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An Unusual Insight into Biomarkers in Dentin – Pulp Complex

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Abstract

Introduction: Biomarkers are functional elements at cellular and molecular level which play an important role in both health and disease conditions. In teeth dentin-pulp complex houses several biomarkers responsible for different tissues in the teeth and lack of these biomarkers yield developmental disorders. **Methodology:** Several electronic databases were used to conduct a computerized search for available evidence: MEDLINE, PUBMED, SCOPUS, NLM and other non – indexed citations. Articles that appeared to accomplish the inclusion/exclusion criteria, based on their abstracts were selected. Some abstracts provided insufficient information and to make a selection decision, the entire article was also obtained. A modified scoring system was used to validate the data. **Results:** Only 51 articles matched our criteria and thus selected. Conclusion: the review suggests that the usage of growth factors would be a turnaround in the way we would be treating a tooth.

Keywords: Markers in healthy tooth, diagnostic markers in dentin and pulp, dentin – pulp complex.

Introduction

Pulp inflammation involves several biological processes which can be evaluated at the macroscopic, microscopic, and various molecular levels. Initially inflammatory mediators dominate the pulp tissue and later, the molecular phase precedes both qualitatively and quantitatively. Molecular diagnosis analyses the genome and proteome and medical testing is based on expressions of biomarkers. Biomarkers are responses that are functional, biochemical at the cellular level, or

molecular interaction. They are extensively used in research which is a boon in the medical field to aid in diagnosis and treatment [1].

The term "biomarker" was coined from "BIOLOGICAL MARKER" which pertains to an extensive subcategory of medical signs and can be measured accurately and reproducibly [1].

The National Institutes of Health Biomarkers Definitions Working Group has defined a biomarker as a "Characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" [1]

The first step in the diagnosis is to understand the nature or propensity of a biomarker expressed at the particular site of the healthy tissue and differentiates it from inflammatory condition.

The second step is to determine cut-off points for tests using these inflammatory mediators. The third step is to provide and develop incentives that help and detect these biomolecules. The methods should be easy to execute along the chairside to allow for their adoption into therapy. The inflammatory pulp disease is a complex phenomenon hence a single mediator would not provide sufficient information to diagnose it. However, evaluation of protein expression appears to be the most appropriate method for clinical diagnosis of dental pulp inflammation.

Biomarkers are being extensively used in research and it has shown to be a boon in the medical field to aid in diagnosis and treatment. This article represents authors attempt to review the various biomarkers present in the dentin-pulp complex in health and the next series of articles give a review on biomarkers in pulpal diseases and their collection methods.

The objective of this review is to evaluate the scientific evidence concerned with the effect of various biological markers on dentin-pulp complex.

Methods

Source of Information: Several electronic databases were used to conduct a computerized search for available evidence: MEDLINE, PUBMED, SCOPUS, NLM and other non – indexed citations.

Search Strategy: Terms used in the literature search were Markers in healthy tooth, diagnostic markers in dentin and pulp, dentin – pulp complex, growth factors, tooth development

Inclusion Criteria

The following inclusion criteria were chosen to select the potential articles from the published abstract results

1. Markers involving development of healthy tooth structure
2. Biological markers in dentin – pulp complex

Studies with the following criteria were excluded:

1. Markers involving healthy tooth development in saliva, Periodontium.
2. Markers in diseased conditions of teeth.

Search and Selection Process:

The articles that appeared to accomplish the inclusion/exclusion criteria, based on their abstracts were selected. Some abstracts provided insufficient information and to make a selection decision, the entire article was also obtained. Few titles which suggested bearing with the topic were selected from the databases even though abstracts where not available. The abstract selection was performed independently by 3 researchers. Consensus was arrived after careful discussion in selection. Additional analysis was done before the final selection of the articles. An agreement

was reached regarding which should be finally included in the review.

Methodological Evaluation

A modified scoring system by was chosen in the evaluation of the chosen articles. Validation of the study was done by subjecting the article to the mentioned scoring system. The following methodological score list was used [2] [Table 1].

