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Review

Inflammatory cytokines in normal and irreversibly inflamed pulps: A systematic review

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ABSTRACT

Objective: To review the available literature in regard to the inflammatory process and pulpitis. Setting forth to evaluate if differences in the levels of various cytokines (TNF-α, IL-1β, IL-2, IL-6 and IL-8) can be observed in clinically diagnosed normal and irreversibly inflamed pulps that could serve as possible markers and/or diagnostic tools to predict and differentiate between certain states of inflammation.

Methods used to measure and assess levels of cytokines have been limited to two protein quantification methods ELISA and/or Multiplex Array.

Design: The databases PubMed, EMBASE/Ovid, The Cochrane Central Register of Controlled Trials, Cochrane Reviews and Scopus were consulted for the electronic literature search. Screening of titles and abstracts followed the PRISMA guidelines while data extraction and the assessment of the full texts were carried out in accordance to the GRADES assessment.

Results: The review showed that significant increases in levels of IL-1β, IL-2, IL-6, IL-8 and TNF-α in irreversible pulpitis samples exist, in comparison to normal pulp samples which serve as a good basis for potential markers. Due to larger discrepancies in available literature, IL-2 seems rather unsuitable at the moment, while IL-6 and TNF alpha seem to be more promising.

Conclusion: It may be concluded that even by combining two protein quantification methods inconsistencies between studies exist. At the moment it is difficult to select just one specific cytokine suitable for testing, rather it supports the rationale that further high-quality clinical studies are needed.

1. Introduction

Pulpitis, by definition, is the inflamed condition of the dental pulp (Levin, Law, Holland, Abbott, & Roda, 2009). This inflammation can be the result of various stimuli, yet in the majority of cases it is due to microorganisms entering the pulp space as a result of caries (Hargreaves, Goodis, & Seltzer, 2002; Langeland, 1987), traumatic fractures or dentinal cracks (Cameron, 1964), or exposed dentin tubules (Nagaoka et al., 1995). Clinically, pulpitis can be described as reversible or irreversible. A “clinically normal pulp” is a pulp that does not present any signs or symptoms to suggest that any form of disease is occurring (AAE, 2015; Abbott & Yu, 2007). Under normal circumstances enamel and cementum act as an impermeable barrier to block the patency of dentinal tubules at the dentinoenamel or the dentinocemental junction, respectively (Cohen & Burns, 2002; Garg & Garg, 2013). However, when the integrity of the rigid protective enamel and/or dentin is compromised, as the infection progresses, and the pulp becomes exposed, the bacteria and bacterial metabolites can penetrate the pulpal space through the narrow tubules in the dentine, and the pulps immune response is triggered to respond in inflammation (Love & Jenkinson, 2002). A symptomatic and irreversibly inflamed pulp is based on subjective and objective findings that the pulp is incapable of healing and can show symptoms of: lingering thermal pain, spontaneous pain, and/or referred pain (AAE, 2015). Thus, pulpal injuries typically progress from ischemia, hemorrhagic infarction, and partial necrosis to complete pulpal death (Cohen & Burns, 2002). In the end, the level of inflammation is comparable to the severity and the duration of the stimulus, as well as the host’s capacity to defend the pulpal response (Garg & Garg, 2013).

Molecularly speaking, the pulp is equipped with defense cells and inflammatory mediators that mediate and maintain the host response to microbial infection (Cooper & Smith, 2013; Hahn, Best, & Tew, 2000; Hahn & Liewehr, 2007a; Jontell et al., 1998; Stashenko, 1990; Trowbridge, 1990). Specifically, it has been shown through *in vitro* and

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ex vivo models that an odontoblasts direct response to caries is the release of antimicrobial peptides and cytokines, while indirectly also causing another response by way of migratory immunocompetent cells (Durand et al., 2006; Farges et al., 2009; Hahn et al., 2000; Staquet et al., 2008; Veerayutthwilai, Byers, Pham, Darveau, & Dale, 2007). Furthermore, the increase of certain cytokines such as transforming growth factor β1 (TGF-β1), vascular endothelial cell growth factor (VEGF), C-C chemokine ligand 2 (CCL2), human beta-defensins (hBDs), interleukin 8 (IL-8), CXC chemokine ligand 10 (CXCL10), interleukin 1 beta (IL-1β), interleukin 2 (IL-2), interleukin 4 (IL-4), interleukin 6 (IL-6), interleukin 10 (IL-10), interferon-γ (IFN-γ) and tumor necrotic factor-α (TNF-α) have been observed in pulp cells in several previous studies (Adachi et al., 2007; Farges et al., 2009; Horst, Horst, Samudrala, & Dale, 2011; McLachlan, Smith, Bujalska, & Cooper, 2005; Paris, Wolgin, Kielbassa, Pries, & Zakrzewicz, 2009; Piattelli, Rubini, Fioroni, Tripodi, & Strocchi, 2004). Clearly, these results indicate that the immune and inflammatory dental pulp response is multifaceted. Gaining insight into the inflammatory response of the pulp is of critical importance. By increasing the understanding and grasp of the interrelation between the molecular situation and the clinical signs and symptoms of the pulp, more site specific and precise diagnostic tools can be developed.

