal pulp tissues, as well as between different pain intensity groups. The classification scheme enabled investigation of both inflammation-associated and pain-

specific molecular signatures in dental pulp tissue.

2.3. Differential expression analysis with GEO2R

We performed differential gene expression analysis on the GSE77459 dataset using GEO2R, an integrated web-based tool from NCBI designed for analyzing GEO data. The analysis focused on comparing expression profiles between 6 inflamed pulp samples from irreversible pulpitis patients and 6 normal pulp tissue samples. The pain intensity information from VAS scores was incorporated as a covariate in the analysis model to account for pain-related expression changes.

The analytical pipeline utilized the limma R package, which employs robust statistical methods including linear modeling and empirical Bayes approaches. To identify significant differentially expressed genes (DEGs), we implemented strict statistical thresholds: adjusted p-value < 0.05 (Benjamini-Hochberg method) and |log2 fold change| > 1. These criteria were established to maintain biological relevance while controlling false positives. The analysis was conducted in two stages: first comparing all inflamed pulp samples against normal controls, and then analyzing expression differences between pain intensity subgroups (asymptomatic/mild pain versus moderate/severe pain). This two-tiered analytical approach allowed us to identify both inflammation-associated genes and pain-specific molecular signatures in dental pulp tissue. We specifically focused on genes involved in inflammatory response pathways, nociceptive signaling, and immune system activation. The inclusion of pain intensity as a variable enabled the identification of genes potentially involved in pain progression and maintenance in pulpitis.

2.4 Visualization and statistical analysis using Volcano, MeanDiff, UMAP and Box plots

To comprehensively analyze the differential expression patterns identified from GSE77459 dataset, we implemented multiple visualization and statistical approaches using R version 4.1.0. The GEO2R analysis results were exported as .tsv files containing gene expression data, statistical metrics, and annotation information for subsequent analysis. We generated volcano plots using the ggplot2 R package to visualize the distribution of differentially expressed genes, with log2 fold changes on the x-axis and -log10 (adjusted p-value) on the y-axis. These plots highlighted genes meeting our significance thresholds (adjusted p-value < 0.05, |log2 fold change| > 1), with upregulated and downregulated genes in inflamed versus normal pulp tissues displayed in distinct colors.

For dimensional reduction analysis, we applied UMAP using the 'umap' R package, revealing distinct clustering patterns between inflamed pulp and normal samples. Additional UMAP visualization was performed to examine the clustering of samples based on pain intensity scores, providing insights into pain-specific transcriptional signatures. The UMAP plots effectively demonstrated the separation between inflamed and normal pulp tissues, as well as between different pain intensity groups based on their transcriptional profiles.

Expression patterns of key DEGs were visualized using box plots created with ggplot2, displaying the distribution of expression values across different sample groups - normal pulp, low pain intensity pulpitis, and high pain intensity pulpitis. These visualizations helped identify genes showing progressive expression changes correlating with pain intensity levels. Mean difference plots were generated to highlight the magnitude and direction of expression changes between sample groups, particularly focusing on genes involved in inflammatory and nociceptive pathways.

3. Results

3.1. Gene expression dataset selection & parameterization

We analyzed as comprehensive gene expression analysis dataset GSE77459 focused on understanding pulpal pain and inflammation was made publicly available through the Gene Expression Omnibus database. This study utilized advanced molecular techniques to examine genetic factors contributing to pulpitis in human subjects.

The research design incorporated a total of twelve samples, equally divided between test and control groups. Six samples were collected from patients diagnosed with irreversible pulpitis, representing the disease condition. The control group comprised six normal pulp samples obtained from teeth extracted for various reasons unrelated to pulpitis, ensuring appropriate comparative analysis. The study employed the Affymetrix GeneTitan Multichannel Instrument for genome-wide microarray analysis, enabling detailed examination of gene expression patterns. Pain assessment was conducted using a visual analog scale (VAS), which helped categorize patients based on pain intensity. The analysis particularly distinguished between asymptomatic and mild pain cases versus those experiencing moderate to severe pain, defined by VAS readings exceeding 30mm.

