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# Expression of K-19 Cytokeratin in Gingival Epithelial Cells in Periodontal Health and Disease - An Immunohistochemical Study

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## ABSTRACT

**Background and aim:** CK19, a secondary keratin of simple epithelial cells, is the smallest cytokeratin. Its expression was observed in basal and suprabasal stratified squamous epithelial cells of oral mucosa, especially in case of inflammatory conditions and epithelial dysplasia. The aim of this analytical cross-sectional study was to investigate the expression of CK 19 in the gingival epithelial cells of healthy and periodontitis subjects and to correlate the findings with the clinical parameters.

**Materials and methods:** The tissue samples were collected from a total of 30 patients from the healthy and periodontitis groups, during orthodontic/ 3rd molar extractions and during periodontal surgeries respectively. The tissues were then processed for immunohistochemistry analysis and the results were obtained.

**Results:** The data obtained were statistically analysed (Chi square and ANOVA tests) and the results suggested that, there was no statistically significant difference in the expression of CK-19 between healthy and periodontitis groups.

**Conclusion:** The results of this study further affirm the varied responses to destruction (breakdown and repair) from individual to individual during the host-microbial interactions. The mixed outcome could also be due to the suppression of overt inflammation following phase I therapy.

***Keywords:*** basal layers, cytokeratin, immunohistochemistry, inflammation, oral epithelium.

## INTRODUCTION

Cytokeratins (CKs) are keratin containing intermediate filaments, located in the intra-cytoplasmic cytoskeleton of epithelial tissue. They are known to maintain the cell's structural integrity. [1] They are of two types, the high weight/basic that includes the subtypes from CK-1 to CK-9 and the low weight/acidic includes the subtypes from CK-10 to CK-20. [2] Among these, in the junctional epithelium, which was the site of interest in this study, the cells expressed cytokeratins 5, 13, 14, and 19, and rarely CK-8, CK-16, and CK-18. In periodontal

health and disease, there is rapid turnover of junctional epithelial cells, in response to any local stimuli, for the maintenance of tissue homeostasis. In these conditions, CK-19 was observed in basal and suprabasal epithelial layers. [3-5]

In this cross-sectional short study, we attempted to assess and understand the pattern of expression of CK-19 in various forms of Periodontitis.

## MATERIALS AND METHODS

### *Study protocol and Ethical statement*

Thirty subjects were selected from the outpatient clinic of our Institution, and the subjects enrolled in the study were grouped as follows, group I consisted of 11 subjects with clinically healthy periodontium (probing depth ≤3 mm and no clinical attachment loss), and group II consisted of 19 subjects with periodontitis (across all stages and grades- AAP 2017).

The study included systemically healthy individuals between the ages 18-55 years, with no history of any adverse habits or use of any medications that may affect the study. Smokers, pregnant or lactating women were excluded. The research protocol was approved and clearance was obtained from the Institutional Scientific Committee ***(RMDCHISC2020PG02PERIO)***, and informed consent was also obtained from each individual included in this current short study. A brief history and baseline clinical periodontal parameters (plaque index, gingival bleeding index, probing pocket depth and clinical attachment loss) along with the demographic data were recorded for all the subjects included in the study. This is a single blinded study, in which those processing the samples were blinded, by numbering the samples belonging to both groups, which was known only to those involved in samples collection.

### *Tissue Collection*

Gingival samples were harvested for subjects in Group I from sites going for therapeutic extraction or surgical removal of third molar and from subjects in Group II during periodontal surgery subsequent to completion of phase I therapy. The tissues thus obtained from the subjects were stored in 10% neutral buffered formalin and then subjected to immunohistochemistry staining technique subsequently for expression of cytokeratin – 19.

### *Immunohistochemistry staining process*

Tissue sections of 3-4µm thickness obtained from paraffin blocks were mounted on APES (3-aminoporpyltriethoxysilane) coated slides (ABDOS, INDIA).

### *The following steps were performed for the staining technique*

1) Deparaffinization of the sections in xylene, followed by hydration through graded alcohol was performed (MOLYCHEM, INDIA).
2) Antigen retrieval was done by heating the slides in a coplin jar containing Tris-EDTA (SIGMA ALDRICH) buffer (pH 9), and was then cool down rapidly to room temperature.
3) All the incubations were done at room temperature using a Humidifying chamber (Pathnsitu Biotechnology Pvt. Limited, USA).

***The step-by-step addition of reagents is mentioned below*** (Pathnsitu Biotechnology Pvt. Limited, USA)***:***

A) The tissue sections were covered with 3% Hydrogen peroxide for 5 minutes, to avoid non-specific background staining due to endogenous peroxidase activity.
B) Rabbit monoclonal ready to use primary antibody CK-19 were flooded over tissue sections separately for 30 minutes.
C) The slides were treated with Target binder for 10 minutes.
D) The slides were then covered with Horse Radish Peroxidase (HRP) polymer solution for 10 minutes.