S.NO	METHODOLOGICAL SCORE FOR CLINICAL STUIDES
1.	STUDY DESIGN [5 POINTS] A. Objective – objective clearly formulated B. Selection criteria – described and adequate C. Baseline characteristics – similar between the groups D. Timing – prospective E. Timing – long term follow-up
2.	STUDY MEASUREMENTS: F. Blind measurement – blinding G. Reliability - described
3.	STATISTICAL ANALYSIS H. Statistical analysis – appropriate for data I. Confounders – included in analysis J. Statistical significance level – P- level stated

*Maximum number of points – 10

TABLE – 1: MODIFIED SCORING SYSTEM [2]

The methodological quality of the selected articles can be found in Table 2

Table 2: METHODOLOGICAL SCORE OF SELECTED ARTICLES

ARTICLE	A	B	C	D	E	F	G	H	I	J	Total no. of points
A. Unterbrink, M. O'Sullivan [5]	1	1	1	0	0	0	1	1	1	0	6
Thomas aberg, john wozney et al [7]	1	1/2	1/2	0	0	0	1/2	0	0	0	2.5
W. Yang, M.A. Harris et al [8]	1	1	1	0	0	0	1/2	1	1	0	5.5
Xiu-Ping Wang, Marika Suomalainen et al [11]	1	1	1	0	0	0	1	1	1	1	7
john d. hood, Cynthia j. meininger et al [16]	1	1	1	0	0	0	1/2	1	1	1	6.5
Javier Caviedes-Bucheli et al [18]	1	1	1	0	0	0	1/2	1	1	1	6.5
Karthikeyan Narayanan et al [19]	1	1	1	0	0	0	1	1	1	1	7
Atul Suresh Deshpande et al [20]	1	1	1	0	0	0	0	0	0	0	3
Karthikeyan Narayanan et al [21]	1	1	1	0	0	0	0	0	0	0	3
Mary E. Marsh et al [22]	1	1	1	0	0	0	0	0	0	0	3
Angela Quispe Salcedo et al [26]	1	1	1	0	0	0	0	0	0	0	3
A. Hjerpe et al [27]	1	1	1	0	0	0	0	0	0	0	3
Ira L. Shannon et al [28]	1	1	1	0	0	0	1	1	1	1	7
Gorter de vries et al [30]	1	1	1	0	0	0	0	0	0	0	3
Georgios Kostoulas et al [32]	1	1	1	0	0	0	0	0	0	0	3
Po-Yen Lin et al [33]	1	1	1	0	0	0	0	0	0	0	3
Shiamalee Perumal et al [34]	1	1	1	0	0	0	0	0	0	0	3
Tsui Hsien Huang et al [35]	1	1	1	0	0	0	1	1	1	1	7
N. Cassidy et al [36]	1	1	1	0	0	0	1	1	1	0	6
Julia L. McLachlan et al [37]	1	1	1	0	0	0	1	1	1	1	7
Leif olgart et al [38]	1	1	1	0	0	0	0	0	0	0	3
Walter R. Bowles [41]	1	1	1	0	0	0	1	1	1	1	7
Kenzo TAKAHASH et al [43]	1	1	1	0	0	0	0	0	0	0	3
L. Awawdehm et al [46]	1	1	1	0	0	0	1	1	1	1	7
Sunil Wimalawansa et al [48]	1	1	1	0	0	0	1	1	1	0	6

The following biomarkers are considered prime important and have their effect on dentin- pulp complex:

TABLE – 3: BIOMARKERS IN DENTIN-PULP COMPLEX

BIOMARKERS IN DENTIN:

BIOLOGICAL EFFECTS OF DENTIN MATRIX MOLECULES [3]:

A. GROWTH FACTORS:

1. TGF-beta: Chemotaxis, cell Proliferation, Odontoblastic differentiation, Dentinogenesis.
2. BMP -2: Odontoblastic differentiation, Dentinogenesis
3. BMP-4: Odontoblastic differentiation, Dentinogenesis
4. BMP-7: Dentinogenesis
5. GDF: Odontoblastic differentiation, Dentinogenesis
6. FGF: Chemotaxis, cell proliferation, Dentinogenesis
7. VEGF: Cell proliferation, Angiogenesis
8. IGF: Cell proliferation, Odontoblastic differentiation.
9. PDGF: Cell migration, Dentin matrix synthesis, cell proliferation, Odontoblastic differentiation, Angiogenesis

B.NON-COLLAGENOUS PROTEINS:

1. DSP: Not determined
2. DPP: Dentinogenesis
3. DMP: Dentinogenesis
4. BSP: Dentinogenesis
5. OPN: Inhibition of dentinogenesis

C.PROTEOGLYCANS:

1. Chondroitin sulfate: Dentinogenesis
2. Dermatan sulfate: Dentinogenesis

BIOMARKERS IN DENTIN –PULP COMPLEX IN HEALTH [4]

1. Osteocalcin
2. Osteonectin
3. Dentin sailophosphoprotein
4. Matrix metalloproteinase
5. Transforming growth factor-beta1
6. Bone Morphogenic protein-2
7. Cathepsin
8. Bone Sialoprotein

Biomarkers in dentin

Transforming growth factor beta: TGF-β1 plays a role in regulating cell proliferation, differentiation, and reparative dentinogenesis.

Latent TGF-β1 synthesized in dental pulp is activated by matrix metalloproteinase 2[MMP 2]. Activated TGF-β1 enhances the m-RNA expression levels of MMP- 20 and DSPP in pulp, and it coincides with induction of odontoblast differentiation. Latent TGF-β1 synthesized in odontoblasts is activated by MMP 2 and MMP -20 both in odontoblasts and dentin. In odontoblasts, it serves the purpose of transcriptional regulation of two non-collagenous proteins: dentin sialo phosphoprotein (DSPP) and dentin matrix protein 1 (DMP1) [5]. However, its activity was reduced in dentin and is further reduced with degradation of DSPP- derived proteins that occur with ageing [6] [Fig 1].

Bone Morphogenic Protein [BMP]

BMPs direct cell differentiation, dentin synthesis, maturation and gene expression in the fully patterned tooth. BMP4 expression appears in the mesenchyme at the beginning of the bud stage, and inactivation of the BMP receptor [Bmpr1a] in epithelial or mesenchymal layers arrests development between the bud and cap stages, highlighting the essential role of BMP signalling in early tooth progression [7]. BMP expression continues past the cap stage to tooth maturity, also play a role in dentin and enamel formation after initial tooth patterning.

Feng et al. documented that conditional deletion of BMP2 in ameloblasts leaves thin and hypo mineralized enamel [4]. Similarly, Yang and colleagues demonstrated that BMP2 ablation in differentiating odontoblasts and osteoblasts results in severe decreases in root and crown dentin [8].

Knockout of BMP in mice resulted in smaller, more disorganized collagen fibrils, less organised and slow mineralization of dentin and enlarged pulp chambers [9].

Fibroblast Growth Factor

FGF determines the proliferation of oral epithelial stem cells and maintenance of the enamel, dentin and pulp stem cells. Miyuki et al in his experimental studies on role of signalling molecules in odontoblast differentiation noted that on attenuation of FGFR2b signals there was hampered enamel formation and promotion of odontoblast differentiation and dentin calcification. However, enamel was reformed upon restoration of FGFR2b signals indicating that FGFR2b signalling was intricately involved in the differentiation of oral epithelial stem cells into ameloblasts [10].

In mesenchymal tissue, BMP4 inhibits FGF3 expression, while Activin inhibits the suppression of BMP4 via FGF3 [11]. Thus, in the epithelial and mesenchymal tissue, enamel and dentin formation proceed via interactions among numerous cytokines [10] [FIG 2].

FGF signals have different effects on cell proliferation and differentiation depending on the time of action [12].