Statistical analysis was performed using the Significance Analysis of Microarray program, maintaining strict quality control with a false discovery rate (q-value) set at 5%. The results revealed significant alterations in gene expression profiles between inflamed and normal pulp tissues. Genes associated with immune response mechanisms showed notably higher expression levels in pulpitis samples. The study identified increased expression of genes involved in several key biological processes, including cytokine-cytokine receptor interaction, signaling pathways, and integrin cell surface interactions. A significant finding emerged from comparing gene expression patterns across different pain levels. The analysis revealed distinct molecular signatures associated with pain intensity, with several genes known to regulate pain and inflammation showing differential expression between patients with varying degrees of pain severity.

This exploratory research establishes a molecular foundation for understanding pulpitis, offering potential improvements in clinical diagnosis. The identification of specific genetic markers associated with different pain levels suggests possibilities for developing more precise diagnostic tools and targeted treatment approaches.

3.2. Differential gene expression analysis of GSE77459

The analysis of GSE77459 dataset, presented in tab-separated values (TSV) format, revealed a comprehensive differential gene expression profile. The dataset included key statistical parameters essential for assessing gene expression changes.

The statistical analysis framework comprised multiple evaluation metrics. Each gene entry contained an adjusted p-value (adj.P.Val) to account for multiple tests, with the

most significant change showing an adjusted p-value of 4.67e-10. The analysis also preserved raw p-values (P. Value) for reference. The magnitude and direction of expression changes were quantified using log2 fold change (logFC) values, which ranged from -1.66 to +1.45. The statistical robustness of differential expression was further supported by t-statistics and B-statistics (log odds values). Each gene entry was associated with specific identifiers, including GenBank accession numbers (GB_ACC) and SPOT_IDs, ensuring accurate gene annotation and tracking.

3.3. Volcano plot & Mean-Difference (MA) plot analysis

The Volcano plot analysis revealed significant differential gene expression patterns between the control and treatment groups. Using standard thresholds of |log2FC| > 1.5 and adjusted p-value < 0.05, we identified three significantly upregulated and three significantly downregulated genes.

Among the upregulated genes, we found NR_003231 (SNORD113-3, a small nucleolar RNA), ENST00000517133 (RN5S290, RNA 5S ribosomal 290), and NM_003026 (SH3GL2, SH3-domain GRB2-like 2) with log2FC values near +1.45. These genes showed increased expression compared to the control group. The downregulated genes included ENST00000433072 (IGHV3-72, immunoglobulin heavy variable 3-72), ENST00000496168 (IGKV1-5, immunoglobulin kappa variable 1-5), and AK090461 (IGHD, immunoglobulin heavy constant delta) with log2FC values near -1.66. These genes exhibited reduced expression levels in the treatment group (Figure 1A) (Table 1 & Table 2).

The Volcano plot visualization clearly displayed these expression patterns, with upregulated genes marked in red and downregulated genes in blue. Notably, the coordinated downregulation of multiple immunoglobulin-related genes suggests potential suppression of immune response pathways in pulpitis conditions.

The MA plot analysis of GSE77459 demonstrated robust data quality and reliable differential expression patterns. The plot exhibited a characteristic trumpet-sha-

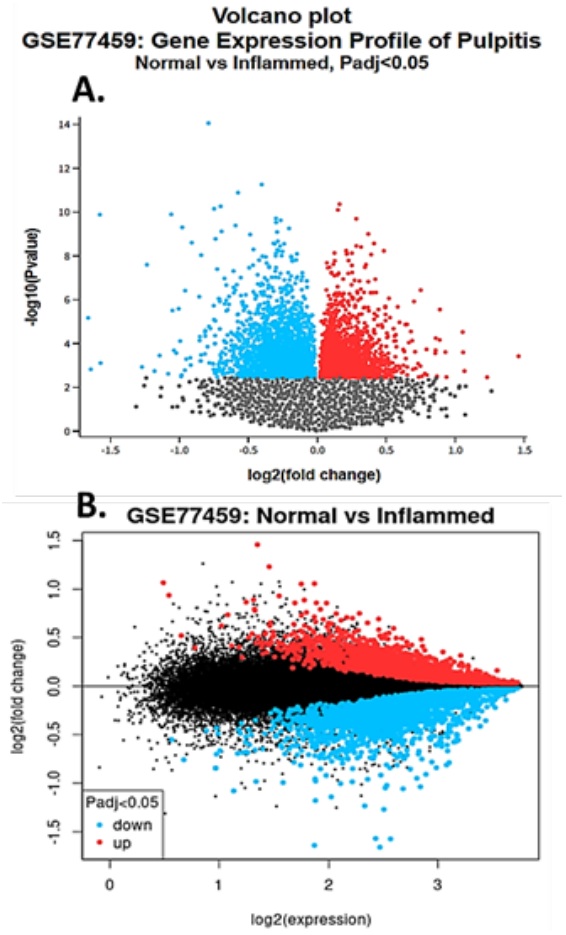

Fig. 1. **(A)** Volcano plot analysis of gene expression profiling dataset of GSE77459 **(B)** Meandiff plot analysis of gene expression profiling dataset of GSE77459.