*** The slides were washed gently treated with PBS (Phosphate buffer saline) from steps A-D.*

E) The slides were treated with freshly prepared DAB (3,3Diaminobenzidine tetra hydrochloride) solution to remove the excess chromogen.
F) Then the slides were subsequently, counterstained with Mayer's Hematoxylin and were dehydrated using graded alcohol and xylene, before mounting using DPX (NICE, INDIA).

***Interpretation of staining***

The immune stained slides were observed for positivity under 40X/100X/400X magnifications. The presence of brown coloured precipitate indicated positive expression and absence indicated negative for CK-19 expression (Figures 1 and 2).

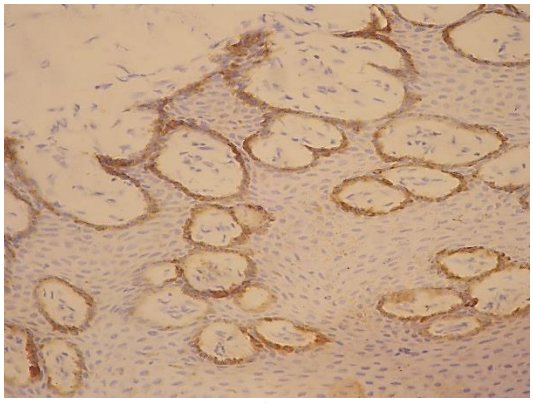


**Figure 1: Positive for CK-19 expression**

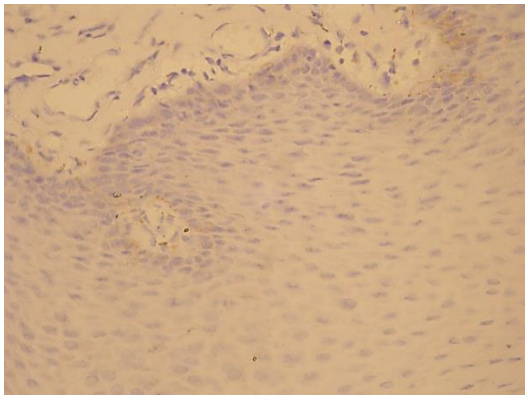


**Figure 2: Negative for CK-19 expression**

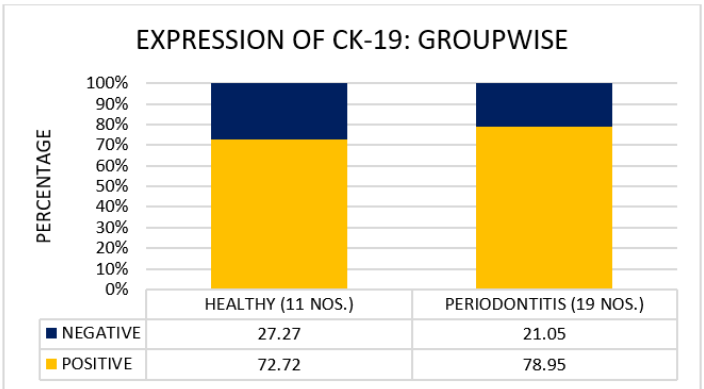
**Statistical Analysis**

The required sample size was calculated using G-power sample size calculation software. The power of the study was 80%. The level of confidence interval was 95%.

All the data obtained were analysed using SPSS 16 statistical software. Chi-square test was done to evaluate the percentage and intensity of CK-19 expression. ANOVA test was performed to evaluate the relationship between the age and clinical periodontal parameters and the positive and negative CK-19 expression. P-value $\leq 0.05$ was considered significant.

## RESULTS

The tissue samples when observed under the microscope, revealed the expression of CK-19 in the basal and suprabasal layers.




**Graph 1: Expression of CK-19: Groupwise**

Graph 1 reveals that, there was no statistically significant difference (Chi square value: 0.151 and P value: 0.698) in the expression of CK-19 between the two groups. 72.72% of the healthy samples and 78.95% of the periodontitis samples expressed CK-19 while 27.27% of the healthy samples and 21.05% of the periodontitis samples were negative.

**TABLE 1: INTENSITY SCORE FOR EXPRESSION OF CK-19: GROUPWISE**

| INTENSITY SCORE | HEALTHY (11 NOS.) | | PERIODONTITIS (19 NOS.) | | CHI SQUARE TEST | |
|---|---|---|---|---|---|---|
| | NUMBER | PERCENTAGE | NUMBER | PERCENTAGE | TEST VALUE | P VALUE |
| 0 | 3 | 27.27 | 4 | 21.05 | 0.523 | 0.914 |
| 1 | 3 | 27.27 | 4 | 21.05 | | |
| 2 | 4 | 36.36 | 8 | 42.11 | | |
| 3 | 1 | 9.09 | 3 | 15.79 | | |

There was no statistically significant difference (P-value=0.914) in the intensity score of CK-19 expression between the two study groups (Table 1). Out of the 7 negative samples, 3 belonged to healthy group and 4 belonged to periodontitis groups. A score of 1 was given to mild staining which included 3 healthy and 4 periodontitis samples. A score of 2 was given to moderate staining which included 4 healthy and 8 periodontitis samples. A score of 3 was given to severe staining which included 1 healthy and 3 periodontitis samples.

