



## Antimicrobial Peptide Cathelicidin and Vitamin D Receptor Gene Polymorphism in Oral Health

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### ABSTRACT

**Background:** Anti-microbial peptides and vitamin D levels in saliva, GCF plays an important role in oral health and its maintenance. The peptide levels are regulated by the Vitamin D cathelicidin pathway and their association and influence on oral health is of utmost importance. The aim of the present review article is to establish a relationship and possibly the role of Cathelicidins and Vitamin D receptor gene polymorphism in relation to oral health.

**Methodology:** The (PRISMA) Preferred Reporting Items for Systematic Review and Meta-Analysis were used for reporting this systematic review. The study protocol has been approved by all co-authors. The study has been registered at the (NHR) National Institute for Health Research under the Prospero ID: CRD42020200946.

**Results:** A total of 102 articles were retrieved through electronic database search. After evaluating the title, abstract and full text of these articles only 7 were selected for the present systematic review. However 3 articles were excluded because they were classified as high risk of bias according to PRISMA guidelines. Out of 7 studies selected, 4 were classified as low risk bias and 3 were classified as moderate risk bias.

**Conclusion:** The current evidence suggests anti-microbial peptide LL-37 and vitamin D receptor gene polymorphism plays an important role in caries prevalence and periodontal health.

**Highlight:** Understanding the role of antimicrobial peptides in saliva helps in enhancing focus on the effect of these components on oral health and its maintenance.

**Key words:** Saliva, Anti-Microbial peptide, Vitamin D, Dental caries, Periodontitis

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### INTRODUCTION

Organisms possess very strong defence system to combat the invading pathogens and microorganisms [1]. One of the principal defence system which plays a major role against microorganism is innate immunity system. The anti-microbial functionality of innate immunity modulates the disruption of gram negative, gram positive, fungi and virus principally by disruption of bacterial cell membrane [2]. Anti-microbial peptides enhances the chemical and physical barrier in mucous membrane and skin. Various recognized anti-microbial peptides are highly conserved and expressed in organisms such as insects (drosophila) rabbits, mice, crabs. They are expressed in response to inflammatory conditions by phagocyte cells like monocytes, macrophages and granulocytes [3].

Oral cavity inspite of these numerous bacterial colonies and has high chances of invasion to the body through GIT, epithelium, gut, surgical procedure rarely leads to infection. Thus, indicates a strong and efficient host defensive mechanism that is in action [4]. Production of antimicrobial peptides from neutrophils, salivary gland makes saliva rich in its anti-microbial, anti-fungal and anti-bacterial properties. They perform various functions like stimulation of epidermal growth factor thereby promotes wound healing, stimulates keratinocytes, chemotaxis and immune activation. The binding ability of LL-37 to lipopolysaccharides and neutralizing its activity facilitates a strong positive correlation between inhibition of lipopolysaccharides induced cytokine secretion. LL-37 influence epidermal growth factor levels, migration of keratinocytes and mediates angiogenesis. This

quality of these AMP's make them an area of interest and they are also extensively studied [5]. These anti-microbial peptides destroyed by attraction, attachment and insertion. Attraction is electrostatic attraction between cationic and anionic moieties on bacterial membrane, these antimicrobial peptides attach in parallel/perpendicular manner to the membrane thereby stretching the bacterial membrane further thinning it down leads to pore formation, further insertion of these antimicrobial peptides takes place by three different model systems barrel stave model, carpet model leading to bacterial dissociation. Several types of anti-microbial peptide adverse in their sequence and structure are identified. Among them cathelicidins are peptides which are crucial in inflammatory conditions.

Cathelicidins are transcribed from a single gene CAMP with a N-terminal (30 amino acid residues) a highly conserved pro-sequence and mature anti-microbial peptide name LL-37 at the C-terminal domain. It has been observed that LL-37 play a role in oral inflammatory conditions, few studies show their association of these peptides. The peptide has anti-microbial activity against gram positive and negative bacteria specifically on clinically relevant pathogens such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhimium* at a mic of  $<10 \mu\text{g/ml}$ , also has an effect on lowering release of pro-inflammatory mediators. This peptide is located in azurophil granule, immature neutrophils, NK cells, monocytes, mast cells, squamous epithelium, airway, mouth, tongue, oesophagus, intestine, cervix and vagina. Widely produced in salivary glands, acinar cell of submandibular glands and minor glands [6]. This systemic review will focus on Cathelicidins (LL-37) and its role in oral inflammatory conditions.

