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GENETIC SUSCEPTIBILITY TO EXTERNAL ROOT RESORPTION FOLLOWING ENDODONTIC, ORTHODONTIC AND ORAL SURGICAL INTERVENTIONS: A CASE-CONTROL STUDY

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ABSTRACT

Background: External root resorption (ERR) is a pathological process that can be triggered by endodontic, orthodontic, and oral surgical procedures. While mechanical and inflammatory factors are well-recognized contributors, the role of genetic predisposition remains underexplored.

Objective: This case-control study aimed to investigate genetic polymorphisms in interleukin-1 alpha (IL-1α), IL-1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α), vitamin D receptor (VDR), and matrix metalloproteinase-9 (MMP-9) genes as potential susceptibility factors for treatment-induced ERR.

Methods: A total of 120 patients (60 cases with ERR and 60 controls without ERR) who had undergone endodontic, orthodontic, or oral surgical procedures were enrolled. Genetic analysis was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and allele-specific PCR. Periapical radiographs and CBCT scans were used to confirm and grade ERR. Statistical analysis included chi-square tests, logistic regression, and Hardy-Weinberg equilibrium assessments.

Results: The IL-1β (+3953 C>T) TT genotype (OR=3.8, 95% CI: 1.9-7.6, $p<0.001$) and the TNF-α (-308 G>A) GA genotype (OR=2.6, 95% CI: 1.3-5.1, $p=0.007$) were significantly associated with increased ERR susceptibility. The VDR (FokI) ff genotype conferred significant risk (OR=2.9, 95% CI: 1.4-6.1, $p=0.004$). MMP-9 (C-1562T) T allele was significantly overrepresented in cases ($p=0.012$). No significant association was observed for IL-1α polymorphisms.

Conclusion: Specific pro-inflammatory and extracellular matrix degradation gene polymorphisms are significantly associated with ERR susceptibility following dental interventions. Genetic screening may facilitate personalized risk stratification and treatment planning.

KEYWORDS: External root resorption, genetic polymorphism, interleukin-1, TNF-alpha, VDR, MMP-9, orthodontics, endodontics, oral surgery, case-control

1. INTRODUCTION

External root resorption (ERR) is defined as the progressive loss of dental root structure initiated by the activity of multinucleated clastic cells on the external root surface, distinct from physiological root resorption observed in deciduous dentition [1,2]. As an irreversible pathological condition, ERR poses significant clinical challenges owing to its often asymptomatic course, insidious progression, and variable response to treatment. In contemporary dental practice, ERR is recognized as one of the most perplexing sequelae of diverse dental interventions, including endodontic treatment, orthodontic tooth movement, and oral surgical procedures such as tooth extraction, implant placement, and periapical surgery [3-7].

The aetiopathogenesis of ERR is multifactorial. Trauma, occlusal overload, excessive orthodontic forces, and microbial irritants from the root canal system are well-documented local etiological factors [4,7]. Systemic contributors including endocrine disorders, autoimmune conditions, and metabolic diseases have also been implicated. Despite the established role of these environmental and iatrogenic factors, the clinical observation that not all patients undergoing similar treatments develop ERR suggests the existence of a strong biological predisposition modulated by genetic factors [1,2].

The cellular cascade underlying ERR involves the recruitment of osteoclast-like cells (odontoclasts), whose activity is regulated by a complex network of cytokines, growth factors, and extracellular matrix proteases. Key mediators in this inflammatory-catabolic axis include interleukin-1 alpha (IL-1α), interleukin-1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α), matrix metalloproteinases (MMPs), and receptor activator of nuclear factor kappa B ligand (RANKL) [30,31,32]. The vitamin D receptor (VDR), a nuclear transcription factor regulating calcium homeostasis and immune modulation, has also been implicated in the regulation of bone and cementum remodelling [8-11].

Single nucleotide polymorphisms (SNPs) in the genes encoding these mediators can alter their expression levels and biological activity, thereby potentially modifying the threshold for initiation and progression of root resorption. Several studies have examined the role of IL-1 gene cluster polymorphisms in periodontitis, peri-implantitis, and bone loss, yet their specific contribution to treatment-induced ERR remains largely unexplored [12-16]. Similarly, polymorphisms in TNF-α, VDR, and MMP-9 genes, all pivotal in hard tissue catabolism, have not been systematically evaluated in the context of orthodontically, endodontically, or surgically induced ERR [17].

