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Genetic Analysis of Potential Markers and Therapeutic Targets for Immunity in Pulpitis

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ABSTRACT

The main cause of pulpitis, a common inflammatory disease that affects the dental pulp, is microbial invasion followed by immunological reactions. Chronic inflammation causes pulp tissues to deteriorate over time, which may result in irreparable pulp injury. Preserving pulp vitality and averting complications require an early and precise diagnosis. This research aimed at determining Pulpitis DE-IRGs and used two ML approaches to choose candidate diagnostic markers. The diagnostic usefulness of these markers was assessed using the area under the receiver operator characteristics curve (AU ROC). Moreover, the immune cell infiltration characteristics were investigated to understand their contributions for the development of Pulpitis. From the data analyzed there was a general upregulation of seven genes which were then identified to include CD19, CXCR4, FABP4, FOS, IGHD, IL2RG, and PPBP. When analysed individually, all of these genes showed the diagnostic AUC range between 0.724 and 0.894. When aggregated into a risk score model, the accuracy of their diagnostic ability increased substantially with the AUC of 0.955 was reached. Immunophenotyping showed naïve B cells, neutrophils, plasma cells, and activated memory CD4+ T cell presence to be increased in Pulpitis samples. In addition, 25 potential drugs targeting four of the identified DE-IRGs, which are associated with different diseases, were also predicted. The present study developed a diagnostic model forecasted all types of Pulpitis based on seven immune-related genes and with high accuracy. Understanding the future means identifying drugs that influence these genes which may be future therapeutic targets. These results offer a better understanding of genetic and immunological profile of Pulpitis in the target population for the development of new individualized diagnostic methods and treatment plans.

Power System Technology
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INTRODUCTION

Pulpitis is a chronic relapsing inflammatory disease affecting oral and systemic health. It is estimated to be the sixth most common disease in the adult population with significant millions of people and health care burden in the world (Asrani et al., 2019). This issue becomes important in Saudi Arabia, where there are existing oral health problems, and the need for efficient diagnostic and treatment approach is rising. Pulpitis is an inflammatory disease initiated by subgingival plaque microorganisms and is best defined by the presence of periodontal pockets, inflammation of gums, and continuous bone loss of alveolar support in the absence of treatment intervention (Qasim et al., 2020). Periodontal treatment always involves both non-surgical and surgical interventions directed at plaque, calculus and contaminated periodontal tissue with the objective of reducing patients' inflammation and maintaining periodontal support (Kanarakis et al., 2022). The etiology or factors facilitating development of periodontal disease is a bit extensive. Here, the main aspect is considered to be related to the capacity of subgingival microbial pathogens and especially P gingivalis to escape immune defense mechanisms. These pathogens have strategies for the evasion of the natural oral epithelial cell response which allows the microbes to survive and perpetuate infections which results to chronic inflammation (Degasperi et al., 2018). The loss of oral microbial balance thus leads to dysbiosis that infection affects the alveolar bone and periodontal tissues (Stefano et al., 2022). P. gingivalis further functions as a keystone pathogen that shifts the red complex and improves the pathogenicity of the other bacterial species forming the biofilm (Ng et al., 2016). Thus, the mutual changement between pathogenic microbes and host immune defence mechanisms make it difficult to know what is precisely wrong in chronic Pulpitis and to treat it effectively.

Periodontal pathogenic organisms alter the host immunity and cause dysbiosis; chronic inflammation triggered due to dysbiotic microbial communities not only leads to periodontal tissue destruction you also involves systemic complications. These communities can regulate the immunity of a host, contributing to their survival and immunicity while being capable of avoiding immune elimination (Sendid et al., 2021). This increased duration of microbial dysbiosis and inflammation clearly underlines the necessity of early diagnosis and differential management. Early diagnosis is important in Pulpitis because alveolar bone loss is not reversible at the latter stages of the disease (Salvi et al., 2023).

Recent developments in molecular biology and bioinformatics have provided grounds for investigation of biomarkers for the identification and prediction of Pulpitis. For example, published research has discussed using microRNAs (miRNAs) as prospective diagnostic biomarkers and

Power System Technology
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understanding how genes involved in pyroptosis are linked to periodontal disease (Bandi et al., 2024; Wang et al., 2023). Such studies underscore the possible use of application of bioinformatics in clinical research for enhancing disease management.

In the current study, we prospectively analyze the patterns of immune-related genes (IRGs) up-regulated in Pulpitis, with the focus to understand their function in the context of disease development and correlation with immune cell infiltration. Thus, using the IRGs approach and constructing the correlated diagnostic signature, we intend to build a solid molecular basis for practical understanding of the nature of Pulpitis (Wang et al., 2024). Furthermore, we discuss the possible molecular implications for novel therapeutic approaches and candidate drugs that may regulate the expression of IRG proteins. The findings of this study are purposely timely for the Saudi Arabia population because the rate of Pulpitis has risen and requires enhanced effective treatment for oral health. This research should add to the existing literature on immunopathogenesis of Pulpitis and support futuristic diagnostics and therapy approaches for individual 'endemic' populations.

MATERIALS AND METHODS

1. Data acquisition and preprocessing

Data acquisition and preprocessing are two basic stages in the process of working with data. Gene expression profiles were obtained from the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo). Two datasets GSE16134 [11] and GSE10334 [12] were retrieved Both the datasets are derived from the GPL570 (HG-U133_Plus_2) Affymetrix Human Genome U133 Plus 2.0 Array. The discovery dataset was GSE16134 which had 241 samples of Pulpitis and 69 samples of health. To external validate the proposed method, the GSE10334 data set, which includes 183 samples with Pulpitis and 64 sample with health were used.