#### MATERIALS AND METHODS

The (PRISMA) Preferred Reporting Items for Systematic Review Ad Meta-Analysis were used for reporting this systematic review. The study protocol has been approved by all co-authors. The study has been registered at the (NHR) National Institute for Health Research under the Prospero ID: CRD42020200946.

#### SYSTEMATIC SEARCH STRATEGY

Electronic search strategy consisted of two

stages: Stage 1 included studies that specifies anti-microbial peptide levels [7,8] and vitamin D receptor gene polymorphism, Stage 2 included systemic search to identify various studies evaluating these levels in association with oral health and disease Dental caries and Periodontitis.

#### FOCUSED QUESTION

For identifying various studies the below mentioned focused questions [9] were adapted using PICO criteria (Miller and forest 2001) "What is the role of anti-microbial peptide LL-37 (Cathelicidins) in oral health and the association of vitamin D receptor gene polymorphism and dental caries"

#### POPULATION

Younger adolescence and middle aged with dental caries and periodontitis.

#### INTERVENTION/EXPOSURE

Salivary anti-microbial peptide levels and its protective anti-inflammatory effect on oral health.

#### COMPARISON

Control group with Good oral hygiene (Low caries index and good periodontal health) Case group with poor oral health status (High caries index and poor periodontal health).

#### OUTCOME

Effect of LL-37 levels and vitamin D receptor gene polymorphism on caries prevalence and periodontal health.

#### SCOPE

Clinical studies which involved estimation of LL-37 and vitamin D receptor gene polymorphism conducted in human subjects. Comparison done between healthy individuals and study subjects with dental caries and periodontitis. Animal studies, abstracts, letter to editors, narrative reviews and case reports were excluded.

#### SOURCES

Electronic search was performed in Pubmed

medline, ISI web of science [10] and medline database applying Mesh terms "Dental caries", "periodontal disease", "Cathelicidins", "LL- 37", "Vitamin D", "VDR receptor gene polymorphism", "saliva". The search system reviewed the article published between 2009 to 2019. Also a hand search of references and related articles were performed.

**STUDY SELECTION**

Controlled observational studies comparing and evaluating dental caries and periodontitis with LL-37 and vitamin D levels were selected. Studies concerning permanent dentition individuals with any other [11] systemic disease and under medication that effect the salivary composition were excluded. Case report, review articles were excluded. Studies which presented at least 8 of evaluating criteria were considered as low risk bias and 4 to 7 criteria were considered "moderate risk" less than 3 criteria it was considered as high [12,13] risk bias ad excluded from the study due to low evidence.

**RESULT**

Electronic search retrieved 102 no-duplicate

records. However after checking the titles, abstract and full texts, 9 studies were selected for this systematic review. Two of them [7] were excluded because they were classified has " high risk bias " leaving a total of 7 for systematic review (Figure 1) from the selected studies [4]. classified as low risk,3 classified as moderate risk bias (Table 1). Shows quality assessment,

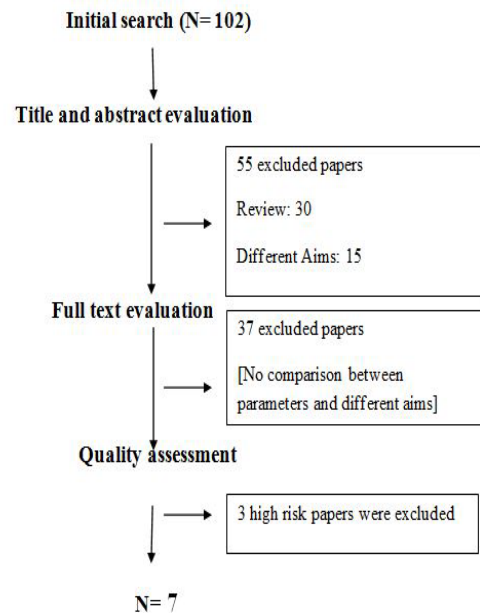


Figure 1: Stages of study selection process.

TABLE 1: Quality assessment of selected articles.