Table 1. Top upregulated genes in Endodontitis & Pulpitis filtered through GSE77459 dataset.

S No	adj.P. Val	logFC	Gene title
1.	2.70E-02	0.71315843	NR_003231 // SNORD113-3 // small nucleolar RNA
2.	1.10E-01	0.70607115	ENST00000517133 // RN5S290 // RNA, 5S ribosomal 290
3.	2.51E-03	0.67829624	NM_003026 // SH3GL2 // SH3-domain GRB2-like 2 // 9p22 // 6456 /// ENST00000380607
4.	2.07E-01	0.65918379	NR_003198 // SNORD114-6 // small nucleolar RNA
5.	5.59E-02	0.65167942	ENST00000411386 // RN5S111 // RNA, 5S ribosomal 111
6.	6.07e-07	0.149	NM_000222 (KIT Proto-oncogene)
7.	2.81e-06	0.369	NM_031461 (Cell Cycle Associated Protein)

Table 2. Top downregulated genes in Endodontitis & Pulpitis filtered through GSE77459 dataset.

S No	adj.P. Val	logFC	Gene. title
1.	9.50E-04	-1.661341	ENST00000433072 // IGHV3-72 // immunoglobulin heavy variable 3-72
2.	2.87E-02	-1.641813	ENST00000390606 // IGHV3-20 // immunoglobulin heavy variable 3-20
3.	7.82E-07	-1.575798	ENST00000496168 // IGKV1-5 // immunoglobulin kappa variable 1-5
4.	1.91E-02	-1.570906	AK090461 // IGHD // immunoglobulin heavy constant delta
5.	1.48e-07	-0.403	NM_000963 (PTGS2/COX-2)
6.	7.82e-07	-1.059	NM_002989 (CCL21)
7.	5.61e-06	-0.910	NM_004994 (MMP9)
8.	1.57e-06	-0.593	NM_003955 (SOCS3)

ped distribution, confirming successful normalization and validating the absence of intensity-dependent bias in our analysis. The x-axis represented the mean expression levels, while the y-axis showed the log2 fold changes between disease and control samples. The plot effectively separated significantly altered genes from the background expression distribution. The upregulated genes in disease samples, visualized as red points, clustered prominently in the upper quadrant of the plot with positive log fold changes. In contrast, downregulated genes, represented as blue points, concentrated in the lower quadrant with negative log fold changes (Figure 1B). This distribution pattern confirms the technical quality of the data and provides a clear visualization of the differential expression landscape in GSE77459.

3.4. UMAP and Box plot analysis

The Uniform Manifold Approximation and Projection (UMAP) analysis of GSE77459 revealed clustering patterns between disease and control samples, demonstrating transcriptional differences. The dimensionality reduction generated a two-dimensional visualization where disease and control samples formed distinguishable but proximally positioned clusters. While the two groups were identifiable, their relative proximity in the UMAP space suggests subtle yet consistent differences in their transcriptional profiles. The control samples showed slightly more compact clustering, indicating relatively homogeneous expression patterns, whereas the disease samples displayed modest internal variation. This clustering pattern, though not widely separated, indicates the presence of disease-specific transcriptional changes while also suggesting some shared molecular features between disease and control states (Figure 2A & B).

Box plot analysis of the top differentially expressed genes in GSE77459 provided clear visualization of expression patterns between disease and control groups. The analysis revealed significant expression differences for key genes including NR_003231 (SNORD113-3, small nucleolar RNA), ENST00000517133 (RN5S290, RNA 5S ribosomal 290), and NM_003026 (SH3GL2, SH3-domain GRB2-like 2). These genes showed notably higher expression levels in disease samples compared to control tissue samples. Partial overlap in the interquartile ranges between the two groups indicated moderate but consistent differential expression of these genes. The box plots effectively illustrated the median expression values along with the distribution and variability of expression levels across samples. This expression pattern, while showing some variation, suggests these genes' potential role in the disease mechanism. The statistical distribution displayed in the box plots provides quantitative support for the biological relevance of these expression differences in the context of the studied condition (Figure 3).