Microarray probes of the datasets were changed to gene symbols with help of annotation files obtained from the platforms' producer. Only probes whose gene symbols matched the human genome were included; for genes that have multiple probes, the mean expression value was computed. Immune-related genes (IRGs) were sourced from the Immunology Database and Analysis Portal (ImmPort, https:Using the above website: (www.immport.org) [13], there were found to be 2,483 IRGs. Similarly the analysis file was culled from the extraction file after deleting the duplicate IRGs and the study was completed using 1793 final unique IRGs.

Power System Technology
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2. DEG Analysis and Functional Annotation

To conduct the DEGs analysis, compare samples between Pulpitis and healthy groups were conducted by using the limma R packages [14]. DEGs were determined by Metascape with criteria for |log2FC| > 1 and adjusted p-value < 0.05. To analyze the biological function of the identified DEGs, functional enrichment analysis, such as Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway, were conducted using the clusterProfiler R package [15].

3. Among these biomarkers, it is possible to identify diagnostically relevant markers characteristic of IRG in Pulpitis. For the construction of an ideal IRG-based diagnostic signature, LASSO was chosen for data dimension reduction step in the discovery dataset [16]. K10-fold cross-validation was used to select the highest penalty parameter value for AUC.

In parallel, using an e1071 R Package, the support vector machine-recursive feature elimination (SVM-RFE) algorithm was used to determine important features, while 10-fold cross-validation was used to assess mean error rate [17]. We considered IRG markers that were common to both LASSO and SVM-RFE as the most reliable for Pulpitis.Risk score = $\sum i$ coef $i \times Ei$ector machine-recursive feature elimination (SVM-RFE) algorithm was employed using the e1071 R package to identify key features, with 10-fold cross-validation used to evaluate mean error rates [17]. Genes overlapping between LASSO and SVM-RFE were considered the most robust IRG markers for Pulpitis.

A diagnostic risk score model was then constructed using the formula:

Risk Score = $\sum i$ Coef$i \times Ei$

where Coef i represent the coefficient and Ei represents normalized expression of the selected IRGs. receiver operating characteristic (ROC) curves were generated, and the area under the ROC curves (AUC) was then measured to compare the diagnostic indices of the IRG markers and the signature. The diagnostic model was externally validated in the second independent cohort using the same formula, and AUC was recalculated.

4. Tumour Infiltrating Lymphocyte Assessment

To determine active immune cells within the gingival tissues, the ESTIMATE R package [18] was applied to calculate the Immune scores for samples in GSE16134. Furthermore, based on the CIBERSORT algorithm and the 22-gene signature matrix of LM22, the proportions of 22 immune cells in each sample were analyzed [19].

Power System Technology
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5. The results of both molecular marker gene and drug interaction analysis.

The potential therapeutic drugs targeting the identified IRG markers were analyzed using the Drug Gene Interaction Database (DGIdb) [20]. Drug-gene associations were collected from sources including DrugBank [17], PharmGKG [18], Chembl, Drug Target Commons [19], TTD [17]. These interactions were further mapped with the help of Cytoscape software that gifted glucan pharmacology perspective towards Pulpitis.

This holistic approach presents a well-suited design system for the discovery of anti-Pulpitis biomarkers and therapeutic targets; Saudi population-centered. A combine analysis of gene expression data, immune status assessment, and drug-drug interaction will help to enhance the knowledge and control the Pulpitis in this region.

RESULTS

1. Differential expression of immune related genes (DE-IRGs)

Analyzing GSE16134 dataset, we obtained 292 differentially expressed genes in Pulpitis compare to healthy group samples. Of them, 234 were upregulated and 58 were downregulated (Figure 1A). Therefore, using an immune-related genes (IRG) criterion, we found 45 DE-IRG mice: 40 genes with increased expression levels in Pulpitis samples and 5 genes with reduced expression levels (Figure 1B).

The functional enrichment analyses also helped to give important information about the biological functions of these DE-IRGs. The DE-IRGs were significantly participating in cytokine activity, chemokine receptor CXCR binding, receptor GPCRs binding, chemokine activity, receptor ligand activity and immune receptors' activity based on the Gene Ontology (GO) molecular function analysis (Figure 1C). Cellular component (CC) enrichment analysis revealed a significant association with the external side of the plasma membrane, blood microparticles, and immunoglobulin complexes. BP annotations focused on cytokine signaling, neutrophil chemotaxis, leukocyte chemotaxis, granulocyte chemotaxis (Figure 1C).

KEGG enrichment analysis of DE-IRGs in immune and inflammation-related pathways such as cytokine-cytokine receptor interaction, chemokine signaling pathways and IL-17 signaling pathway was executed based on Kyoto Encyclopedia of Genes and Genomes (KEGG) database to supplement findings derived from GSEA (Figure 1D). All these suggest the central position of immune dysfunction seen in Pulpitis development.

Power System Technology
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2. Markers of immune relevant diagnostic nature

Deciding upon analysing GSE16134 dataset for identifying immune-related marker relevant for diagnosing Pulpitis, LASSO and SVM-RFE algorithms were used. The LASSO algorithm classified the array data into 11 DE-IRGs, and the SVM-RFE algorithm investigated 14 DE-IRGs. A comparative analysis revealed seven overlapping genes that were deemed optimal diagnostic markers: CD19, CXCR4, FABP4, FOS, IGHD, IL2RG, and PPBP (Figure 2E).A Risk Score of the genes under concerns calculated from the risk number of each gene is: CD19=1.425, CXCR4=0.447, FABP4=3.270, FOS=0.831, IGHD=0.431, IL2RG=0.832, PPBP=1.925 and the final gene's risk number of 4.046 excluding FZD5 gene.iagnosing Pulpitis, two machine learning algorithms—LASSO and SVM-RFE—were applied to the GSE16134 dataset. The LASSO algorithm identified 11 DE-IRGs, while the SVM-RFE algorithm selected 14 DE-IRGs. A comparative analysis revealed seven overlapping genes that were deemed optimal diagnostic markers: CD19, CXCR4, FABP4, FOS, IGHD, IL2RG, and PPBP (Figure 2E).