**TABLE 2: MEAN AND STANDARD DEVIATION OF AGE AND SELECTED CLINICAL PERIODONTAL PARAMETERS**

| VARIABLE | NEGATIVE (7/ 30 NOS.) | | POSITIVE (23/ 30 NOS.) | | ANOVA TEST | |
|---|---|---|---|---|---|---|
| | MEAN | STANDARD DEVIATION | MEAN | STANDARD DEVIATION | F VALUE | P VALUE |
| AGE | 31.57 | 4.79 | 33.83 | 8.99 | 0.399 | 0.533 |
| PLAQUE INDEX | 1.22 | 0.52 | 1.01 | 0.48 | 0.950 | 0.338 |
| GINGIVAL BLEEDING INDEX | 33.00 | 24.32 | 30.83 | 19.95 | 0.058 | 0.812 |
| PROBING POCKET DEPTH | 4.21 | 1.93 | 5.06 | 2.21 | 0.825 | 0.371 |
| CLINICAL ATTACHMENT LEVEL | 4.66 | 2.46 | 5.23 | 2.38 | 0.306 | 0.585 |

There was no statistically significant difference (P-value=0.533, 0.338, 0.812, 0.371, 0.585), in the age and clinical periodontal parameters as assessed using plaque index, gingival bleeding index, probing pocket depth and clinical attachment respectively, between samples in which CK-19 expression were positive and negative (Table 2).

## DISCUSSION

Cytokeratin 19 is the smallest cytokeratin to be discovered and it is also known as a secondary keratin. Keratins in general, have been proposed to be effective diagnostic and prognostic markers, especially in cancerous tissues, as they were also found to be involved in signalling, cell growth and motility and protein synthesis. This observation of its expression was seen, not only during epithelial dysplasia, but also during inflammation. In periodontal disease, CK-19 was observed in the basal and suprabasal stratified squamous epithelial layers, with particular reference to the junctional epithelium. [4, 6]

Increased expression of CK-19 in the gingival epithelium in patients with periodontal disease when compared with patients with healthy periodontium has been widely noted in multiple studies while mechanisms which lead to this exaggerated expression of CK-19 are poorly defined. [7-10] Ouhayoun et al hypothesized that CK-19 is synthesized in epithelial tissue in response to an environmental stimulus and in gingival tissue this stimulus can be the alterations brought by the inflammatory process in the underlying connective tissue due to periodontal disease. [7] In another study, they found that, the fimbrial adhesion to cytoskeletal elements, which is a contributing factor for P. gingivalis' pathogenicity, has been speculated to cause increased expression of CK-19. [11]

The increased proliferation of junctional epithelium, fostered the increase in the depth of periodontal pockets. [12] A study by Yen-Chun Chen et al. stated that, in gingivitis, CK19 was limited to the sulcular epithelium whereas in periodontitis its expression was significantly high in both the oral as well as the pocket epithelia. This particular study revealed that, along with an increased expression of CK-19, there was also an elevation in the expression of TLR9,

collagenolytic MMP-13 and activated NF-kB subunit p65 found in periodontitis tissues than in gingivitis tissues. [13] However, in an earlier study, its presence in tissues undergoing healing was studied, revealing that it may have a role in the attachment of soft tissues to the root surface. [14] This helps us understand that CK-19 is present in a highly proliferative set-up, such as inflammation and healing or repair. In another recent study, CK19 was expressed by healthy tissues in vitro, but this was not replicated in healthy tissues in vivo. [15]

The non-significant statistical values in the current study can be attributed to the fact that gingival tissue samples of periodontitis patients were obtained during surgical periodontal therapy after suppression of overt inflammation which usually follows phase I therapy. [16] It can also be understood that, the unpredictable pattern and progression of this disease, in each individual, as associated with variations in host-microbial interactions and the possible genetic influence, could have also been responsible for these mixed responses. [17, 18]

## CONCLUSION

We need further studies, which recognize the molecular mechanisms contributing to these changes and their effects on various tissues, with a larger sample size. An extended work, with the use of additional relevant markers for future immunohistochemical studies, along with microbial assays, is being contemplated, in order to completely explore the diagnostic and prognostic importance of K-19 cytokeratin molecule in periodontitis.

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**Conflict Of Interest:** There are no conflicts of interest among the authors.

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