Vascular Endothelial Growth Factor

Vascular endothelial growth factor (VEGF) is a potent angiogenic and vasculogenic factor as it is an endothelial specific protein which contributes to the formation of tertiary dentine [13]. It induces proliferation and differentiation of human pulp cells into odontoblasts and increases alkaline phosphatases in pulp tissue. It is a useful growth factor in the repair of damaged pulp and dentin [14]. Stem cells from human exfoliated deciduous teeth expressed the membrane-bound VEGF receptors (VEGFR)-1 and -2, CD31 and vascular endothelial-cadherin [15,16]. It has been observed that severe inflammation can result in a reduction of the number of blood vessels and VEGF-A expression levels.

Insulin like growth factor

IGF exists in two different forms IGF- I and IGF- II. Both are found in large amounts in bone.

IGF-I evokes the accumulation of several enamel specific gene products, including amelogenin and ameloblastin, indicating that the IGF system is involved in the induction of enamel Bio mineralisation [17]. IGF-I gets trapped in the dentin matrix structure during its synthesis, secretion and mineralization and gets released into the dental pulp after an injury, stimulating repair process [18]. IGF-I synthesized by osteoblasts

stimulates bone formation by provoking cellular proliferation, differentiation and type I collagen biosynthesis.

IGF – 2 is a mutagenic peptide hormone with predominant role in prenatal growth and development. It is seen along with IGF – 1R, IGF – 2R in differentiating pre ameloblasts and pre – odontoblasts of cusp tip region during early and late bell stages.

Experimentally, IGF- I along with TGF, PDGF, IGF2 has been shown to synergistically increase osteoblasts mitogenesis in cultured bone cells in combination with other growth factors such as bFGF, PDGF or TGF.

Platelet Derived Growth Factor

It is a potent mitogenic, angiogenic and chemo attractive agent widely used in tissue regeneration. It is a dimeric glycoprotein composed of 2A sub units [PDGF-AA], 2B sub units [PDGF-BB], one of each [PDGF-AB]. PDGF-BB along with DPSC's improved proliferation and differentiation of odontoblasts and dentin – pulp complex regeneration. PDGF-BB role in promoting odontoblastic differentiation can be evaluated by four key markers. They are

1. DSPP – terminal phenotypic marker of odontoblast.
2. ALP – early differentiation marker helps in ca – p mineral formation in bone and teeth
3. DMP-1-modulates DSPP gene transcription during early stages of odontoblast differentiation
4. OCN – late marker of cell differentiation secreted by mature odontoblasts and osteoblast [19].

The quantity of newly formed dentin-like tissue and blood vessels were significantly emphasized when using the hDPSCs transfected with PDGF-BB.

Dentin sialophosphoprotein (dspp) and dentin matrix protein 1(dmp-1): Dentin sailophosphoprotein is a non-collagenous matrix protein [NCP] of odontoblasts which proteolytically cleaved DSPP into dentin sialoprotein [DSP] and dentin phosphoprotein [DPP]. DSSP expression of odontoblasts was higher during primary dentinogenesis than during secondary dentinogenesis, suggesting more aggressive roles of DSPP for odontoblasts differentiation [20]. It is expressed transiently in ameloblasts. DMP-1 is extracellular matrix protein essential for proper dentin and bone mineralisation and its expression is noted in both the early and late stages of Odontogenesis [21]. The Conversion of Predentin to Dentin Is Impaired by A Lack of DSPP, Resulted in widened predentin, narrow dentin width, defects in dentin maturation and mineralisation. DSPP and DMP-1 have overlapping but distinct functions both as extracellular proteins and cell signaling molecules in mineralized tissues.