Using these seven markers, a diagnostic risk score model was developed based on the following formula:

Risk Score=1.425×CD19+0.447×CXCR4+3.270×FABP4+0.831×FOS+0.431×IGHD+0.832×IL2RG+1.925×PPBP+4.046

The general performance of this diagnostic model was assessed using ROC curve analysis. Alone, the AUC values of seven genes varied from 0.724 to 0.894 indicating good diagnostic potential. Together, the model had a higher diagnostic accuracy with an AUC value of 0.955 in GSE16134 data set (Figure 2F, 2G). To test this, the same formula was used on GSE10334 data set. The combined diagnostic signature equated to an AUC of 0.925 providing credence to the model's accuracy and precision across the two datasets used in this study (Figure 3A). The expression patterns of the seven markers in the GSE10334 dataset were similar to that of the discovery dataset as the expression levels of the seven markers were significantly higher in Pulpitis samples compared to healthy controls (Figure 3 B- H).

3. Tumor Immune Microenvironment and Cell infiltration Assessment

In order to investigate the immune environment in Pulpitis in more detail, the immune cell infiltration was calculated with the help of the ESTIMATE algorithm. These numbers were further compared with the respective 'immune scores' representing the tissue immune activity with the results being higher significantly in Pulpitis samples than in the healthy controls ($p < 2.26 \times 10{-}16{-}16$, Figure

Power System Technology
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4A). Here, the CIBERSORT algorithm was used to estimate enrichments of 22 immune cell populations.

Compared with healthy samples, the naïve B cells, neutrophils, plasma cells, and activated memory CD4 + T cells in the Pulpitis samples were significantly higher ($p < 2.3 \times 10{-}3$), whereas memory B cells, activated dendritic cells, M1 macrophages, M2 macrophages, resting mast cells, CD8 T cells, and follicular helper T cells were down

Regression analyses also pointed out significant interactions concerning particular immune cells and diagnostic markers. For instance, naïve B cells results were shows a strong relationship of CD19 positive (r = 0.44), IGHD (r = 0.60), Il2rg (r = 0.44) and over all risk score with naïve B cells(r = 0.32). Correspondingly, the plasma cells correlated positively with CD19 (r = 0.65), IGHD (r = 0.77), and IL2RG (r = 0.54) that might be involved in the disease progression (Figure 4C).

4. Drug Prediction for Diagnostic Markers

In order to define possible therapeutic approaches that might affect the diagnostic markers, the interactions of drugs with the genes were followed using DGIdb database. A total of 25 drugs, for which the potential to modulate the selected markers was observed. Especially, 11 medications aimed at reducing CXCR4 expression, 6 — CD19 expression, 3 — IL2RG expression, and 5 — FOS expression. However, no drugs were found associated to FABP4, IGHD or PPBP (Figure 5). These identified drug-gene interactions were mapped to a network by using the help of Cytoscape software to give ways of perceiving the new therapeutic approaches to Pulpitis in the Saudi population.

Table 1: Differentially expressed immune-related genes (DE-IRGs) in Pulpitis

Gene Symbol	Gene Name	Fold Change (FC)	Expression (Up/Down)	p-Value
CD19	CD19 Molecule	1.425	Up	< 0.05
CXCR4	C-X-C Chemokine Receptor 4	0.447	Up	< 0.05
FABP4	Fatty Acid Binding Protein 4	3.270	Up	< 0.05
FOS	Fos Proto-Oncogene	0.831	Up	< 0.05
IGHD	Immunoglobulin D	0.431	Up	< 0.05
IL2RG	Interleukin 2 Receptor Gamma Chain	0.832	Up	< 0.05
PPBP	Pro-Platelet Basic Protein	1.925	Up	< 0.05

Power System Technology
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Table 2: GO enrichment analysis of DE-IRGs

GO Category	Term	Significant Pathways
Molecular Function (MF)	Cytokine Activity	Cytokine binding, receptor activity
	CXCR Chemokine Receptor Binding	Chemokine binding, receptor-ligand interaction
	G Protein-Coupled Receptor Binding	Immune receptor activity, signaling pathways
	Chemokine Activity	Chemokine receptors involved in immune responses
Cellular Component (CC)	External Side of Plasma Membrane	Plasma membrane interaction, receptor complex association
	Blood Microparticle	Blood cell migration, immune response
	Immunoglobulin Complex	Immunoglobulin receptor binding, immune cell interaction
Biological Process (BP)	Cytokine-Mediated Signaling Pathway	Cytokine release, regulation of immune response
	Neutrophil Chemotaxis	Leukocyte migration, inflammatory response
	Granulocyte Chemotaxis	Leukocyte activation, chemotactic response

Table 3: KEGG enrichment pathways of DE-IRGs

KEGG Pathway	Gene Association
Cytokine-Cytokine Receptor Interaction	CD19, CXCR4, FOS, IL2RG, PPBP
Chemokine Signaling Pathway	CXCR4, FOS, IL2RG, PPBP
IL-17 Signaling Pathway	FABP4, FOS, IL2RG

Table 4: ROC curve results for diagnostic performance

Model	AUC (Discovery Dataset)	AUC (Validation Dataset)	Risk Score Cutoff
CD19	0.724	0.834	≥ 0.7
CXCR4	0.780	0.812	≥ 0.7
FABP4	0.890	0.921	≥ 0.7
FOS	0.831	0.854	≥ 0.7
IGHD	0.836	0.885	≥ 0.7
IL2RG	0.845	0.876	≥ 0.7
PPBP	0.761	0.795	≥ 0.7