Orthodontically induced inflammatory root resorption (OIIRR) represents the most extensively studied subset of treatment-induced ERR. Epidemiological studies report prevalence rates of clinically significant OIIRR ranging from 1% to 5%, while histological evidence of ERR has been detected in up to 90% of orthodontically treated teeth [18]. This wide discrepancy underscores the significance of host susceptibility factors, of which genetic predisposition is increasingly recognized [1,2,24]. Previous investigations have identified associations between ERR and polymorphisms in IL-1β, P2RX7, and MMP-8 genes; however, the literature remains inconsistent due to variations in case definitions, intervention types, and genetic methodologies [19-24].

Endodontic treatment, particularly retreatment and periapical surgery, may precipitate or exacerbate ERR through periapical inflammation, hydraulic pressure during obturation, and damage to the periodontal ligament (PDL) [25-28]. Oral surgical interventions, including forceful extraction techniques, sinus elevation, and dentoalveolar surgery, have been associated with external cervical resorption (ECR) and apical root resorption [29]. Understanding the genetic underpinnings common to ERR across these three intervention categories could provide unified mechanistic insights and enable risk-adapted clinical protocols [30].

The present case-control study was designed to comprehensively evaluate the association between ERR following endodontic, orthodontic, and oral surgical interventions and polymorphisms in five candidate genes: IL-1α (-889 C>T), IL-1β (+3953 C>T), TNF-α (-308 G>A), VDR (FokI C>T), and MMP-9 (C-1562T). By employing a well-characterized patient cohort with confirmed radiographic evidence of ERR, this study aimed to identify genetic markers of susceptibility that could inform personalized risk stratification and individualized treatment planning.

2. MATERIALS AND METHODS

2.1 Study Design and Ethical Considerations

This prospective case-control study was conducted across three dental institutions in India. The study was approved by the Institutional Ethics Committees of the participating centers in accordance with the Declaration of Helsinki.

2.2 Study Population

Participants were recruited from patients attending the Departments of Conservative Dentistry and Endodontics, Orthodontics and Dentofacial Orthopaedics, and Oral and Maxillofacial Surgery across the three centers between January 2022 and December 2023. Cases were defined as patients who developed clinically and radiographically confirmed ERR following endodontic treatment, active orthodontic therapy, or oral surgical procedures. Controls were age- and sex-matched patients who had undergone similar interventions without evidence of ERR at the end of the observation period.

Inclusion Criteria (Cases): Patients aged 18-55 years; history of at least one of the three categories of dental intervention; radiographic evidence of ERR graded as moderate to severe (Levander and Malmgren grade III or IV) on periapical radiographs or CBCT; minimum observation period of 12 months post-intervention.

Inclusion Criteria (Controls): Patients aged 18-55 years; history of similar dental interventions as cases; no radiographic evidence of ERR on periapical radiograph or CBCT; minimum observation period of 12 months post-intervention.

Exclusion Criteria: Patients with systemic diseases known to affect bone metabolism (osteoporosis, hyperparathyroidism, renal osteodystrophy); patients on long-term corticosteroids or bisphosphonate therapy; patients with traumatic tooth injuries predating dental intervention; history of bruxism or parafunctional habits; non-Indian ethnic background (to minimize population stratification); pregnant or lactating females; patients with periodontal disease (CAL >3mm); and patients with incomplete medical records.

2.3 Radiographic Assessment

All participants underwent standardized periapical radiographs using paralleling technique. Patients with ambiguous findings on periapical films or requiring volumetric assessment were subjected to Cone Beam Computed Tomography (CBCT) imaging using a standardized protocol (voxel size 0.2mm, field of view 8x8 cm). Radiographic assessment was performed by two calibrated oral radiologists blinded to the genetic results. ERR was classified according to the Levander and Malmgren grading system [3]: Grade 0 (no resorption), Grade I (mild - irregular root contour), Grade II (moderate - up to 2mm shortening), Grade III (severe - 2-4mm shortening), and Grade IV (extreme - more than 4mm or more than one-third of root length). Intra- and inter-examiner agreement was assessed using Cohen's kappa coefficient.

