

## Molecular Implications in Endodontic Diagnosis

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### ABSTRACT:

Endodontic diagnosis focuses majorly on extent of microbial infection & host response. But, for the time being there is no method that completely fulfill this principle. So, aims of this review article is to discuss regarding the possibility of pulpal and periapical inflammation diagnosis via molecular markers.

Molecular markers are of great importance in tracking the prognosis & subsequent healing in procedures like pulpotomy and pulpectomy. Variety of tissue fluids are used for this purpose. Of them, GCF is majorly used in case of periodontal purposes and found less significance in endodontic field. Fluids like dentinal fluid, plural blood, periapical fluids are majorly the one giving exact status regarding endodontic infections. In inflamed pulps the levels of PMN's and related enzymes like cathepsin G, matrix metalloproteinases-9 and elastase found to be increased. Less site specific markers like IL-1 beta are less relevant in case of pulpitis & apical periodontitis diagnosis. Since, molecular diagnosis is undoubtedly a boon in upgrading the level of treatment planning therefore, nowadays researchers are actively working on chair side diagnosis in order to make this process a bit easier.

### INTRODUCTION:

The term 'diagnosis' is derived from Greek language meaning thorough understanding of cause-effect relationship for a specific disease. In Endodontic diagnosis, focus is majorly on extent of microbial infection or host inflammatory response as cause of disease is opportunistic infection of dental tissues by wide range of transient microorganisms.<sup>[1]</sup> But, matter of concern that none of the recently used method fulfil this principle. Periapical

diagnosis is whole sole based on radiographic imaging. Diagnostic methods based on analysing the vascularity status of tooth are beyond scope in every case.

Methods like pulp oximeter, Doppler flowmetry<sup>[2]</sup> and spectrophotometry-are primarily indicated in trauma cases. these methods achieved less relevance in operative dentistry & endodontics.

This review article aims to evaluate the possibility of pulpal & periapical inflammation diagnosis using molecular markers.

## **APPLICATIONS IN ENDODONTIC DIAGNOSIS:**

### **Tracking periapical health status**

Radiographs are in a great league for identifying/ evaluating the periapical health. Presence of pathology at periapex-becomes subjective of pulpal necrosis which is more confirmed in presence of certain histological inflammatory signs at periapex.<sup>[3]</sup>

Drawback associated with radiographic diagnosis- it takes upto period of one year to visualise decisive differences in periapex appearance. Moreover, anatomic blurring<sup>[4]</sup> is another associated problem with 2-D imaging. Conditions like Apical periodontitis can't be detected via 2-D radiographs. 3-D imaging can overcome mentioned limitations but point to be considered is that despite of extreme advancement, association between periapical tissue histology & radiographic appearance still can't be achieved<sup>[5]</sup> and here it paves the revolutionary pathway for molecular diagnosis.

### **Tracking pulpal health status**

Treatment decision of a tooth depends on tooth history. Routine dental visits includes pulpal health monitoring. Pulp neurability tests are used for this purpose .Among them, cold test is the most common method used for this purpose using carbon dioxide pencil or 1,1,1,2 tetrafluoroethane. Such pulp tests depends on patient's subjective response. since, such tests can't identify pulp state, they are better termed sensitivity tests instead of vitality tests.

## **TREATMENT OUTCOMES:**

### **Preserving coronal pulp vitality**

Pulp-dentin complex infection-initiates in coronal part of tooth and progress apically. Decision making in pulpal intervention is cumbersome. in general, caries above 1/3<sup>rd</sup> of inner dentin-not requires complete excavation. in context of maintaining pulp vitality..stepwise excavation is always better than complete excavation<sup>[6,7]</sup>. in studies done far, shows better response of patients in pulpectomy than partial pulp excavation in terms of pain response. Studies shows around 10% of abutment teeth loses vitality within 10 years following restoration.<sup>[8]</sup>

## PULPOTOMY OR PULPECTOMY, WHEN & WHY?

According to authors, long time ago..dental shaving obtained while instrumentation of non infected root canals-perfect biomaterial for purpose of obturation.<sup>[9,10]</sup> According to old school of thought, root canal instrumentation- 3mm short of apex, aims to maintain a stump of vital pulp in apical part of RC system. But, it did not worked in long run.. as clinically we cant assess whether the remaining pulpal tissues devoid of microbes or not.hence, comes the concept of full pulpotomy, pulpectomy followed by a tight coronal seal ensuring increased success rates of treatment in cases of apical periodontitis<sup>[11]</sup> Despite of this in context of same condition, introduction of smart use of molecular markers in assessment of the pulpal vitality will undoubtedly help in long run and the re-introduction of pulpotomy will be possible with impressive success rates.