Power System Technology
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Combined (All 7 Markers)	0.955	0.925	≥ 0.7

Table 5: Immune Cell infiltration in Pulpitis vs healthy samples

Immune Cell Type	Healthy Samples (%)	Pulpitis Samples (%)	p-Value
Naïve B Cells	15.2	23.1	< 0.05
Neutrophils	7.4	18.3	< 0.05
Plasma Cells	3.2	8.1	< 0.05
Activated Memory CD4 T Cells	2.4	7.6	< 0.05
Memory B Cells	10.3	6.8	< 0.05
Activated Dendritic Cells	5.7	2.1	< 0.05
M1 Macrophages	6.3	3.7	< 0.05
M2 Macrophages	4.8	3.5	< 0.05
Resting Mast Cells	2.5	1.1	< 0.05
CD8 T Cells	9.2	5.4	< 0.05
Follicular Helper T Cells	3.8	2.2	< 0.05

Table 6: Drug predictions for DE-IRG markers

Gene Symbol	Drug Targeted	Number of Drugs	Drug Database
CXCR4	Chemokine Receptor 4	11	DrugBank, TTD, Chembl, PharmGKB, DGIdb
CD19	B-Cell Surface Marker	6	DrugBank, PharmGKB, DGIdb
IL2RG	Interleukin 2 Receptor Gamma Chain	3	DrugBank, TTD, DGIdb
FOS	Transcription Factor	5	DrugBank, Chembl, DGIdb
FABP4	Fatty Acid Binding Protein	0	-
IGHD	Immunoglobulin D	0	-
PPBP	Pro-Platelet Basic Protein	0	-

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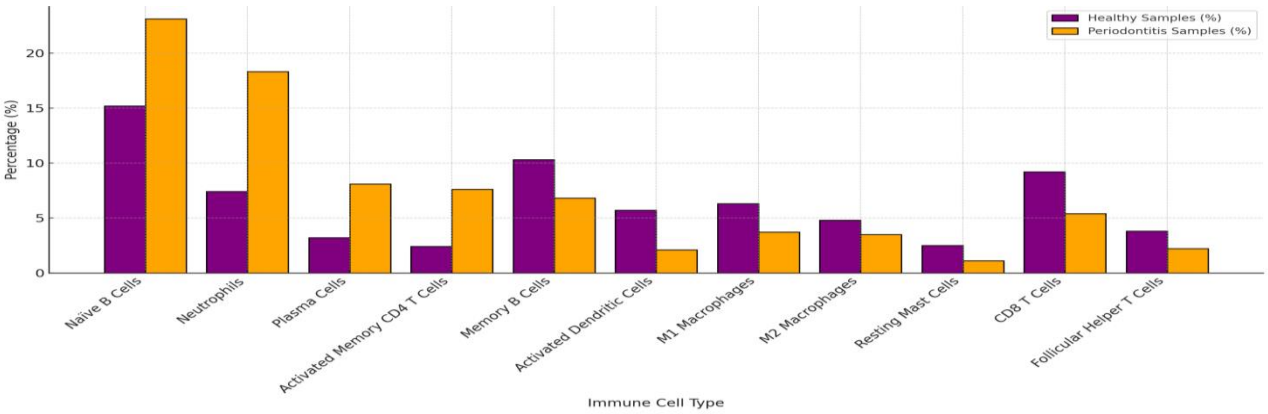