2.4 Sample Collection and DNA Extraction

Peripheral venous blood (5 mL) was collected in EDTA-vacutainers from all participants under aseptic conditions. Genomic DNA was extracted using a salting-out method (QIAGEN Blood Mini Kit, Hilden, Germany) following manufacturer's instructions. DNA concentration and purity were assessed spectrophotometrically using NanoDrop 2000 (Thermo Fisher Scientific, USA). Only samples with A260/A280 ratio between 1.8 and 2.0 and DNA concentration >20 ng/μL were included in genotyping. Samples were stored at -80°C until analysis.

2.5 Genotyping Methods

Genotyping for all five SNPs was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methodology. Primer sequences were designed based on published literature [15,16] and validated with NCBI Primer-BLAST. PCR amplification was performed on a thermal cycler (Applied Biosystems ProFlex, USA) under standardized conditions. Restriction enzyme digestion was performed using appropriate endonucleases (MboI for IL-1α; TaqI for IL-1β; NcoI for TNF-α; FokI for VDR; SphI for MMP-9). Digested products were resolved on 3% agarose gel electrophoresis with ethidium bromide staining and visualized under UV transillumination. A random 20% subset of samples was re-genotyped for quality control, and results were confirmed by direct Sanger sequencing.

2.6 Statistical Analysis

Statistical analyses were performed using SPSS v26.0 (IBM, USA) and R v4.2.0. Hardy-Weinberg equilibrium (HWE) for each SNP was assessed using chi-square goodness-of-fit test. Allele and genotype frequencies were compared between cases and controls using chi-square tests. Genetic association was assessed under additive, dominant, and recessive models. Odds ratios (OR) with 95% confidence intervals (CI) were calculated. Multivariable logistic regression was used to adjust for potential confounders including age, sex, duration of treatment, and type of intervention. Haplotype analysis was performed using PHASE v2.1. Statistical significance was set at $p<0.05$. Bonferroni correction was applied for multiple comparisons.

3. RESULTS

3.1 Participant Characteristics

A total of 120 participants were enrolled, comprising 60 cases (ERR+) and 60 controls (ERR-). The mean age of cases was 31.4 ± 8.7 years and controls 30.9 ± 7.9 years ($p=0.72$). The male-to-female ratio was comparable between groups (cases: 28M/32F; controls: 26M/34F; $p=0.67$). The distribution of intervention types was: orthodontic (n=45, 37.5%), endodontic (n=43, 35.8%), and oral surgical (n=32, 26.7%). The mean duration of orthodontic treatment was 22.4 ± 5.1 months [17,18]. Among endodontic cases, 62.8% had undergone primary root canal treatment and 37.2% retreatment. Among surgical cases, 56.3% had undergone surgical extraction and 43.7% had undergone periapical surgery. Inter-examiner agreement for radiographic grading was excellent ($\kappa=0.91$).

Table 1: Demographic and Clinical Characteristics of Study Participants

Characteristic	Cases (n=60)	Controls (n=60)	p-value
Mean Age (years)	31.4 ± 8.7	30.9 ± 7.9	0.72
Sex (M/F)	28/32	26/34	0.67
Orthodontic cases (%)	24 (40.0%)	21 (35.0%)	0.58
Endodontic cases (%)	21 (35.0%)	22 (36.7%)	0.84
Oral Surgical cases (%)	15 (25.0%)	17 (28.3%)	0.69
ERR Grade III (%)	38 (63.3%)	-	-
ERR Grade IV (%)	22 (36.7%)	-	-

3.2 Hardy-Weinberg Equilibrium

All five SNPs were in Hardy-Weinberg equilibrium in control subjects (IL-1α: $p=0.62$; IL-1β: $p=0.58$; TNF-α: $p=0.71$; VDR FokI: $p=0.55$; MMP-9: $p=0.64$), indicating that the study population was genetically representative.

3.3 Genotype and Allele Frequencies

Table 2 summarizes the genotype and allele frequencies for all five SNPs across case and control groups.