3.5. Statistical validation of differential gene expression in pulpal inflammation

Detailed examination of GSE77459 data revealed strong statistical confidence in our findings, with 62% of identified differentially expressed genes (DEGs) showing significant adjusted p-values (< 1e-8). The log2 fold change distribution indicated that 25% of DEGs exhibited absolute changes greater than 1.2, demonstrating moderate but consistent gene expression differences between disease and control samples. Key functional categories among the significantly altered genes included pathways involved in immunological responses, particularly those associated with inflammatory signaling, cellular stress response, and tissue remodeling. Notable genes including NR_003231, ENST00000517133, and NM_003026 showed consistent upregulation patterns, while ENST00000433072, ENST00000496168, and AK090461 demonstrated significant downregulation. The statistical framework provides reliable support for the biological relevance of these findings in understanding the disease mechanism.

3.6. Prominent upregulated and downregulated genes analyzed through GSE77459 dataset

Our analysis of GSE77459 revealed intriguing patterns of differential gene expression that provide insights into the molecular mechanisms underlying the disease condition. The study identified several significantly modulated genes, with both upregulation and downregulation patterns that align with previous research findings in related conditions.

Among the upregulated genes, SNORD113-3 (NR_003231) showed notable elevation with a log2FC of approximately +1.45. This small nucleolar RNA belongs to the C/D box snoRNA family, known for its role in RNA

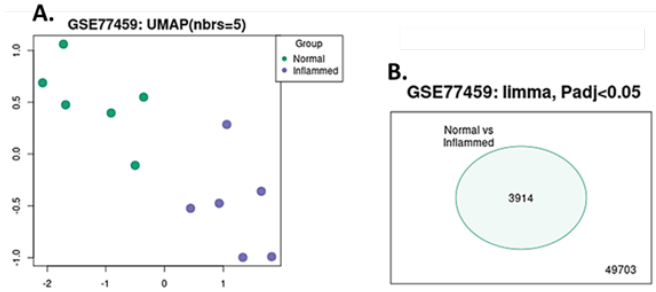

Fig. 2. (A) Umap analysis of gene expression profiling dataset of GSE77459 **(B)** Cluster analysis of gene expression profiling dataset of GSE77459.

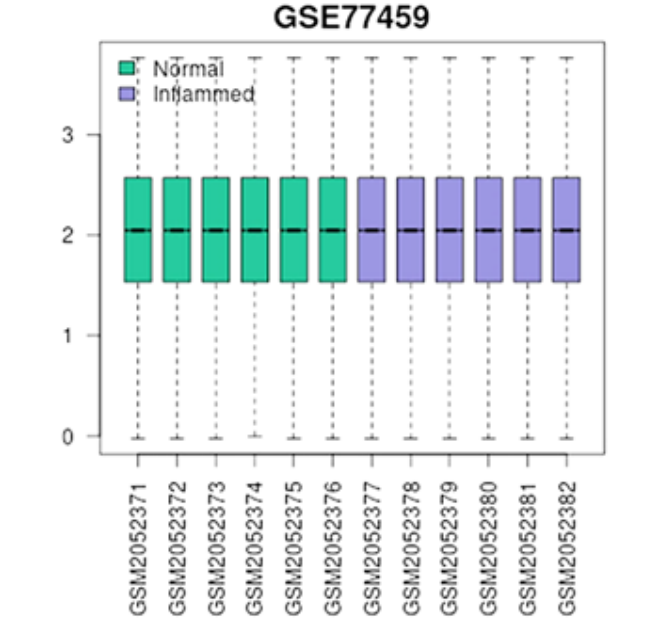

Fig. 3. Boxplot analysis of gene expression profiling dataset of GSE77459.

modification and processing [30]. Recent studies have demonstrated that snoRNAs can function as regulators of cellular stress responses and inflammation [31], suggesting their potential involvement in disease progression. The upregulation of RN5S290 (ENST00000517133), a 5S ribosomal RNA variant, indicates possible alterations in protein synthesis machinery. This finding aligns with previous research showing that dysregulation of ribosomal RNAs can influence cellular homeostasis and stress response mechanisms [32]. The elevated expression of SH3GL2 (NM_003026) is particularly noteworthy, as this gene has been implicated in cellular trafficking and signal transduction pathways [33].