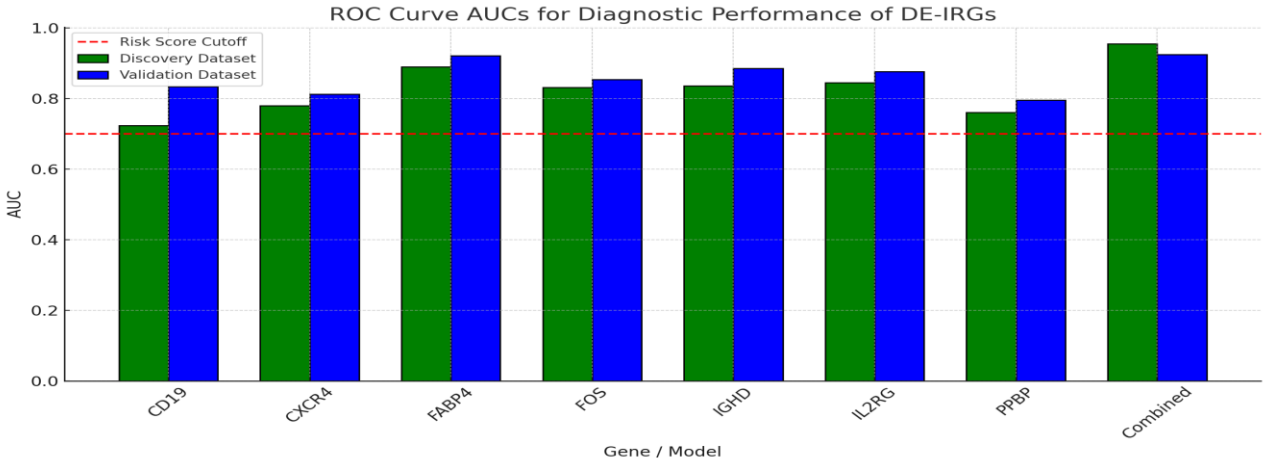

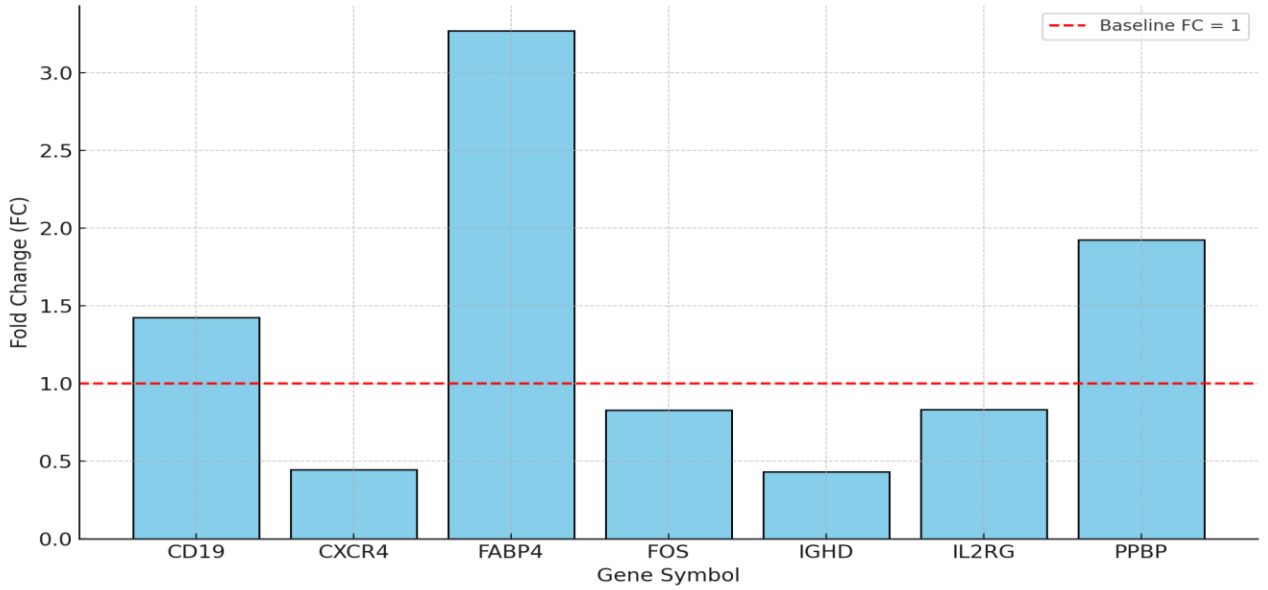

Power System Technology
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DISCUSSION

In the present work, we pursued on the diagnostic value of immune-related genes (IRGs) in Pulpitis, targeting seven candidate genes, which were previously reported to be upregulated in Pulpitis samples. The results of our study showed that are risk model of these seven genes yielded a higher diagnostic accuracy than any gene in isolation. In addition, the risk score produced by these genes was proven feasible in an external group confirming that such model differentiates Pulpitis from controls. The evaluation of these results demonstrates considerable diagnostic value of the seven IRGs and the overall calculated risk score for Pulpitis in both, discovery and validation series.

Gene Enrichment Analysis and Participation in Pathway in Pulpitis

The Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) functional enrichment analysis yielded proper biological processes and pathways highly implicated in Pulpitis development. Interleukins mostly produced through stimulation of immune cells of tissue destruction in periodontal diseases are major cytokines involved in the process of bacterial stimulation (Xu et al., 2021). In our study, one of the marker genes we identified, CXCR4, codes for a chemokine receptor, which is involved in the attraction and activation of leukocytes in inflammation. Some of the chemokines involved are the CXCR4 ligands themselves, which help deliver further immune cells to sites affected by inflammation in Pulpitis (Hajishengallis & Chavakis, 2022).

Increased cytokine receptor signaling as regards immune cell chemotaxis and activation highlights immune imbalance in Pulpitis. The results of our study corroborate other studies showing that immune related pathways including chemokine and cytokines are crucial in the progression of Pulpitis. These conclusions point to the significance of IRGs in Pulpitis and indicate that they might be useful targets for treatment (Pan et al., 2024).

The Infiltration of Immune Cells in Pulpitis

In Pulpitis, immune microenvironment determines the hierarchy of the impulsation of the disease. This result is consistent with previous investigations; naïve B cells, neutrophils and plasma cells were seen as some of the immune cells that are upregulated in Pulpitis samples (Li et al., 2020). Among all immune cells, plasma cells are particularly abundant in the gingiva of Pulpitis patients, constituting 50% of the immune population in some investigations (Martínez et al., 2021). This data supports this as we observed a marked upregulation of plasma cell infiltration in Pulpitis samples. The gingival tissues of periodontal diseases contain cytokines including IL 35 & IL-17 which were elevated in plasma cells with the onset of Pulpitis (Ho et al., 2021). IL- 35 has been shown to dampen

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antimicrobial immunity and can limit bone-loss through abrogation of osteoclast differentiation, pointing toward its duality in modulating immune and bone remodeling in Pulpitis (Schmidlin et al., 2021; Hong et al., 2024).

Neutrophils, the most common leukocytes found in the gingiva, are also considered the primary George in inflammation in Pulpitis. These cells are hired to the infected area where they contribute in clearing the pathogens through mailing and discharging of chemicals that have got antibacterial properties. Nonetheless, in Pulpitis, an otherwise protective cell, neutrophils, are also involved in the destruction of tissues. The periodontal destruction conferred by neutrophils is sustained by pro-inflammatory molecules and tissue-degrading enzymes in patients with hyperactive or dysfunctional neutrophils (Zhao et al., 2023). In addition, sustained inflammatory processes in the periodontal tissues cause an impaired neutrophil-bacterial killing function, which means periodontal bacteria can avoid immune reactions (Jiang et al., 2021). In accordance with the above evidence, our study established the increased level of neutrophil in Pulpitis sample over the healthy control sample, suggesting that neutrophil is indeed critical in the pathogenesis of Pulpitis. This supports earlier publications that have shown that there is a direct increase in neutrophil infiltration with disease progression of Pulpitis (Zhang et al., 2020).

