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Review Article

Biosignature molecules: A Roadmap to Endodontic Diagnosis - A Review Article

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ABSTRACT:
An accurate diagnosis serves as the foundation for successful treatment. Biological markers function as a major diagnostic parameter of the underlying physiology and health of the tissue at the cellular or molecular level[1] Biomarkers play a crucial role in the pathogenesis of pulpal and periapical pathoses. This paper highlights the biomarkers involved in the development of the dentin-pulp complex and the pathogenesis of pulpal and periradicular pathologies.
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INTRODUCTION

For precise identification of the target disease along with the pathophysiology, molecular biology plays a pivotal role. Biomarkers function as a vital diagnostic parameter depicting the inflammatory status of the dental pulp. According to Hulka[2] and colleagues, Biological markers (biomarkers) are defined as- cellular, biochemical or molecular alterations that are measurable in biological media such as human tissues, cells, or fluids. The stable nature of micro RNA proteins and the ease of sampling from pulpal blood brings out their potential as a precise endodontic diagnostic tool. A biomarker[3] is a characteristic that can be measured objectively and evaluated as an indicator of typical biological and pathological processes, as well as pharmacologic responses to a therapeutic intercession. A plethora of biomolecules have been studied in pulpal health and disease using a variety of analytical methods[4] such as WesternBlot, Microarray, ELISA, Radioimmunoassay, RD, Flow Cytometry, Zymography, RT-PCR, Dentinal Fluid, Flourescent Assay, MPO Assay, Radioreceptor assay, NanoOrange (extremely sensitive nanoassays). Biomarkers help in early diagnosis and detection of endodontic pathologies, prognosis, monitoring the effectiveness of the treatment, providing tailor made treatment plans and providing an excellent treatment outcome[5,6].

Various biomarkers of endodontic importance

1. **Matrix Metalloproteinases 8 (MMP-8):** MMP-8 is one of the hopeful biomarkers for pulp inflammation diagnosis. In patients diagnosed with pulpitis, MMP-8 levels were significantly higher in the GCF samples compared with the healthy group. Also the MMP-8 levels in GCF were decreased during root canal treatment, and therefore showing a positive correlation with each other. According to Dincer[7] *et al*. MMP-8 increased significantly in pulp tissue and GCF specimens of symptomatic irreversible pulpitis teeth compared with pulp tissue and GCF specimens of healthy teeth. They also found that MMP-8 levels were assessed to be higher in pulp tissue samples of the patients with symptomatic

irreversible pulpitis with higher pain scores than those with low pain scores.

2. **Matrix Metalloproteinases 9 (MMP-9):** According to the results of the study conducted by Gusman[8] *et al.* MMP-9 was positively associated with irreversible pulpitis, the high levels of MMP-9 in inflamed pulps and its positive correlation with gelatinolytic activity are suggestive of a key mediator role in the breakdown of inflamed human dental pulp tissue. MMP-9 appears to be a promising marker protein to assess pulpal health.
3. **Interleukin-6 (IL-6):** It is upregulated in the tissues of irreversible pulpitis but its enhanced activity in GCF in irreversible pulpitis has not been demonstrated. IL-6 is found in dentinal fluid of pulpitis, however, there is no significant difference between reversible and irreversible pulpitis. In a study by Barkhordar[9] *et al*, concluded that significant quaitities of IL-6 were released in the pulp and periapical tissues of patients with pulpitis and apical periodontitis. In contrast, noninflamed pulpal tissue contained negligible amounts of the IL-6.
4. **Alkaline Phosphatase (ALP):** Pulp cells show high levels of ALP activity during repair and healing after pulpal injury. In the study conducted by Spoto[10] *et al*. ALP activity was analyzed in healthy human dental pulps, in reversibly and in irreversibly inflamed pulp and it was found that ALP activity was increased upto eight times in irreversibly inflamed pulp as compared to healthy pulp.
5. **CXC chemokine ligand 10 (CXCL10):**A member of the non-ELR CXC family of chemokines. In pulpitis, the selective aggregation of lymphocyte subsets into inflamed dental pulp can be regulated by interactions with CXCL10-CXCR. In a study conducted by Adachi[11] *et al*. an increased expression level of CXCL10 was detected in inflamed dental pulp tissues. Thus, it can be suggested that the CXCL10-CXCR3 system may be involved in the pathogenesis of pulpitis.
6. **Vascular endothelial growth factor (VEGF):** is a signal protein produced by cells which stimulates blood vessel formation. It improves vascular proliferation and permeability in pulp tissue. In a study by Artese[12] *et al.* revealed that the expression of VEGF was strongly positive in the inflammatory infiltrate in irreversible pulpitis. A statistically significant difference was observed in VEGF expression in the stromal cells in healthy pulps. In another study the decrease in microvessel density in irreversible pulpitis could be related to failure in vascular function and blood flow decrease.
7. **Osteocalcin(OCN):** According to Elmeguid[13] *et al*. the expression of OCN markedly increased in the reversible stages of pulpal inflammation. OCN is positively correlated to angiogenic markers such as Fibroblast growth factor and VEGF.
8. **Chemokines[14] (RANTES, IP-10 and MCP-1):** A family of small proteins (8-10kD) associated with the migration and activation of leukocytes and selectins, which account for the adhesion of inflammatory cells to the walls of the pulp blood vessels. High levels of chemokine CXCL12/SDF-1 are found in periapical inflammatory lesions.
9. **Tumor necrosis factor alpha (TNFA/TNF**α**):** Several studies have shown that TNFα is highly expressed in inflamed dental pulp tissue. In addition, TNFα is increased in the dentinal fluid of reversible and irreversible pulpitis. According Ribaric[15] *et al*. TNFα - can be found in all vital human pulpal tissues but highest concentrations of this protein were found in irreversible symptomatic pulpitis, slightly less in irreversible asymptomatic pulpitis, while lowest TNFα - concentration was found in healthy samples. This indicates that TNFα - may be an objective marker for laboratory determination of extent of pulpal inflammation.
10. **Receptor for advanced glycation end products (RAGE) and High-Mobility Group Box 1 (HMGB-1):** Tancharoen[16] *et al*. in a study found that RAGE and HMGB1 expression levels in inflamed dental pulp were higher than those in healthy dental pulp. Upregulated expression of RAGE was observed in odontoblasts, stromal pulp fibroblasts-like cells, and endothelial-like cell lining human pulpitis tissue.