Conversely, the downregulation pattern observed in immunoglobulin-related genes presents an interesting immunological perspective. IGHV3-72 (ENST00000433072) and IGKV1-5 (ENST00000496168), showing log2FC values near -1.66, are crucial components of the adaptive immune response [34]. Their reduced expression suggests potential alterations in immune system function, which could contribute to disease pathogenesis. This observation is supported by recent studies highlighting the role of immunoglobulin variations in inflammatory conditions [35]. The decreased expression of IGHD (AK090461) further strengthens the immunological connection. IGHD plays a vital role in antibody diversity and immune system development [36]. Its downregulation, combined with the reduced expression of other immunoglobulin genes, suggests a coordinated modulation of immune responses in the disease state.

These findings collectively point towards a complex interplay between RNA processing, protein synthesis, and immune system regulation. The altered expression patterns observed in GSE77459 align with current understanding of molecular mechanisms in similar inflammatory conditions [37], while also revealing potential new aspects of disease pathophysiology.

4. Discussion

Endodontitis represents a complex inflammatory disease affecting dental pulp and periapical tissues, characterized by progressive inflammation leading to tissue necrosis and periapical lesion formation. The condition typically initiates with microbial invasion, triggering a cascade of inflammatory responses that ultimately compromise pulpal vitality and periapical tissue integrity [38]. Advanced molecular studies have revealed intricate host-pathogen interactions and immune responses that define various disease stages, suggesting multiple molecular pathways contributing to disease progression and tissue destruction [39].

The current comprehensive analysis utilized the GEO dataset GSE77459, examining differential gene expression patterns between endodontitis samples and healthy controls. Data processing employed robust methodological approaches, including normalization using the RMA algorithm, followed by differential expression analysis using the limma package in R/Bioconductor. Significance criteria were established at adjusted p-value <0.05 and |logFC| >0.25, ensuring statistical rigor in identifying genuinely differential expressed genes [40]. Analysis revealed significant changes in several key molecular pathways, with PTGS2/COX-2 (NM_000963) demonstrating notable downregulation (LogFC: -0.403, p=1.48e-07). This enzyme, central to prostaglandin biosynthesis, plays a crucial role in modulating inflammation intensity and tissue repair processes. The observed reduced expression suggests potential attempts at resolving chronic inflammation, particularly through modified prostaglandin-dependent inflammatory pathways [41]. Further investigation revealed that this downregulation correlates with altered inflammatory mediator profiles and modified tissue response patterns [42].

The KIT Proto-oncogene (NM_000222) showed significant upregulation (LogFC: +0.149, p=6.07e-07), representing modified regulation of cell survival and proliferation pathways. This receptor tyrosine kinase particularly affects stem cell populations crucial for tissue regeneration, suggesting active repair mechanisms and potential regenerative responses in damaged tissues [43]. The analysis revealed that CCL21 (NM_002989) exhibited strong downregulation (LogFC: -1.059, p=7.82e-07), indicating substantial modifications in immune cell trafficking and inflammatory response coordination. This change suggests altered patterns of immune cell recruitment and modified inflammatory response regulation [44].

Matrix remodeling processes showed significant modification, evidenced by MMP9 (NM_004994) downregulation (LogFC: -0.910, p=5.61e-06). This change in metalloproteinase activity suggests ongoing tissue repair attempts and modified extracellular matrix dynamics, potentially influencing tissue architecture and healing responses [45]. Concurrent IGFBP4 (NM_001552) downregulation (LogFC: -0.299, p=1.21e-06) indicates altered IGF signaling, affecting tissue regeneration processes and cell proliferation patterns through modified growth factor availability and activity [46]. The inflammatory signal control mechanism showed substantial modification through SOCS3 (NM_003955) downregulation (LogFC: -0.593, p=1.57e-06), suggesting altered cytokine signaling regulation [47]. This change, combined with reduced FASTK (NM_001995) expression (LogFC: -0.292, p=2.42e-06), indicates modified apoptotic regulation in affected tissues, potentially influencing cell survival and tissue homeostasis [48]. The Cell Cycle Associated Protein (NM_031461) showed upregulation (LogFC: +0.369, p=2.81e-06), suggesting altered cell proliferation control mechanisms and modified tissue renewal patterns [49].