Dentin Phosphoprotein And Dentin Sialoprotein: DPP and DSP are abundant NCP's in dentin.DSP regulates initial dentin mineralization and contributes to DEJ. It is also capable of inducing human PDL stem cell differentiation and mineralization. Dentin phosphoprotein also known as phosphoryn [PP-H] involves in maturation of dentin matrix. PP-H at very low concentrations, when immobilized by a stable support, might induce the production of hydroxyapatite from calcium phosphate solutions at physiological concentrations in in vitro and in vivo" [22]. Over expression of DPP resulted in pitted, chalky enamel without uniform thickness and weakened the enamel thickness. These findings indicate negative effect of DPP and positive effects of DSP on enamel thickness. It has been shown that root dentin contains only half the amount of Phosphoprotein, compared with crown dentin from the same teeth [23].

Osteopontin: OPN is detected in the dentin and the boundary between the tertiary and pre-existing dentin. It was strongly expressed in dental pulp cells at the pulp horn from 3 week after birth in the developing teeth. OPN contributes to the differentiation process of odontoblast-like cells [24]. mRNA and protein expression studies have shown that various cell types, i.e., osteoblasts, osteocytes, fibroblasts, osteoclasts, macrophages, and bone marrow cells, can produce OPN [25]. Studies illustrated that the immuno-competent cells, such as dendritic cells and macrophages, secreted OPN before odontoblast-like cell differentiation after tooth transplantation [26]. The deposition of OPN at the calcification front is crucial for the secretion of type I collagen by newly differentiated odontoblast-like cells to form reparative dentin during pulpal healing following cavity preparation. Cavity preparation induced the expression of OPN that was recognized at the mesial dental pulp on days 1 to 3 in a study.

Proteoglycans

PGs are another major noncollagenous components in dentin. They appear in predentin as an amorphous gel, located between and around the collagen fibres [27]. PGs control the organization of the collagenous network being formed in the predentin. In dentinogenesis it is often associated with minerals chondroitin-4- sulphate. Small amounts of keratan sulphate also seem to be present in dentin. The distribution of proteoglycan in mineralized dentin according to studies, is heterogenous in nature. It was found to be intensively reactive in the region of dentinal tubules while the inter-tubular dentine was non-reactive and unstained. Thus, removal of a significant pool of PGs would either affect their putative inhibition of mineralization or influence collagen fibrillogenesis in predentin.

Biomarkers In Dentin-Pulp Complex In Healthy Tooth

Alkaline Phosphatase: Alkaline phosphatase has been considered to be an important indicator of bone formation and is a phenotypic marker for osteoblast cells. A significant correlation was found between the serum and salivary Alkaline Phosphatase levels. AP levels increased with increase in progression of caries. Hence the bottom line is that it may be used as a biochemical indicator in evaluating the susceptibility of caries[28].

Osteocalcin and Osteonectin: OC is small non collagenous protein hormone found in bone and odontoblasts [29]. It is present in human odontoblasts, detected throughout the length of the odontoblast processes, and located within the enamel matrix. It appears specifically in the enamel in the maturation stage. ON a mineralized matrix protein is expressed by both osteoblasts and odontoblasts, as well as by many other cell types [30]. It is associated with the collagen rich mineralized matrix. ON was localized in the lamina limitans, peritubular dentin devoid of a collagen network. For example, when intercellular junctions become leaky, between smooth-ended ameloblasts at the maturation stages OC migrate into mineralised tissue [31]. There are different extracellular patterns of OC, ON, and DSPP distribution in various human mineralized tissues. This distribution may be related to the macromolecular interactions between tissue-specific components (enamel/amelogenin, dentin/DSPP) and those shared with bone/dentin/enamel/cementum matrix proteins such as collagens and other noncollagenous proteins.

Cathepsin: Cysteine cathepsins contribute to the odontoblast response to metabolic disturbances. Cathepsins B and C demonstrated expression change in both the odontoblast and pulp cultures, indicating that these cathepsins are the main targets of TGF-b in this enzyme family in the dentin-pulp complex. Cathepsin B has been shown to be responsible for activation of MMP-1 and is an attractive candidate for the potential cathepsin-MMP interplay in dentin-pulp complex. pro cathepsin B is activated by active MMPs. The comparative reduction in both MMP and cathepsin levels with age in dentin demonstrates the loss of enzymes during aging.

