

Original Research Article

Detection of Porphyromonas Gingivalis in the Deeper Tissues of Gingival Squamous Cell Carcinoma Using Polymerase Chain Reaction

Dr. P L Ravishankar¹, Dr. Priyankar Chakraborty^{2*}, Dr. Y. Pradeep Kumar³, Dr. A. V. Saravanan⁴, Dr. E N Anila⁵, Dr. Maharshi Malakar⁶

¹Professor and HOD, Department of Periodontics, SRM Kattankulathur Dental College and Hospital, Potheri, Chennai-603203, India

²Post Graduate Student, Department of Periodontics, SRM Kattankulathur Dental College and Hospital, Potheri, Chennai-603203, India

³Professor, Department of Periodontics, SRM Kattankulathur Dental College and Hospital, Potheri, Chennai-603203, India

⁴Reader, Department of Periodontics, SRM Kattankulathur Dental College and Hospital, Potheri, Chennai-603203, India

⁵Post Graduate Student, Department of Periodontics, SRM Kattankulathur Dental College and Hospital, Potheri, Chennai-603203, India

⁶Post Graduate Student, Department of Periodontics, SRM Kattankulathur Dental College and Hospital, Potheri, Chennai-603203, India

*Corresponding Author:

Dr. Priyankar Chakraborty

Email: cpriyankar65@gmail.com

Abstract: Periodontal disease has been recently linked to a variety of systemic conditions such as diabetes, cardiovascular disease, preterm delivery, and oral cancer. Oral Squamous Cell Carcinoma is a lethal disease whose incidence is increasing. The most common bacteria associated with periodontal disease, Porphyromonas gingivalis (*P. gingivalis*) has not yet been significantly studied in the malignant gingival tissues. The objective of this study was to investigate the presence of *P. gingivalis* in specimens taken from the deeper tissue biopsy in squamous cell carcinoma patients. We have performed Real Time Polymerase Chain Reaction (PCR) technique to investigate the presence of *P. gingivalis* in deeper tissue biopsy samples of gingival squamous cell carcinoma. *P. gingivalis* is abundantly present in malignant oral epithelium suggesting a potential association of the bacteria with gingival squamous cell carcinoma. These observations may help to explain, in part, the predominant role that *P. gingivalis* plays as an infectious agent in oral malignant patients.

Keywords: Periodontal disease, Porphyromonas gingivalis, squamous cell carcinoma, Polymerase chain reaction.

INTRODUCTION

In recent years periodontal disease has been linked with many systemic conditions such as cardiovascular disease, low-birth weight complications in pregnancy, diabetes, pulmonary disease and cancer. The scientific rationale behind this proposed association is the long chronic nature of the inflammatory process underlying periodontitis [1, 2]. Recent studies supports a strong relationship between certain micro-organisms and various forms of cancer. The link between viruses and cancer is well documented in various number of studies. Some bacteria have been associated with gastric cancer as well such as Helicobacter pylori [3]. More recently, a link between cancer and periodontal disease has been suggested through various studies performed [4]. Oral cancer, gingival squamous cell carcinoma in particular has been shown to mimic advanced periodontal disease in clinical appearance presenting

clinically similar symptoms such as swelling, bleeding, tooth mobility, deep periodontal pockets and bone destruction [5]. In some cases the lesions are probably overlapping. Porphyromonas gingivalis (*P. gingivalis*), a gram negative anaerobic bacteria is a major etiological agent for the initiation and progression of the chronic periodontal disease. The virulence of *P. gingivalis* has been attributed to a variety of potential factors associated with its cell surface, including fimbriae, lipopolysaccharides, capsules, proteases, haemagglutinins and major outer membrane proteins. *P. gingivalis* is known to invade and penetrate various epithelial cells. It has sophisticated mechanisms to modify the host cell defense by altering some of the host specific genes. In the past literatures *P. gingivalis* is found to be associated with oral squamous cell carcinoma. According to the literatures when an epithelial cell is exposed to *P. gingivalis* it triggers TLR

signaling. Once the TLR gets activated it results in Interleukin-6 production which further activates STAT-3 gene. After the STAT 3 gene activation cyclin D an important biomarker in the initiation of oncogenic cells expresses itself along with MMP-9 and heparinase.