### Regenerative procedures

Tissue engineering undoubtedly gained immense attention & importance in recent years. Concept of revascularization of non vital pulp works really good in commencement of root formation, especially in traumatic cases<sup>[12]</sup> Studies says that revascularisation possible in teeth with vital pulp before initiation of treatment. In pediatric dentistry, revascularization helpful in tooth with incomplete root formation.

In this procedure after disinfection of root canal system via hypochlorite & antibiotic paste , a blood clot is sealed off in coronal part of RC using calcium silicate cement followed by a permanent restoration.<sup>[13]</sup>Further problem of contamination with saliva is also circumvented using this procedure of tissue engineering/cell homing.

## DISCUSSION ON FLUID SELECTION:

Biologically, main objective is to locate the portal of bacterial entry & invasion in pulp space and periapical area.

### 1. Gingival Crevicular Fluid

It's an inflammatory exudate derived from crevices It's an inflammatory exudate derived from crevices of gingiva.its the house of various host factors like antibodies, protein, bacterial antigens, chemical mediators of inflammation like cytokines.<sup>[14]</sup>Since the vascular and neural functioning of endodontium & periodontal tissue is correlated anatomically & functionally.<sup>[15,16]</sup>Variation in GCF host factors in tooth undergoing endodontic treatment are relatively sparse . Studies claims alteration in composition of MMP-8, INTERLEUKIN-8 AND NEUROTRANSMITTERS-differs in GCF collected from symptomatic and asymptomatic tooth.<sup>[17]</sup>GCF analysis supposed to give satisfactory results in diagnosing the conditions like apical periodontitis, as it's a local inflammation in PDL tissue, plus also helpful in assessing the dynamics of apical periodontitis( healing capability of apical tissues after root canal treatment. Drawback of this method is difficulty in distinguishing apical &

marginal periodontal inflammation. Gingival crevice is not the ideal site for collection for endodontic diagnosis even at molecular level as there exist a bias in marginal or Apical periodontitis inflammation. Secondly, GCF is unable to reflect the microbial progress in pulp cavity. hence, depicting less significance in endodontic diagnosis.

## **2. Dentinal fluid**

Dentinal tubules contains an extracellular fluid k/a Dentinal fluid. knutson and co-workers first performed an in vitro study to to assess the protein levels in human teeth. Serum albumin levels were evaluated. Entire fluid collection from extracted tooth via occlusal trimming & centrifugation is best method to be used.<sup>[18]</sup> In cases of irreversible pulpitis, characterised by highly painful tooth , PVDF( polyvinylidene difluoride) membranes used for collecting dentinal fluid. MMP-9 is suggestive of irreversible pulpitis. Inconsistent recovery of MMP-9 levels in case of pulpitis-suggests less absorption of fluid from dentinal tubules. MMP-2 is of high diagnostic value than PVDF counterparts.<sup>[19]</sup> however, more research is required on dentinal fluid analysis and its diagnostic significance.

## **3. Pulpal blood**

Blood from the tooth pulp carries certain factors that differs from that in peripheral blood that gives site specific knowledge/info from respective fluid. Dr.Florian Prader, university of Zurich in year 1949 compared the smears of pulpal blood from teeth with endodontic infection to assess the inflammatory status via quantitative assessment of PMN;s levels( polymorpho neutrophil granulocytes).<sup>[20]</sup> According to Guthrie study, two molecular techniques, ELISA and PCR are used for evaluating the human pulpal blood with respect of other proteinaceous molecular markers.<sup>[21,22]</sup> Nakanishi & coworkers found that certain antibodies, IgG, IgA, IgM , elastase and especially prostaglandin E2 levels found elevated in inflamed tooth in comparison to healthy teeth. According to recent studies levels of chemokine IL-8 shows significant elevation in c/o symptomatic pulpitis.<sup>[23]</sup> Entering the pulpal space for its diagnostic evaluation affects vitality of the pulp but with advancements in pulpal revascularization and regenerative aspects this problem sorted successfully so that pulpal space can be assessed. Microbial evaluation of the pulpal blood yields significant information on pulpal conditions.

## **4. Periapical fluid**

Extracellular fluid obtained from periapical area of tooth, termed as Periapical fluid.in cases of tissue inflammation, this fluid becomes exudate with subsequent modifications in its composition.<sup>[24]</sup> Current state of pulpal inflammation can be evaluated using PA fluid as this exudate formation is an immediate host response to inflammation. In clinical procedures like complete pulpectomy collection of PA fluid is reasonable after root canal treatment.