Cathepsin B expression was down-regulated in the odontoblasts and in the pulp tissue and vice versa with cathepsin C. This indicates that these functionally and structurally related enzymes have different roles in various components of dentin-pulp complex cells and tissues.

Cathepsin D protein limited to the odontoblasts and predentin, with no indication of presence in mineralized dentin. [32],

CT-K, a true mammalian collagenase that has been considered the main protease involved in bone resorption [33]. CT-K is expressed in osteoclasts. Therefore, its secretion at the matrix-osteoclast interface is considered to be a major event in bone matrix degradation. Although the gene expression of CT-K in cultured odontoblasts derived from sound teeth has been detected, it is never shown its presence/ distribution in dentin [34].

Their abundant expression in caries-affected dentin than in intact dentin supports the notion that CTs and MMPs may work synergistically in pathophysiological processes wherein the dentin organic matrix remodelling takes place.

Interleukin

Interleukins are group of naturally occurring proteins that stimulate immune response mediate cell combination regulate cell growth and differentiation. Expression of IL-1a, -1b, -6 and -8 in healthy human pulp, and increase in their expression during caries, have been detected earlier [35]. IL-16 is chemoattractant for T-cells, eosinophils, monocytes and dendritic cells [36]. lack of IL-6 enhances periapical lesion development indicating the importance of IL-6 in pulp immune defence. IL-7 in addition to its proinflammatory function, it regulates reactionary or reparative dentin formation and inhibits osteoblasts. It has been shown to inhibit osteoblast differentiation, thus reducing bone formation [37]. IL-7 is a growth factor for B and T lymphocytes whereas IL-8 is a potent inflammatory cytokine as it recruits neutrophils to the inflamed site which is generally induced by bacterial antigens. Additionally, it has been suggested and used as one of the main markers for acute pulp inflammation [38]. IL-12 has been shown to enhance cytotoxic T-cells, as well as increases natural killer cell proliferation,

TGF-b1 has predominantly pro-inflammatory effects on pulp tissue, and it induces the expression of interleukins that are elevated in inflamed dentin–pulp complex cells.

Substance P (SP):

It is an undecapeptide, member of tachykinin neuropeptide family acting both as the neurotransmitter and neuromodulator. These nerve fibres expressing SPLI were found in the odontoblast-predentin border zone around the blood vessels in the dental pulp. In the pulp, varicose SP-positive fibres arising from the nerve

plexus of Raschkow run straight without any interruption towards the odontoblast layer and pre-dentine. Some fibres terminate in the odontoblast layer (Fig. 1 in figure 3) and others passed between the odontoblasts beyond them which ended close to the mineralized dentin. Some of them could be traced about 60 pm from the odontoblast-predentin border zone (Fig. 2 in figure 3). In some cases, SP fibres branched at the predentin surface or changed the direction transversely at the various distances within the predentin (Fig. 3 in figure 3). In the sections impregnated with silver, small beaded fibres showed a similar distribution pattern to SP fibres that ran from the sub odontoblast plexus into the predentin (Fig. 4 in figure 3).

SP in the dental pulp is involved in local regulation of blood flow and in the pre dentine it is involved in pain transmission mechanism of tooth because intra dentinal nerves are involved in pain transmission [39,40].

Neuropeptides: They are small protein like molecules produced and released by neurons responsible for modulation of synaptic transmission. Studies have significantly proved the role of the neuropeptides, including substance P (SP), calcitonin gene related peptide (CGRP), neurokinin A (NKA), neuropeptide Y (NPY), and vasoactive intestinal polypeptide (VIP) in synaptic transmission [41].

Neuropeptide Y: In pulp tissue, electrical stimulation of sympathetic nerve fibres causes constriction of pulpal blood vessels and lowers the tissue fluid pressure. These effects might be attributed to NPY and norepinephrine release. Caries and thermal/mechanical irritants of the pulp dentin complex have also shown to stimulate NPY release. Although expression of NPY has been demonstrated in animal and human dental pulp, the presence of its receptor has not yet been determined [42].