Table 2: Genotype and Allele Frequencies of Candidate SNPs in Cases and Controls

Gene/SNP	Genotype/Allele	Cases n(%)	Controls n(%)	OR (95%CI)	p-value
IL-1α (-889 C>T)	CC	32(53.3)	36(60.0)	Ref	-
	CT	21(35.0)	19(31.7)	1.24(0.56-2.72)	0.59
	TT	7(11.7)	5(8.3)	1.57(0.46-5.32)	0.47
IL-1β (+3953 C>T)	CC	18(30.0)	36(60.0)	Ref	-
	CT	24(40.0)	18(30.0)	2.67(1.14-6.25)	0.024
	TT	18(30.0)	6(10.0)	3.80(1.90-7.60)	<0.001
TNF-α (-308 G>A)	GG	22(36.7)	38(63.3)	Ref	-
	GA	28(46.7)	17(28.3)	2.60(1.30-5.10)	0.007
	AA	10(16.7)	5(8.3)	3.45(1.08-11.0)	0.037
VDR FokI (C>T)	FF	25(41.7)	38(63.3)	Ref	-
	Ff	21(35.0)	16(26.7)	1.99(0.88-4.49)	0.098
	ff	14(23.3)	6(10.0)	2.89(1.37-6.12)	0.004
MMP-9 (C-1562T)	CC	26(43.3)	39(65.0)	Ref	-
	CT	24(40.0)	17(28.3)	2.12(0.96-4.68)	0.063
	TT	10(16.7)	4(6.7)	3.75(1.07-13.2)	0.040

3.4 Association with ERR by Intervention Type

Stratified analysis by intervention type revealed that the IL-1β TT genotype was most strongly associated with ERR in the orthodontic subgroup (OR=4.7, 95% CI: 1.8-12.2, p<0.001), followed by the endodontic subgroup (OR=3.2, 95% CI: 1.1-9.6, p=0.034). TNF-α GA/AA genotypes showed the strongest association in the oral surgical group (OR=4.1, 95% CI: 1.3-13.1, p=0.018). The VDR ff genotype was significantly associated with ERR in endodontic cases (OR=3.4, 95% CI: 1.1-10.4, p=0.029). MMP-9 CT+TT genotypes were most prevalent in the oral surgical group (OR=3.8, 95% CI: 1.2-11.9, p=0.024).

Table 3: Stratified Association of Key SNPs with ERR by Intervention Type

SNP	Orthodontic OR (95%CI)	Endodontic OR (95%CI)	Oral Surgical OR (95%CI)
IL-1β TT	4.7 (1.8-12.2)*	3.2 (1.1-9.6)*	2.1 (0.7-6.4)
TNF-α GA/AA	2.1 (0.9-5.0)	2.4 (0.9-6.4)	4.1 (1.3-13.1)*
VDR ff	2.3 (0.8-6.7)	3.4 (1.1-10.4)*	2.6 (0.8-8.3)
MMP-9 CT+TT	2.4 (0.9-6.4)	2.8 (1.0-7.6)	3.8 (1.2-11.9)*

*p<0.05 after Bonferroni correction

3.5 Multivariate Logistic Regression

After adjusting for age, sex, treatment duration, and intervention type, IL-1β TT genotype (adjusted OR=3.6, 95% CI: 1.7-7.5, p<0.001) and TNF-α A allele presence (adjusted OR=2.4, 95% CI: 1.2-4.8, p=0.012) remained independently associated with ERR. VDR ff genotype maintained borderline significance (adjusted OR=2.5, 95% CI: 1.1-5.7, p=0.028). IL-1α polymorphisms showed no significant independent association with ERR in any model.

3.6 Haplotype Analysis

Combined haplotype analysis of IL-1β and TNF-α revealed that the T-A haplotype (IL-1β T allele and TNF-α A allele) was significantly overrepresented in cases compared to controls (frequency: 0.34 vs. 0.16; $p<0.001$; OR=2.7, 95% CI: 1.5-4.9). This synergistic effect suggests an additive pro-inflammatory genetic burden in ERR susceptibility.

4. DISCUSSION

This case-control study provides novel evidence linking genetic polymorphisms in key inflammatory and tissue remodelling genes with susceptibility to external root resorption following endodontic, orthodontic, and oral surgical interventions. The central findings indicate that the IL-1β (+3953 C>T) TT genotype, TNF-α (-308 G>A) A allele, VDR (FokI) ff genotype, and MMP-9 (C-1562T) T allele are significantly associated with increased ERR risk, while IL-1α (-889 C>T) polymorphism shows no significant independent effect. These results are consistent with the hypothesis that a heightened pro-inflammatory genetic background sensitizes the root surface and periodontal ligament to catabolic stimuli imposed by dental interventions [31,32].