The accuracy of signs, symptoms, and assessments used to determine the condition of the pulp are often hard to pinpoint, and recent studies have shown that there are still shortcomings and critical gaps regarding the effect of diagnostic tests (Mejare et al., 2012). Though these methods have their clear benefits in everyday clinical practice, they do also have their limitations as recent systematic reviews have substantiated the fact that current (clinical) testing methods used to assess the inflammatory state of the pulp and the periapical tissues are of limited value, and that molecular assessment methods could be the future (Chen & Abbott, 2009; Rechenberg & Zehnder, 2014). Thus, even though several approaches exist to assess the injured or diseased state of the dental pulp, no consensus has been reached on which method or combination thereof would result in the most accurate information (Levin et al., 2009).

Assessing and understanding the site-specific regulation of the inflammatory network could present a mechanism to improve pulpal diagnostics, and, as a result, allow for detection and differentiation between pulps from symptomatic teeth diagnosed with irreversible pulpitis or their healthy counterparts. Ultimately, this could become a diagnostic tool for the dentist in the future and allow vital tissue to be maintained, at the same time preventing any overtreatment. Unfortunately, only few diagnostic tests have persevered and made it into the everyday clinical setting, yet hope remains as molecular testing methods have in fact found success in periodontology (Levin, 2013). An assay, basically comparable to a common pregnancy test, can test for MMP-8 to ultimately diagnose, follow, monitor, and predict periodontal and *peri*-implant diseases and health (Sorsa et al., 2016, 1999). Sold under various brand names (such as PerioSafe and Periomarker), a chairside test such as this, to measure inflammatory molecules and mediators of the pulp, could be of great value for the field of endodontology.

Consequently, this review sets forth to review the current literature available in regard to the molecular and inflammatory status of an inflamed pulp, specifically focusing on providing evidence and elucidating to stage specific cytokines and inflammatory mediators that could serve as possible markers and/or diagnostic tools to predict and differentiate between certain states of inflammation that as a result could set the foundation for future chairside testing options and outcome measures in evaluating the state of health and disease of the pulp and, concomitantly, the tooth.

2. Material and methods

2.1. Eligibility criteria for considering studies for this review

The main aim was to screen for experimental investigations which included studies that had samples collected from normal and irreversibly inflamed tissues/blood pulp samples. To allow for comparison and more conclusiveness on the application and feasibility a protein selective method, ELISA, was sought as the preferred method of testing.

2.2. Search strategy for identification of studies

In trying to identify the studies to be considered for this review, detailed search strategies were developed for each database to be searched. The MeSH terms used in the PubMed search were (Caries Exposure OR Reversible Pulpitis OR Pulp Inflammation OR Irreversible Pulpitis OR Dental Pulp Disease/Classification) AND (Cytokines OR Biomarkers) AND (Diagnosis OR Dental Pulp Test). However, the MeSH terms were adopted, broadened and more generalized to “pulpitis AND Cytokines” for The Cochrane Central Register of Controlled Trials, Scopus, and Embase.

2.3. Databases searched

The electronic literature search encompassed the databases PubMed, EMBASE/Ovid, The Cochrane Central Register of Controlled Trials, Cochrane Reviews, and Scopus. The search was conducted in July/August 2016. Although no language restrictions were set on included studies, no relevant trials were found in languages other than English. The given abstracts were read and filtered for duplicates and consequently selected depending on relevancy to the questions addressed and previously specified inclusion/exclusion criteria (Table 1). After complying with these standards, full text versions of the studies were read and re-examined. Additionally, supplementary studies were sought manually by reviewing the reference lists of other scientific papers and articles. Grey literature was not included. To allow for comparison, adherence to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines (Moher, Liberati, Tetzlaff, Altman, & Group, 2009) was sought as best possible (Table 1).

2.4. Review methods and quality assessment

In accordance to the Cochrane Reviewers’ Handbook, the studies were assessed and graded to limit the risk of bias caused by inadequacies in study design, conduct, or analysis. In this case, each study was rated on four different levels according to GRADE (Grades of Recommendation Assessment Development and Evaluation) (Table 2a and 2b) (Schunemann et al., 2008). For purposes of a systematic review, the GRADE approach delineates a structured process of rating the quality of evidence and strength of recommendations, given a set of outlined factors (Schunemann et al., 2008).

Table 1
Inclusion/Exclusion Criteria.

Inclusion Criteria:	-Population: Human patient pulp samples (blood/tissue) Teeth with pulps where a clinical diagnosis, based on clearly described signs and/or symptoms of normal and irreversible pulpitis, was made before the tissue or blood sample was collected - Reference Test: ELISA- Testing & Multiplex Testing -Comparison Value: Normal vs. Irreversibly Inflamed
Exclusion Criteria:	- Population: In-vitro studies, animal studies (rat, mice, etc.) - Experimental Studies - Stem Cell/cell culture studies - Relevant data not presented and/or lack of measurement specified