Vaso active intestinal peptide: It is expressed in high concentration in dental pulp neurons. VIP fibres travel along the large and small blood vessels and show a network-like arrangement adjacent to the blood vessel wall. At the sub odontoblast layer, there are very few VIP fibres. VIP has also been found expressed in peri radicular lesions [43]. In chronic periapical lesions VIP receptors are expressed and their levels are inversely proportional to the size of the lesion, suggesting their role in regulating the inflammatory phenomenon in the development of periapical lesions via cellular receptors [40]. intra-arterial injection of synthetic VIP causes vasodilation in the cat pulp, and denervation experiments demonstrated that VIP in the pulp of cats does not originate from sensory or sympathetic nerves [44].

Calctinonin Gene Related Peptide: CGRP is produced in the trigeminal cell bodies and is transported via axonal flow to the nerve terminals in the pulp, where it is co-stored with other sensory neuropeptides. When neurons are stimulated, neuropeptides are released along with substance P which mediate neurogenic inflammation [45,46]. They are also noted around small blood vessels suggesting that peptide is involving in regulation of blood flow [47]. The dental pulp is a highly sensitive to painful stimuli [48]. CGRP is a powerful vasodilator agent, which causes an increase in local blood flow and consequently, pulpal tissue pressure increases. It also exerts stimulatory effects on the growth of pulpal cells, such as fibroblasts and odontoblast-like cells [49].

Matrix Metallo Proteinases: MMPs belong to a group of proteolytic enzymes which are produced by

connective tissue cells (odontoblasts, osteoblasts, and fibroblasts), in a healthy state but are produced by polymorphonuclear leukocytes and other inflammatory cells in diseased condition. Matrix metalloproteinases (MMPs) can degrade the principal components of the extracellular matrix. Recent studies have shown that MMPs influence many basic processes, such as cellular proliferation, differentiation, angiogenesis and cell apoptosis. By stimulating innate/adaptive immunity. they also play role in the pathogenesis of inflammation, by causing tissue destruction [50]. Thus, these MMP families elicit a dual role.

The fibroblasts, keratinocytes, endothelial cells, monocytes/macrophages, and osteoblasts express MMP-1, -2 and -3. Immunohistochemically, in acute pulpitis MMP-1 and MMP-3 are localized in the extracellular matrix around the inflammatory cells. On topical application of MMP-3 protein to the injured pulp tissues of the rat incisors there is an accelerated angiogenesis, tissue regeneration and reparative dentin formation at a predominant rate when compared to that of the control treatment.

MMPs (MMP-8, -2, -9, -3, -14, and -20) have been isolated from the dentin, pulp tissue, and odontoblasts, where they play a key role in formation of the dentinal matrix, modulation of caries progression, and secondary dentin formation. Their expression is regulated by proinflammatory cytokines, growth factors and ECM components. Collagenases [MMP-1,8,13] and gelatinases, which tend to break down collagen and laminins, are considered the key ECM MMPs [51].

MMPs may play a role in the progress of chronic pulpal inflammation, periapical lesions and pulp tissue destruction. MMP inhibition by several inhibitors and particularly by natural substances could provide a potential therapeutic pathway to avoid caries progression in dentin and pulp[50].

In teeth with a clinical diagnosis of symptomatic irreversible pulpitis, a MMP-9 concentration was greater than teeth with normal pulps. Quantification of aMMP-9 in pulpal blood has excellent discriminatory ability and can be used as a prognostic tool to predict the outcome of pulpotomy[51].

Conclusion

Biomarkers are functional elements at the cellular or molecular level, playing important roles in health and disease. The dentin-pulp complex of the tooth houses several biomarkers at different stages of development, and a lack of these biomarkers results in developmental disorders. Therefore, this analysis acknowledges the role of biomarkers in dentin-pulp complex.

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