Criteria	Author, Year					
	Turkaglu, et al. 2017	Davidipoulou, et al. 2014	Simon Takeuchi, Sara, Y, et al. 2018	Turkoglu O, et al. 2009	Yu M, et al. 2017	Kong YY, et al. 2017
Inclusion criteria	X	X	X X	X	X	X
Exclusion criteria	X		X	X		X
Dental caries diagnosis criteria	X	X	X		X	X
Periodontal condition and evaluation criteria			X	X		
Saliva/GCF/Buccal swab collection description	X	X	X X	X	X	X
Statistical analysis description	X	X	X X	X	X	X
Risk of Bias	Low	Moderate	moderte low	low	moderate	low
Level of evidence	High	Moderate	moderate high	high	moderate	high

TABLE 2: Description of selected studies.

Author, Year	Sample	Parameter	Methodolgy	Results
Turkaglu, et al. 2017	GCF	LL-37	ELISA	LL-37 in GCF increase in the presence of gingival inflammation, however, this does not vary according to subjects being young or middle-aged
Davidipoulou, et al. 2014	Saliva	LL-37	ELISA	Significantly lower concentrations of LL-37 were found in children with high caries activity, compared to caries free children or to children with low to moderate caries activity
Simon Sara, et al. 2018	Saliva	LL-37	ELISA RT-PCR	Children with low caries at the baseline al 2018 RT-PCR evaluation showed increased LL-37 even during follow up. LL-37 and several genera of bacteria did not correlate with either the health or caries associated dimensions but instead were located in the dimension that appeared to define the pre-transition from healthy to caries status
Takeuchi Y, et al. 2012	Saliva	LL-37	ELISA	Salivary LL-37 was positively correlated with chronic periodontal destruction, lower levels of LL-37 lead to increased risk of periodontitis
Turkoglu O, et al. 2009	GCF	LL-37	ELISA	Elevated levels of GCF LL-37 in chronic Periodontitis suggested their major role in innate immunity system during periodontal

Yu M, et al.	Buccal epithelial Cells, saliva	Salivary Vitamin D levels and VDR receptor gene	PCR-RFLP	VDR-FokI gene polymorphisms may be associated with susceptibility to permanent tooth caries in Chinese adolescent
Kong YY, et al. 2017	Buccal epithelial Cells, saliva	Salivary Vitamin D levels and VDR receptor gene	PCR-RFLP	VDR BsmI polymorphism was associated with the risk of deciduous tooth decay in Chinese children aged 4-7 years.

gives description of study group. Simon Sara included 33 children's grouped based on caries at baseline and developing caries were further evaluated [8]. Turkaglu et al. conducted a study including 4 participants with gingivitis and healthy periodontal tissue was included and presence/absence of caries was not of concern [6]. Another study conducted by Davidopoulou included 49 systemically healthy individuals evaluated for Decay index, periodontal index and gingival index and were divided into 3 groups based on caries experience [7].

Takeuchi conducted a study among 69 patients, comparison between them was done based on probing depth, clinical attachment levels, Bleeding on probing, Plaque record (Table 2) No comparison with healthy individuals was done [9]. Turkoglu in 2009 evaluated IL-18 and LL-37 in 18 healthy 41 individuals with chronic periodontitis and comparison of these levels was done [10]. BsmI and FokI gene role in VDR receptor gene polymorphism was analyzed by [11] among 400 and 380 consequently. Both the studies included caries free group for comparison [11,12]. Based on the results of selected studies 5 studies showed statistical difference between groups with/without caries and with/without periodontitis. Two of the studies showed association between BsmI and FokI gene polymorphism and dental caries.

## DISCUSSION

The selected studies were those which satisfied the minimum criteria that would be able to evaluate the role of LL-37, Vitamin D in caries and periodontal conditions. Rolf Kotsmann in 1956 first reported condition called morbus Kotsmann syndrome (infantile genetic agranulocytosis) with a missing serum factor. These patients were highly susceptible to repetitive infections primarily gingivitis and periodontitis persisted even after antibiotic treatment. Later absence of LL-37 in saliva and load were reported. LL-

37 is tested as an anti-bio film compound, due to its broad spectrum effect, rare resistance development by bacteria's, bacterial growth inhibition and bactericidal activity. These Anti-microbial peptides help in maintaining oral health by allowing more resistant nonpathogenic bacteria to dominate the colonization. The conducive factor for anti-microbial activity is the low ionic strength of saliva, Therefore evaluation of LL-37 plays a major role in caries risk assessment and development of treatment strategies.