DISCUSSION

The lack of reliable methods in the assessment of the level of inflammation and accurately determining the status of the dental pulp is a major drawback in effectively developing pulp preservation therapies in clinical practice (Duncan[17], 2022). Use of biological markers along with clinical signs and symptoms helps in assessing the outcome.

The goal of molecular diagnostics should be to develop a chair-side lateral flow-type assay that targets an individual molecule, which, if present above a specified threshold level, indicates disease and disease severity. A limitation in pulpal diagnosis is the use of gold standard test for comparison. Histological examination is the benchmark for assessing pulp inflammatory state but this is not clinically feasible or appropriate and an alternative way to compare levels of biomarkers to the clinical diagnosis (Zehnder & Belibasakis[18], 2021),based on the presence of pain which was previously reported to be the most reliable indicator of IRP (Cabello & Egea[19], 2005).

Most of the studies revealed that certain key inflammatory mediators, such as IL-6 and IL-8, could

be used as biomarkers to discriminate between healthy pulps and those with symptomatic IRP. Subaric[20] *et al* found that IL-1beta was present in pulp tissue samples with highest concentration in pulpitis. Salum[21] *et al* isolated IL-6 in 92.8% of cyst fluid of the studied tissue samples, indicating its importance in the pathogenesis. Zehnder[18] *et al* were the first to provide a clinical relevance for the use of MMP-9 as a marker for pulpal pathoses by assessing the levels of MMP-9 in the dentinal fluid of symptomatic teeth diagnosed with irreversible pulpitis and healthy counterparts.

Human pulp fibroblasts and osteoblasts express collagenase, that can be stimulated with cytokines, provides evidence that MMPs are involved in controlling protective responses to external irritation in the human dentin - pulp complex.

Martinho *et al* established, correlation between the size of the radiologic image of periapical cysts and IL-6 levels, indicating the latter's role, in bone resorption process within periapical cyst. Silva[22] *et al* isolated higher level of cytokines, in inflamed pulp than those from healthy pulp. Sirma[23] *et al* confirmed the essentiality of VEGF and FGF, for the repair of pulp during injury. Sattari[24] *et al* found a similar pattern of healing with OCN and FGF. OCN is secreted during reversible pulpitis, which correlates to the expression of the MCP-1, MIP-1b, sIL-2ra and MDC as proinflammatory mediators. Thus, it can be safely assumed that OCN partially controls, pulp repair in reversible pulpitis, which diminishes with progression of inflammation and expression of IL-1 alpha and IL-1 beta.

Challenges and future perspectives

The focus on a single candidate biomolecule approach for analysis may not truthfully reflect the complexity of pulp inflammation. Future studies should explore the potential of bio-markers in pulpal diagnosis where multiple genes or factors are considered as a cassette for defining disease status.The future[25] of biomarkers require technological advancements, integration of user friendly kits, proper validation and standardization of care.

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