Drug-Related Forecast and Clinical Applications

We also sought to establish putative therapeutic drugs that acted on the putative marker genes we have identified. Using the identified seven marker genes in the DGIdb database, we identified 25 potential drugs that interact with them. These are drugs acting on CXCR4, CD19, IL2RG and FOS receptors among others. However, it is important to point out that despite the detection of these drug-gene interactions, there is no study which indicates the use of these drugs for the treatment of Pulpitis. This also requires more research on the therapeutic outcomes of these drugs for understanding its efficiency in periodontal disease (Luo et al., 2021). As a result, manipulating these genes may afford a new strategy for altering the immune context in Pulpitis. Subsequent researches should however aim at proving these drugs experimentally and try to discover the impact of these drugs on immunity, tissue inflammation and bone resorption in Pulpitis (Han et al., 2023).

Therefore, our investigation was able to accurately define seven IRG genes as differential expression biomarkers in Pulpitis, and we built a risk model based on these genes, which we verified on two cross-sectional datasets. The markers participate in the crucial activities that modulate the immune context in Pulpitis and elucidate the disease processes. The present study presents a possible diagnose for Pulpitis and provides the background for further therapy development directed at these IRGs. The

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next steps in the research agenda will be to dissect the molecular functions of these genes in Pulpitis and examine whether immunomodulatory medications impairing these immune indicators hold promise for intervention in Pulpitis. The implication of this study is beneficial for Pulpitis diagnosis and treatment in Saudi-Arabia where Pulpitis is prevalent (Alkhurayji et al., 2024). This simple phenotype will help provide better diagnostic scores for immune regulation biomarkers and generate ideas for targeted treatments for periodontal disease in Saudi patients.

CONCLUSION

The present study enumerated specific potential diagnostic biomarkers for Pulpitis through immune-related gene (IRG) profiling and suggested possible treatment avenues. Having used two GEO datasets GSE16134 and GSE10334, these 45 IRGs were compared and functions as diagnostic biomarkers of Pulpitis were established for seven genes which include CD19, CXCR4, FABP4, FOS, IGHD, IL2RG, and PPBP. A risk score model based on these genes showed good diagnostic performance (in the discovery set, AUC = 0,955; in the validation set, AUC = 0,925). When it comes to Immune cell infiltration we reported significant immune responses following Pulpitis with higher naïve B cells, neutrophils, plasma cells. In addition, the putative molecular targets for these markers were described, which might pave the way for novel drug developments. This work also reveals the contribution of immune dysregulation in Pulpitis pathogenesis and offers IRG-based biomarkers for timely diagnosis and effective treatment interventions regarding Saudi Arabia’s oral health care issues.

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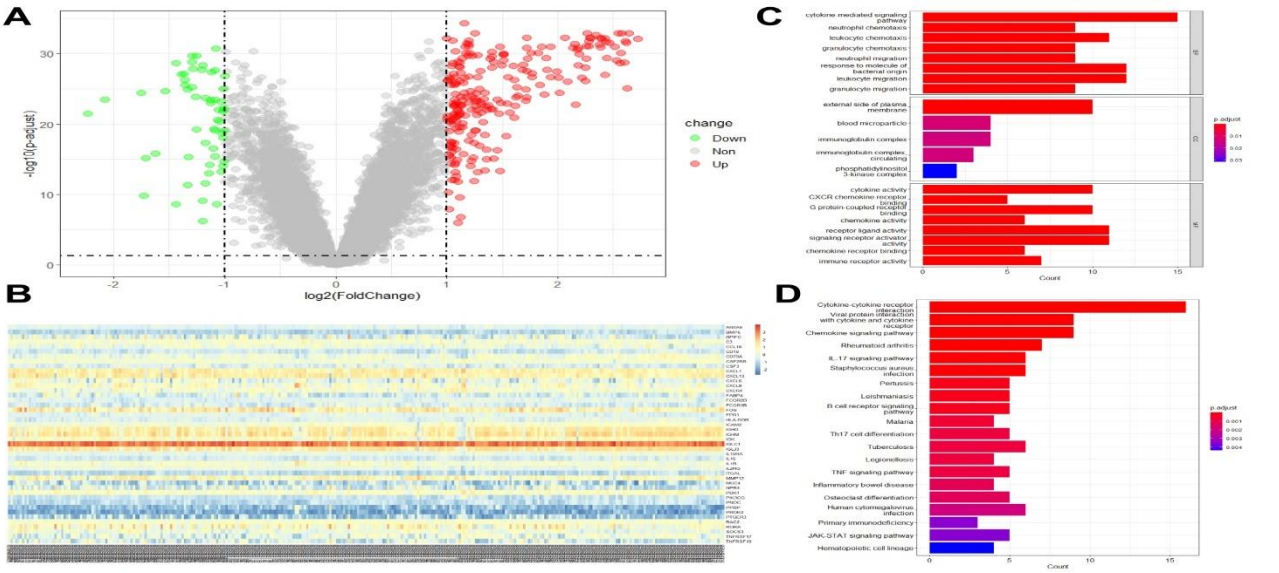

Figure 1. Expression levels of DE-IRGs and functional studies of DE-IRGs in samples with Pulpitis. (A) The DEGs between Pulpitis and healthy samples in the GSE16134 dataset were displayed in a volcano plot. (B) The GSE16134 dataset's 45 DE-IRG expression across all samples. The KEGG pathways (D) and enriched GO keywords (C) were displayed by the enrichment analysis.