Majority of studies showed relationship between salivary LL-37, periodontitis and dental caries. In a study microbial composition was compared with LL-37 levels in children significant increase in LL-37 levels in healthy individuals throughout the study. This longitudinal study concluded that both antimicrobial composition of saliva and bacterial composition predispose development of dental caries [8]. In another study LL-37 levels were evaluated in different age group in patients with gingivitis showed that inflammation of gingiva as an increasing effect on LL-37 levels, independent of individual age group [6].

Caries experience and LL-37 were evaluated in relation to age, gender, type of dentition *i.e.*, primary, mixed and permanent in a study which showed that there is low peptide at early childhood and 100 fold increase in adolescence in ng/ml. Variation in gender was served based on differences in immuno-competency. Thus suggested that LL-37 may serve as prognostic tool for disease and efficient therapeutic agent [7]. A study compared LL-37 in chronic periodontitis shows increase in LL-37 which may be attributed to response of innate defence mechanism against perio-odontopathic bacteria.

In contrast LL-37 was elevated in chronic periodontitis compare to healthy individuals, suggesting an important role of LL-37 in pathogenesis of chronic periodontitis they



concluded that LL-37 in gingival crevicular fluid was sufficient to decrease or prevent periodontal destruction. IL-18 was evaluate to whether both LL- 37 and IL-18 contribute to innate immunity in periodontal destruction. IL-18 is a pro-inflammatory cytokine stimulates mediators of inflammation and activate neutrophil's thus a part of host defence it was showed that LL-37 can stimulate production of IL-18. But in this study there was no correlation between the LL-37 levels and IL-18 in chronic periodontitis [10].

These peptides being one of the important effector molecules of innate immunity is produced by the circulating cells, bone marrow progenitor cells, various epithelial surfaces, inflamed gingival tissues. Vitamin D is critical for regulation of CAMP gene and DEFB [4] gene which codes for cathelicidin and defensins in both normal and hematopoietic cells. Therefore vitamin D boosts innate immunity system and helps in wound healing and infection control. Presence of pathogen stimulates (TLR) Toll-Like Receptor's of macrophages inducing expression of CYP27B1 (25-hydroxyvitamin D-1 $\alpha$ -hydroxylase) which, in turn, leads to the production of bioactive 1,25(OH)<sub>2</sub>D from circulating inactive 25(OH)D. Locally increase in 1,25(OH)<sub>2</sub>D activates CAMP gene which codes for cathelicidin. The releases of cytokines are also mediated through stimulation of toll-like receptors through vitamin D. Thus, Vitamin D levels play a major role in modulating immunity Kong YY and Yu m et al studied the possible correlation between genetic polymorphism of vitamin D receptor and occurrence of dental caries. Vitamin D regulates the calcium metabolism and promotes calcium depositing on enamel, also in a meta-analysis they mentioned that supplementation with vitamin D decreases he occurrence of caries by 40%. Both these studies showed correlation between vitamin D and caries pattern even though the specific mechanism is not understood. A single variable like LL-37 is difficult to establish as a marker because dental caries is multifactorial as a maker in nature. Understanding the complexity of peptide and their role in innate immunity makes it complex to understand the role of these LL-37 peptides in dental caries [13]. They target a broad spectrum of microbial activity, the ease of synthesis they are ideal for formulation of peptide based oral health care products along with routine maintenance of oral hygiene methods.

Since they help to kill bacteria selectively in mixed culture, they can be used as an adjuvant to standard hygiene care in immune-compromised patients, control biofilms, progression of caries, biofilms on implants also prevention of plaque mediated dental diseases. These Anti-microbial peptides can be idealized has biomarkers for dental caries assessment, the up regulation and down regulation of these peptides may help in monitoring treatment outcome pre and post treatment. They are highly conserved and show relatively lower potential to resistance against microbes.

### CONCLUSION

Theselectedstudiesshowedasignificantvariation in LL-37 levels in dental caries, periodontitis, gingivitis and vitamin D polymorphism with dental caries. Still the complexity in mechanism of action of LL-37 and its relationship with Vitamin D levels makes it difficult to conclude. Only with more evidence and analysis we will be able to establish vitamin D and L-37 have a major role to play in occurrence and progression of dental caries.

### ETHICAL STATEMENT

Not relevant.

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