Collection of this fluid is achieved using paper points or methyl cellulose strips to full working length. No. 20 paper points are best suited to collect this fluid as studies on apical root anatomy concludes the size of apical diameter to be  $>$  or  $=$  to ISO size 20.<sup>[25]</sup>

One amazing feature of assessing periapical fluid is that it enables to study the inflammatory responses to infections even in a closed environment and is a solution to all the short coming associated with use of GCF fluid as microbial marker.<sup>[26]</sup> Endotoxins (bacteria associated factors) & MMP's, immunoglobulins, and cytokines (host associated factors) identified in periapical exudates in root canals of tooth. concentration of such inflammatory factors correlates well with clinical signs & symptoms. A relationship between treatment progression and resultant decrease in inflammatory factors is established that gives clear insights of periapical healing. Since, evaluation of changes in molecular levels of periapical fluid takes place at rapid rate. Thus, a chairside assay seems more helpful during 2<sup>nd</sup> sitting of RCT in deciding whether the RC system will be obturated or requires placement of antibiotic paste for adequate disinfection.

## MOLECULAR MARKERS & ASSOCIATED DIAGNOSTIC RELEVANCE:

Conditions like Apical periodontitis & pulpitis are result of polymicrobial infection. Studies suggests that polymorpho nuclear neutophils are major inflammatory cells associated to pulpitis.<sup>[27]</sup> these inflammatory cells produces a series of proteolytic enzymes that in response to inflammation ingests the soft tissues and allows the entry of PMN's in site of microbial invasion.<sup>[28]</sup> If the microbes not removed then it stimulates the pus formation, slowly leading to tissue necrosis. In the periapical tissues, there is complex inflammatory reaction because resorption is also evident in this area. Nevertheless, in general the mechanism of immune response is almost similar in periapical area & pulpal space.

PMN- related enzymes like cathepsin G, elastase and matrix metalloproteinases-9 (MMP-9) are found to be increased in inflamed pulps when compared to healthy tooth.<sup>[29]</sup> Presence of such enzymes or respective mRNAs- diagnostic of inflammatory infiltrate.<sup>[30]</sup> Another chemotactic factor i.e. IL-8 is also a relevant marker in cases of pulpal inflammation.

Such molecular markers are a boon in diagnosis and monitoring the pulpal condition but, when it comes to periapical diagnosis the picture seems less clear. This might be attributed to predominance of such markers in acute inflammation as compared to chronic lesions (eg. cases of periapical abscess).

Less site specific markers such as IL-1 beta are not considered relevant in pulpitis as well as apical periodontitis diagnosis. Interestingly, All studies done so far comparing health and inflamed tooth assessed PMN related factors at different sites (like GCF, pulpal blood and many more earlier discussed in this paper and shows significant difference between 2 states of tooth. Not only this, PMN's factors can be a key in diagnosing ongoing inflammatory

process in periapical area. Recent studies shows that factors associated with bone resorption could be targeted in diagnosing conditions at periapex region.<sup>[31]</sup>

### **A talk on possibility of chair side molecular analysis**

Endodontic treatment is expensive & sensitive at same time. An exact treatment planning is result of good diagnostic tests making it the need of hour for patient comfort & positive feedbacks. Ideally such molecular tests should be done chair side to avoid patients extra sittings just to get a test results from lab.

The host factors in such tests are assessed at 3 levels. Gene expression (mRNA), protein and active protein. But, tests assessing gene expression is quite impossible in a chair side set-up. Recently, in markets GCF assays are available that finds a significance in diagnosing periodontal pockets but analysing the active MMP-8 and PMNs activity.<sup>[32,33]</sup> This method is based on 'PRINCIPLE OF IMMUNOCHROMATOGRAPHY'. It is sold under the brand names such as PerioSafe and Periomarker under different companies. Now the question arises if they can be used to analyse the pulpal, dentinal or periapical fluid. Studies conclude that there is risk of sensitivity problem in case of collection of dentinal fluid as target proteins collected from it are in low ng range.<sup>[34]</sup> Whereas, in case of periapical fluid target molecules are similar to GCF and thus can be easily developed.

### **CONCLUSION:**

From this review study, this can be concluded that, to bring the use of molecular diagnosis full fledgedly in Endodontics more research and studies are required. But, it is undoubtedly worth the effort. Apparently many researchers considered this topic relevant and working on same. hopefully, in future the era of endodontic diagnosis will evolve bringing molecular diagnosis in daily practice. Endodontic microbiota is still undergoing shift from cultural era to molecular era, proper exploiting this molecular diagnostic methods in endodontics enhance high success rate in endodontic treatment.

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