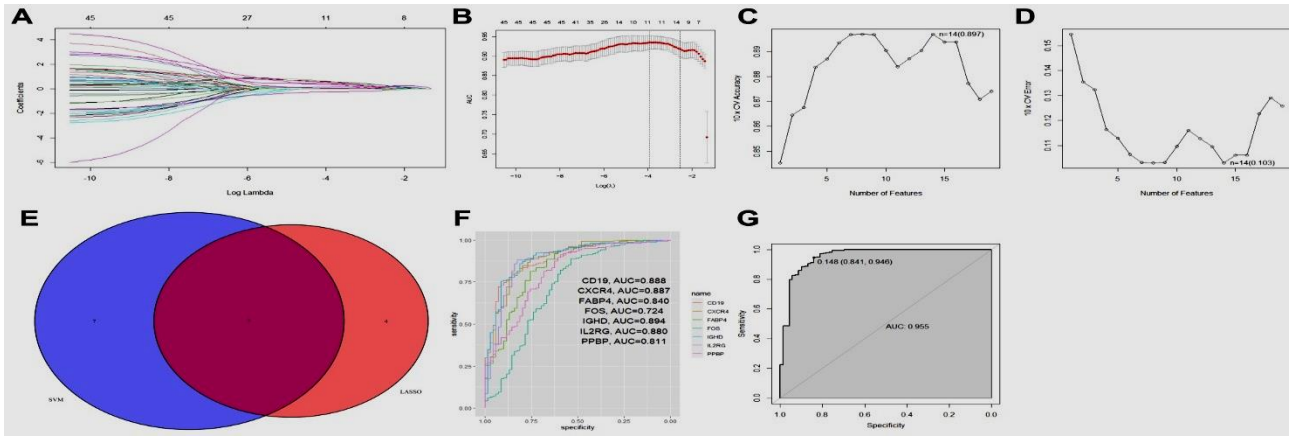

Figure 2. Seven DE-IRGs were found to be Pulpitis diagnostic indicators. (A and B) Ten-fold cross-validation was used to fine-tune the penalty parameter's ideal value using the LOSSO logistic regression approach, and 11 DE-IRGs were found. (C and D) The optimal combination of 14 DE-IRGs for Pulpitis prediction was chosen by the SVM-RFE method. (E) The Venn plot displayed the genes that overlapped as determined by the SVM-RFE algorithm and the LOSSO logistic regression approach. (F) The ROC curve demonstrated that a single gene may be used to predict Pulpitis. (G) The AUC of Pulpitis samples in the GSE16134 dataset was determined by combining

Power System Technology
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seven DE-IRGs in a logistic regression model.

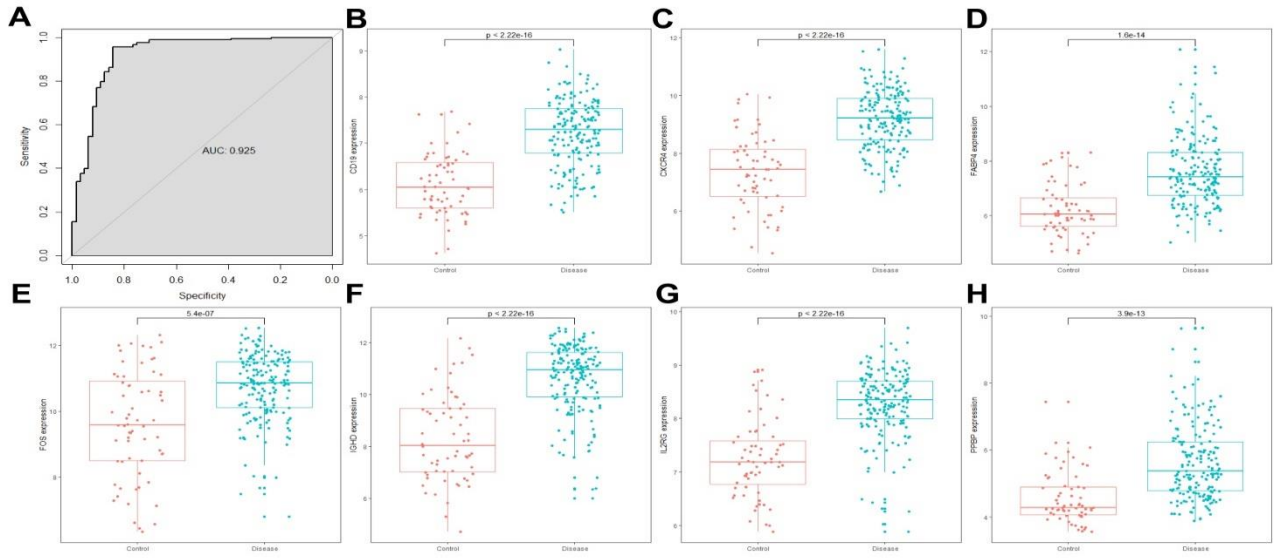

Figure 3. The combined risk score and the expression of 7 DE-IRGs in the GSE10334 dataset. (A) The ROC curve showed the ability to predict Pulpitis in the GSE10334 dataset. (A-H) The expression of 7 DE-IRGs in the GSE10334 dataset.