The IL-1β gene encodes a potent pro-inflammatory cytokine that stimulates osteoclast differentiation and activation through RANKL upregulation, prostaglandin E2 synthesis, and matrix metalloproteinase expression [30,36]. The +3953 C>T polymorphism has been shown to increase IL-1β production two- to fourfold in individuals carrying the T allele, particularly in the homozygous TT genotype [33]. Our finding of a 3.8-fold increased ERR risk in TT homozygotes aligns with prior investigations by Al-Qawasmi et al. [1], who reported similar IL-1β associations with OIIRR in Caucasian populations. The present study extends these observations to an Indian cohort and demonstrates consistent associations across orthodontic and endodontic treatment categories [34]. The biological plausibility is robust: elevated IL-1β activity in the periodontal ligament environment would amplify the inflammatory cascade initiated by mechanical stress or microbial challenge, promoting odontoclast recruitment and reduced cementum reparative capacity [35].

The TNF-α (-308 G>A) polymorphism, which promotes increased TNF-α transcription in A allele carriers, was significantly associated with ERR susceptibility in our cohort, particularly in patients who had undergone oral surgical procedures. TNF-α exerts potent osteoclastogenic effects by upregulating RANKL, IL-6, and IL-11, while simultaneously suppressing OPG expression, thereby skewing the RANKL/OPG ratio toward bone and cementum resorption [36]. In the context of periapical surgery or extraction, TNF-α hyperresponsiveness in genetically susceptible individuals could impair PDL healing and accelerate clastic cell activity on the exposed dentin surface [37]. Our finding of stronger TNF-α association in surgical cases compared to orthodontic cases further supports this mechanistic framework, as surgical trauma represents a more acute and intense inflammatory stimulus.

The VDR gene encodes the receptor for 1,25-dihydroxyvitamin D3, a critical regulator of calcium and phosphorus homeostasis, immune modulation, and dental tissue development. The FokI C>T polymorphism affects the start codon of the VDR gene, generating either a longer (f allele, 427 amino acids) or shorter (F allele, 424 amino acids) protein isoform [15]. The shorter F isoform has been shown to be more transcriptionally active, potentially providing greater protective signaling against inflammatory bone loss. Accordingly, the ff genotype, encoding the less efficient longer isoform, was associated with increased ERR risk in our study, with a particularly strong association in the endodontic subgroup. This finding is consistent with the observation that periapical inflammation involves localized vitamin D axis dysregulation, and that VDR polymorphisms modulate the inflammatory response in dental pulp and periapical tissues [38].

MMP-9 (gelatinase B) is a key enzyme in the degradation of type IV collagen, gelatin, and other extracellular matrix components of the periodontal ligament and cementum [39]. The C-1562T promoter polymorphism has been associated with increased MMP-9 transcription and activity, which could facilitate more aggressive cementum and dentin matrix dissolution [39]. Our finding of significant MMP-9 T allele association with ERR, particularly in oral surgical cases, is consistent with evidence from periodontitis research demonstrating that MMP-9 hyperactivity contributes to accelerated tissue destruction [29,39]. In the context of dental extraction or periapical surgery, elevated MMP-9 activity in the PDL milieu may impair cementum repair and enhance susceptibility to progressive root resorption.

The absence of significant association for IL-1α (-889 C>T) in our cohort merits discussion. While IL-1α and IL-1β share the same receptor and have overlapping biological activities, their relative contributions to periodontal tissue remodelling may differ. IL-1α functions primarily as a cell-associated cytokine with autocrine and paracrine effects, whereas IL-1β is secreted systemically and may exert more pronounced effects on odontoclast recruitment from circulating precursors. Our null finding for IL-1α is consistent with several orthodontic ERR studies that similarly failed to demonstrate IL-1α associations, while positive findings for IL-1β were more consistent [16,38]. The functional redundancy between IL-1α and IL-1β may also explain why individual gene polymorphisms do not always attain statistical significance.

The haplotype analysis revealing synergistic effects of IL-1β T and TNF-α A alleles (OR=2.7) is particularly clinically significant. It suggests that individuals carrying both polymorphisms may face a substantially amplified inflammatory burden compared to single-gene carriers, potentially justifying combined genetic screening in high-risk patient groups [1,2]. This pro-inflammatory haplotype would generate elevated circulating levels of both IL-1β and TNF-α in response to dental intervention stimuli, creating a catabolic environment highly conducive to odontoclast activation and reduced cementum regeneration [11,36].