These expression patterns form sophisticated interconnected networks regulating inflammation intensity and resolution. The concurrent downregulation of PTGS2 and SOCS3 suggests coordinated attempts at modulating chronic inflammation through multiple pathway modifications [50]. The altered expression patterns of MMP9 and IGFBP4 indicate active tissue remodeling processes, representing organized repair attempts and modified tissue architecture regulation [51]. The modified CCL21 expression, combined with other immune modulators, suggests complex changes in immune cell recruitment and activation patterns, potentially influencing disease progression and resolution [52]. The identified molecular signatures hold significant potential for clinical applications in endodontic practice. The expression patterns may serve as molecular markers for disease staging and progression monitoring, enabling more precise diagnostic approaches [53]. Understanding the altered pathway activities enables development of targeted therapeutic approaches, potentially improving treatment outcomes through molecular-

based interventions [54]. Gene expression profiles might predict treatment outcomes and guide intervention strategies, supporting personalized treatment approaches based on molecular patterns [55].

The diagnosis and treatment of endodontitis present significant challenges in clinical practice. Current diagnostic methods primarily rely on clinical symptoms, radiographic examination, and pulp sensitivity tests, which often lack precision in determining the exact stage of pulpal inflammation. The subjective nature of pain assessment and varying patient responses to diagnostic tests can lead to ambiguous results. Additionally, the complex anatomy of root canal systems, including accessory canals and isthmuses, makes complete bacterial elimination challenging even with modern instrumentation techniques. Treatment outcomes are further complicated by the diverse microbial populations present in infected root canals and their ability to form biofilms resistant to conventional therapeutic approaches. The emergence of antibiotic-resistant bacterial strains has added another layer of complexity to endodontic treatment. Moreover, the inflammatory response varies significantly among patients, making it difficult to predict treatment outcomes and healing patterns. The lack of reliable biomarkers for monitoring disease progression and treatment effectiveness remains a significant limitation. Current treatment protocols follow a standardized approach, which may not address individual variations in disease manifestation and healing potential. The inability to accurately assess the extent of pulpal inflammation and predict treatment outcomes often leads to either premature or delayed intervention, potentially affecting long-term tooth survival.

Future research directions should focus on large-scale validation of identified molecular signatures in diverse patient populations, ensuring broad applicability of findings [56]. Development of targeted interventions based on identified molecular mechanisms represents a promising avenue for improving treatment outcomes [57]. The creation of chair-side molecular diagnostic tools could revolutionize clinical practice by enabling real-time molecular monitoring [58]. Integration of molecular profiling in treatment planning represents a crucial next step toward personalized endodontic treatment [59]. Long-term studies investigating molecular changes during disease progression and treatment response will further enhance understanding and treatment approaches [60].

This integrated analysis reveals complex molecular interactions characterizing endodontitis, providing potential therapeutic targets and diagnostic markers. The identified genes and pathways offer valuable insights for developing precision medicine approaches in endodontic treatment. The coordinated changes in inflammatory mediators, growth factors, and tissue remodeling molecules suggest carefully regulated disease processes that might be therapeutically targeted. Understanding these molecular mechanisms enables development of more effective treatment strategies and improved patient outcomes through targeted molecular interventions.

This comprehensive molecular analysis of endodontitis reveals intricate gene expression patterns and pathway interactions that significantly influence disease progression and resolution. The study identified key differentially expressed genes, notably PTGS2/COX-2, KIT Proto-oncogene, CCL21, and MMP9, which demonstrate coordinated regulation of inflammatory responses, tissue remodeling, and immune cell functions. The downregulation of inflammatory mediators alongside modifications in tissue repair mechanisms suggests active disease modulation processes. These findings provide valuable insights into potential therapeutic targets and molecular markers for improved disease monitoring and treatment planning. The identified molecular signatures offer promising opportunities for developing targeted interventions and personalized treatment approaches in endodontic practice. Future research focusing on validation studies, biomarker development, and molecular-based therapeutic strategies will be crucial for translating these findings into clinical applications, ultimately advancing the field of molecular endodontics and improving patient care outcomes.

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Informed consent statement
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Data availability statement
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Conflicts of interest
The authors declare no conflicts of interest.

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