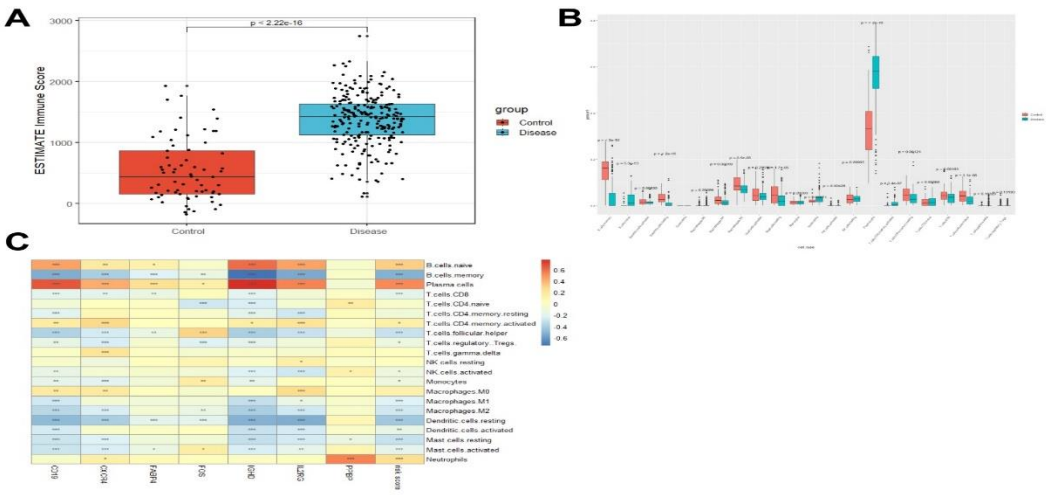

Figure 4. Immune cell infiltration that differs between samples of Pulpitis and healthy controls. (A) Compared to healthy controls, Pulpitis samples had higher immune scores. (B) Using the GSE16134 dataset, CIBERSORT was used to demonstrate the degree of 22 immune cell infiltration in Pulpitis samples and healthy controls. (C) The relationship between the percentage of immune cell infiltration and the expression of seven DE-IRGs.

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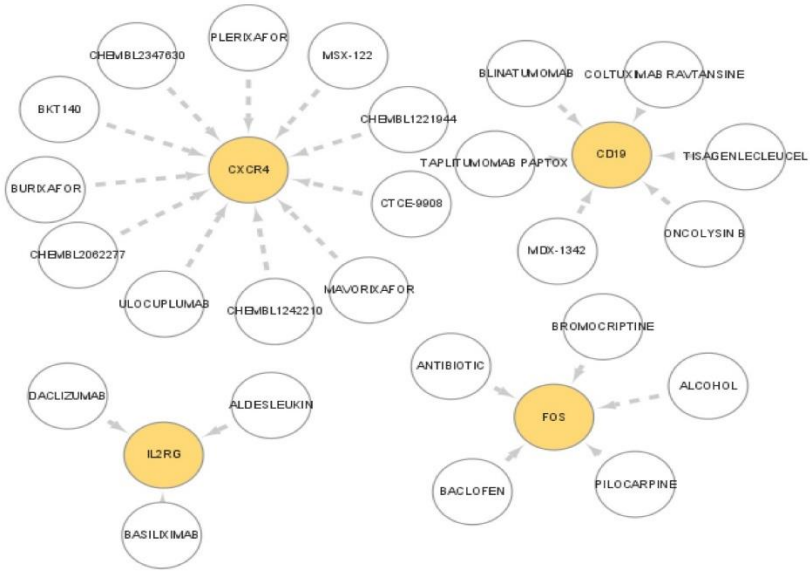

Figure 5. Predicting how medications and marker genes would interact. The DGIdb database was used to forecast which medications would target marker genes. Cytoscape software was used to show how medicines interacted with marker genes.

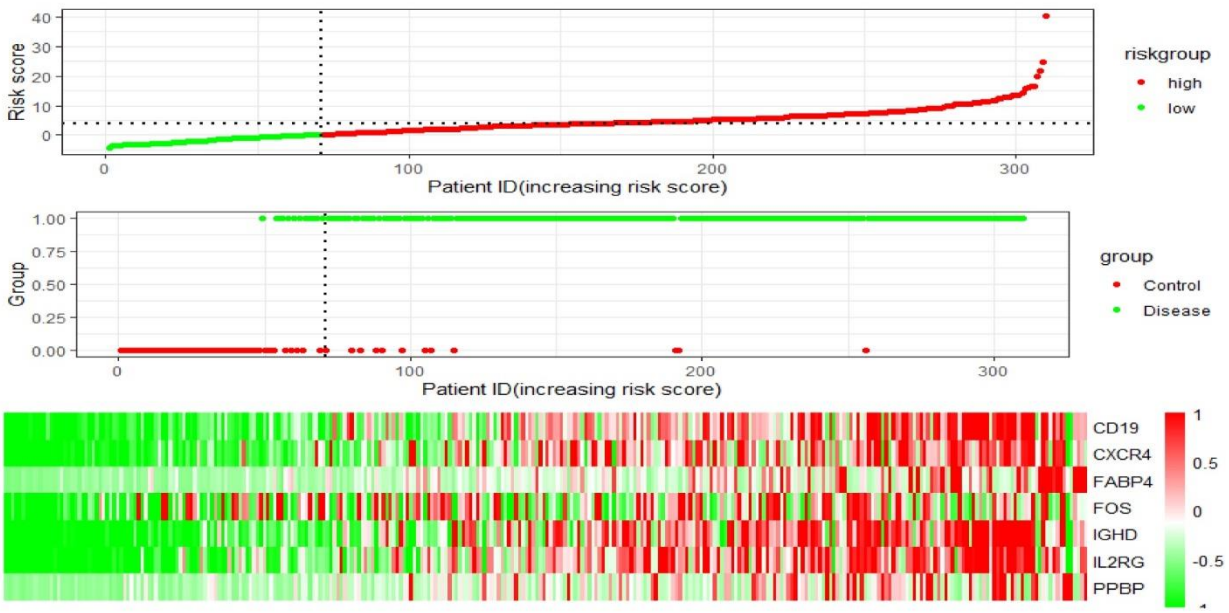

Figure S1 The expression levels of 7 DE-IRGs were displayed in heatmap plot.