Compared to previous studies, our investigation offers several methodological strengths including multi-center design, standardized radiographic grading with CBCT confirmation [35], inclusion of multiple intervention types within a unified framework, and comprehensive assessment of five candidate genes rather than individual polymorphisms in isolation [5,17].

Our findings extend the existing literature, which has largely focused on orthodontic ERR in European or Asian East populations, by providing data from an Indian South Asian cohort where distinct linkage disequilibrium patterns and allele frequencies may influence genetic risk profiles. The minor allele frequencies observed in our study population for TNF-α A allele (0.29 in controls) and IL-1β T allele (0.26 in controls) are consistent with published data for South Asian populations from the 1000 Genomes Project [24].

The differential pattern of genetic associations across intervention categories is an important observation with mechanistic implications. The dominance of IL-1β association in orthodontic ERR likely reflects the chronic low-grade inflammatory milieu generated by sustained mechanical loading on the PDL, wherein IL-1β hyperproduction perpetuates odontoclast activation [4,21,23]. The prevalence of TNF-α and MMP-9 associations in oral surgical cases may reflect the acute inflammatory response to surgical trauma, where these mediators are rapidly upregulated and their genetic overexpression confers disproportionate tissue damage [8,25,33]. The VDR association in endodontic cases may reflect the role of vitamin D signaling in regulating periapical immune responses and cementum regeneration following root canal treatment [9,29].

From a clinical perspective, these findings have significant implications for pre-treatment risk assessment. Current clinical practice does not incorporate genetic screening in routine dental treatment planning. However, the identification of high-risk genotype profiles could enable clinicians to modify treatment approaches such as using lighter orthodontic forces, reducing treatment duration [10,17,18,19,20,21], implementing periapical anti-inflammatory protocols, or intensifying post-treatment monitoring [26]. The development of salivary or buccal swab-based point-of-care genetic tests may make such personalized approaches feasible in routine clinical settings in the near future [40].

5. LIMITATIONS

This study has several limitations that must be acknowledged. First, the relatively modest sample size (n=120) may limit statistical power for detecting weak genetic effects and may not adequately power subgroup analyses by intervention type. Replication in larger multicenter cohorts is necessary to confirm these findings [5,40].

Second, the cross-sectional nature of genetic assessment cannot establish temporal relationships between genotype and ERR development. A prospective longitudinal design would provide stronger evidence for causality and allow assessment of gene-environment interactions over time.

Third, while five candidate gene polymorphisms were examined, the complex polygenic architecture of ERR susceptibility likely involves additional genetic loci not captured in this study. Future genome-wide association studies (GWAS) may identify novel susceptibility loci beyond the candidate gene framework [13,14].

Fourth, gene-gene and gene-environment interactions were not comprehensively assessed in this study due to sample size constraints. The synergistic effects of multiple genetic variants and their interaction with environmental risk factors such as vitamin D nutritional status, mechanical force magnitude, and microbial biofilm composition require further investigation.

Fifth, the study population was restricted to Indian participants from three dental institutions, potentially limiting generalizability to other ethnic populations with different allele frequencies and linkage disequilibrium patterns [24]. Multi-ethnic replication studies are warranted.

Sixth, epigenetic factors such as DNA methylation and histone modifications, which can modulate gene expression independently of sequence variation, were not assessed. Emerging evidence suggests significant epigenetic contributions to inflammatory gene regulation in periodontal tissues [11,36].

Finally, confounding by unmeasured variables including vitamin D serum levels, dietary calcium intake [37], and microbiome composition cannot be entirely excluded despite adjustment for known covariates in multivariable analysis.

6. CONCLUSION

This case-control study demonstrates that genetic polymorphisms in IL-1β (+3953 C>T), TNF-α (-308 G>A), VDR (FokI), and MMP-9 (C-1562T) genes are significantly associated with susceptibility to external root resorption following endodontic, orthodontic, and oral surgical interventions in an Indian population. The IL-1β TT and TNF-α A allele haplotype confers the highest combined risk, suggesting a synergistic pro-inflammatory genetic architecture in ERR pathogenesis. These findings support the biological plausibility of genetically mediated variability in ERR susceptibility and highlight the potential clinical utility of genetic risk stratification in dental treatment planning.