MATERIALS AND METHODS

Tissue Acquisition

After an institutional review board approval, we randomly selected 10 number of biopsy specimen of oral squamous cell carcinoma lesion from patients admitted in our Oral and maxillofacial surgery department and Adyar Cancer Institute under given consent. The samples were kept in normal saline solutions.

Sample Processing

Samples were then transferred to Tris EDTA (TE) buffer solution. Washed in TE buffer for three times. Samples were then processed for centrifugation in Department of Biotechnology. Centrifuge was set at 12,000 RPM with timer set for 10 minutes at 4°C temperature. After centrifugation tissues were taken out and crushed and again centrifuged under the same parameters. The supernatant was taken in a fresh tube and saved it under -20°C.

Polymerase Chain Reaction

Polymerase Chain Reaction (PCR) was done in the Department of Genetic Engineering. PCR was set for 32 cycles. Time was set for 5 minutes, 10 minutes, 10 minutes, 10 minutes for temperatures 95°C, 95°C, 55°C and 72°C respectively (Table 1).

Table 1: PCR settings

Time	Temperature
5 minutes	95 °C
10 minutes	95°C
10 minutes	55°C
10 minutes	72°C

Conventional PCR had been done under these given parameters. Gel electrophoresis of the samples had been completed for the PCR results. Samples were placed in 2% Agarose gel electrophoresis.

RESULTS

After 25 minutes of gel electrophoresis we detected the staining of *P. gingivalis* DNA amplified by conventional PCR. *P. gingivalis* DNA was detected with in 400 and 500 base pairs marking. Normal length of *P. gingivalis* is 404 base pairs (Fig.1).

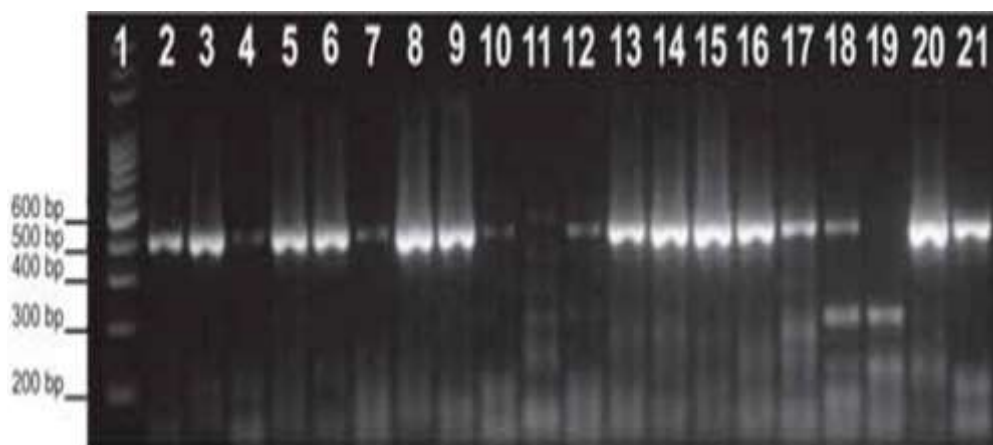


Fig-1: Fluorescent bands of Base pairs of *P. gingivalis* detected under gel electrophoresis

DISCUSSION

In the current study we had identified *P. gingivalis* in gingival squamous cell carcinoma specimens. This finding indicates the bacteria have the capacity to invade the neoplastic cells. Connections between oral cancer and tooth loss or periodontal disease have been evaluated in several studies. Most found a significant increase of oral cancer risk in patients with increased tooth loss or other parameters of periodontal disease even after adjustment for tobacco and alcohol. The significance of our finding of *P. gingivalis* in gingival squamous cell carcinoma is not clear. Although there is a possibility that *P. gingivalis* may be involved in tumorigenesis by inhibiting host cell death and inducing cell proliferation.

CONCLUSION

It may be presumptuous to conclude its carcinogenic potential without further direct evidence. Co-morbidity factors like age, gender, smoking alcohol consumption may affect the ability of the bacteria to invade the gingival tissues and potentially impact the malignant process. Further studies are required to determine its significance in gingival squamous cell carcinoma etiology.

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