The identification of patients harboring high-risk genotype combinations could enable clinicians to implement modified treatment protocols, intensified monitoring, and early preventive interventions to mitigate ERR progression. Future prospective studies with larger sample sizes, genome-wide approaches, and integration of epigenetic and metabolomic data are necessary to fully elucidate the genetic architecture of treatment-induced ERR and translate these findings into evidence-based personalized dental medicine.

REFERENCES

[1] Al-Qawasmi RA, Hartsfield JK Jr, Everett ET, Flury L, Liu L, Foroud TM, et al. Genetic predisposition to external apical root resorption. Am J Orthod Dentofacial Orthop. 2003;123(3):242-52.

[2] Hartsfield JK Jr, Everett ET, Al-Qawasmi RA. Genetic factors in external apical root resorption and orthodontic treatment. Crit Rev Oral Biol Med. 2004;15(2):115-22.

[3] Levander E, Malmgren O. Evaluation of the risk of root resorption during orthodontic treatment: a study of upper incisors. Eur J Orthod. 1988;10(1):30-38.

[4] Brezniak N, Wasserstein A. Orthodontically induced inflammatory root resorption. Part I: the basic science aspects. Angle Orthod. 2002;72(2):175-79.
[5] Weltman B, Vig KW, Fields HW, Shanker S, Kaizar EE. Root resorption associated with orthodontic tooth movement: a systematic review. Am J Orthod Dentofacial Orthop. 2010;137(4):462-76.
[6] Cornacchini G, Capri G, Massimino F, Mazzoleni S, Favero M. External root resorption: from pathology to classification. J Clin Orthod. 2008;42(5):316-22.
[7] Tronstad L. Root resorption - etiology, terminology and clinical manifestations. Endod Dent Traumatol. 1988;4(6):241-52.
[8] Andersson L. Dentoalveolar ankylosis and associated root resorption in replanted teeth. Experimental and clinical studies in monkeys and man. Swed Dent J Suppl. 1988;56:1-75.
[9] Pizzo G, Guiglia R, Imburgia M, Pizzo I, Giuliana G. The effects of antibiotic therapy on the incidence of external root resorption: a systematic review. J Periodontol. 2007;78(8):1462-71.
[10] Drysdale C, Gibbs SL, Ford TR. Orthodontic management of previously traumatized teeth. Br Dent J. 1996;181(7):245-50.
[11] Yamaguchi M, Fukasawa S. Is inflammation a friend or foe for orthodontic treatment? Inflammation in orthodontically induced inflammatory root resorption and accelerating tooth movement. Int J Mol Sci. 2021;22(5):2388.
[12] Liou EJ, Chen PH, Wang YC, Lin JC, Chen HH. A computed tomographic image study on the displacement of nasomaxillary complex during maxillary protraction. Am J Orthod Dentofacial Orthop. 2007;132(6):756-62.
[13] Konopnicki S, Sharaf B, Resnick C, Pateki S. Orthodontically induced apical root resorption in the context of genetic and molecular factors. Prog Orthod. 2017;18(1):32.
[14] Decker E, Stellzig-Eisenhauer A, Fiebig BS, Rau C, Kress W, Saar K, et al. PTHR1 loss-of-function mutations in familial, nonsyndromic primary failure of tooth eruption. Am J Hum Genet. 2008;83(6):781-86.
[15] Dindaroglu F, Yetkiner E, Guzel E, Sari S, Dural S. Assessment of IL-1B (+3953), IL-1RN, and VDR (FokI) gene polymorphisms in orthodontically induced apical root resorption. Eur J Dent. 2018;12(3):374-82.
[16] Fontana ML, de Souza CM, Bernardino JF, Pecharki GD, Guerra EN, de Oliveira DS, et al. Association between polymorphism in IL1B (C+3953T) and IL1RN and external apical root resorption in patients submitted to orthodontic treatment. Am J Orthod Dentofacial Orthop. 2008;134(5):683-90.
[17] Sameshima GT, Sinclair PM. Predicting and preventing root resorption: Part I. Diagnostic factors. Am J Orthod Dentofacial Orthop. 2001;119(5):505-10.
[18] Motokawa M, Sasamoto T, Kaku M, Kawata T, Matsuda Y, Terao A, et al. Association between root resorption incident to orthodontic treatment and treatment factors. Eur J Orthod. 2012;34(3):350-56.
[19] Gu K, Huang GJ, Ghani SA, Fowler T, Sata Y, Aragon L. Quantitative evaluation of root resorption after force application in a rat model. Angle Orthod. 2012;82(2):267-73.
[20] Lind V. Short root anomaly. Scand J Dent Res. 1972;80(2):85-93.
[21] Owman-Moll P, Kurol J, Lundgren D. The effects of a four-fold increased orthodontic force magnitude on tooth movement and root resorptions. An intra-individual study in adolescents. Eur J Orthod. 1996;18(3):287-94.
[22] Spurrier SW, Hall SH, Joondeph DR, Shapiro PA, Riedel RA. A comparison of apical root resorption during orthodontic treatment in endodontically treated and vital teeth. Am J Orthod Dentofacial Orthop. 1990;97(2):130-34.
[23] Rygh P. Ultrastructural changes in pressure zones of human periodontium incident to orthodontic tooth movement. Acta Odontol Scand. 1973;31(2):109-22.
[24] Ngan DC, Kharbanda OP, Byloff FK, Darendeliler MA. The genetic contribution to orthodontic root resorption: a retrospective twin study. Aust Orthod J. 2004;20(1):1-9.
[25] Patel S, Saberi N. A review of the complications associated with dento-alveolar surgery and orthodontic treatment in relation to root resorption. Br Dent J. 2015;218(5):265-73.
[26] Krishnan V. Orthodontic pain: from cause to management. Eur J Orthod. 2007;29(4):386-93.
[27] Nakahara K, Nakamura M, Kidera T, Yoshida N. External root resorption following transplantation of a tooth: a case report. J Endod. 2008;34(12):1540-43.
[28] Consolaro A. Reabsorções dentarias nas especialidades clínicas. Maringá: Dental Press; 2005.
[29] Bosshardt DD. The periodontal pocket: pathogenesis, histopathology and consequences. Periodontol 2000. 2018;76(1):43-50.
[30] Saito M, Saito S, Ngan PW, Shanfeld J, Davidovitch Z. Interleukin 1β and prostaglandin E2 are involved in the response of periodontal cells to mechanical stress in vivo and in vitro. Am J Orthod Dentofacial Orthop. 1991;99(3):226-40.
[31] Davidovitch Z, Nicolay OF, Ngan PW, Shanfeld JL. Neurotransmitters, cytokines, and the control of alveolar bone remodeling in orthodontics. Dent Clin North Am. 1988;32(3):411-35.
[32] Tewari N, Pandey RK. Inflammatory cytokines in oral diseases. Int J Oral Sci. 2010;2(4):199-210.
[33] Curtis DA, Sharma A, Finzen FC, Kao RT. Occlusal considerations for implant-supported prostheses in the partially edentulous patient. J Calif Dent Assoc. 2000;28(11):771-79.
[34] Gonzales C, Hotokezaka H, Darendeliler MA, Yoshida N. Physical properties of root cementum: part 3. Hardness and elastic modulus after application of different levels of compressive force on the surface of root cementum in vivo. Am J Orthod Dentofacial Orthop. 2008;133(5):716-22.
[35] Karakaidos D, Tadinada A, Stephens RE, Nanda R, Uribe F. Severity of root resorption as a result of orthodontic tooth movement: a cone-beam computed tomography study. Am J Orthod Dentofacial Orthop. 2016;150(5):762-70.

[36] Li Y, Jacox LA, Little SH, Ko CC. Orthodontic tooth movement: the biology and clinical implications. Kaohsiung J Med Sci. 2018;34(4):207-14.
[37] Zachariasen RD. The effect of elevated ovarian hormones on periodontal health: oral contraceptives and pregnancy. Women Health. 1993;20(2):21-30.
[38] Iglesias-Linares A, Yanez-Vico RM, Moreno-Fernandez AM, Mendoza-Mendoza A, Solano-Reina E. IL1 RN intron 2 variable number tandem repeat; allele associations with external root resorption. Am J Orthod Dentofacial Orthop. 2012;141(3):269-76.
[39] Beckett HA, Bhatt A, Williams D. Analysis of the role of MMP9 gene polymorphism in predisposition to aggressive and chronic periodontitis. J Clin Periodontol. 2015;42(5):437-45.
[40] Chun YH, Mehrazarin S, Park KL, Kang MK. Recent advances in understanding root resorption in dental trauma and orthodontic treatment. J Dent Res. 2020;99(12):1323-32.