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Pathogenic features of *Streptococcus mutans* isolated from dental prosthesis patients and diagnosed cancer patients with dental prosthesis

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ABSTRACT

Though *S. mutans,* virulence, and pathogenesis are characterized, reports are limited to the status of its carriage and virulence in patients with oral cancer and prosthesis. In this present study, we investigated the pathogenic characteristics of twenty strains of *S. mutans* of healthy subjects, fifteen of prosthesis patients, and eleven from oral cancer. The putative virulence gene and other factors, such as the ability to adhere to the oral epithelial cells, production of glycan and lactic acid of these strains were examined. Few of representative isolates of each group were used to see their activity on oral cancer cell line using cell cytotoxicity assay. The isolation rate of *S. mutans* was significantly more in carcinoma than prosthesis patients and control health group. The production of glycan and latic acid was together high in those isolates derived from prosthesis patients and patients with cancer. Adherence ability of strains isolated from prosthesis patients and cancer patients with oral prosthesis were significantly higher, compared to one isolated from a healthy individual. From our study results, we conclude that prosthesis patients and cancer patients with prosthesis carried a high number of *S. mutans* in their oral cavities.

Significance and impact of study: This study report that prosthesis patients and cancer patients with prosthesis carried a high number of *S. mutans* in their oral cavities. However, the *S mutans* are commensals still they have the capability to raise the severity of disease condition due to their ability to produce glycan and lactic acid. In our study, we proved that the adherence to buccal epithelial cells was significantly increased in *S. mutans* isolates of prosthesis patients and cancer patients. These indicate that in prosthesis patients as well as in cancerous patient's microbes had more potential to cause infection and increase the severity.

1. Introduction

Cavities otherwise called tooth decay caused due to specific bacteria which produce acid and other decay factors to destroy tooth's enamel and its underlying layer, the dentin [1]. *Streptococcus mutans* is one of the bacteria associated with tooth decay [1,2], it sounds very simple but remains a largely misunderstood disease despite their prevalence. Dental caries due to *S. mutans* may pass through the horizontal or vertical transmission to another person to colonize itself among human hosts [1,2]. The metabolites of *S. mutans* changes the oral environment as well flora to enhance colonization and formation of dental plaque. *S. mutans* utilizes its receptors to adhere to the tooth, further construct a biofilm. Once established well in dental caries or around the dental prosthesis, it uses three virulence factors associated with the carcinogenicity [1,3]. The first factor is synthesis glycans, ability to become more acid tolerant, and production of lactic acid. Literature reports the role of glycans in tumour development and progression [4]. Glycans affect with cell–cell adhesion of host through influencing the level of functional E-cadherin at the cell–cell border and enhance the process of tumour cell dissociation and invasion. Various studies has indicated altered N-glycosylation of cell surface glycoproteins in cancer and abundant of complex N-glycans during tumour progression [5–7]. Elevated glycolysis is a metabolic symbol of cancer and increased lactate have seen associated with poor prognosis in several types of human

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cancers. Lactic acid is not the only indicator of the glycolytic flux but directly contributes to tumour growth and progression [8].

Failure of the oral prosthesis of any variety particularly in cancer patients might be associated with infection caused by *S. mutans*. High proportions of cancer patients carry *S. mutans* in their oral cavities and are at a greater risk of developing more complex oral implant infections which increase with cancer therapy. *S. mutans* were detected in 82% of the irradiated patients and 100% of the pre radiotherapy patients [3]. Though *S. mutans* virulence and pathogenesis are characterized reports are limited to the status of its carriage and virulence in patients with oral cancer and prosthesis. In this present study, we investigated the pathogenic characteristics of *S. mutans* of the oral origin from cavities of normal subjects, dental prosthesis wearing patients (P group) and diagnosed cancer patients wearing a dental prosthesis (C-P group).

2. Methods and materials

2.1. Study population

The study was conducted at Department of Oral Medicine & Oral Radiology, KVG Dental College, and Hospital, Sullia, D K District, Karnataka, India. All the subjects contacted for participation and only those agreed to participate in the study were enrolled and signed consent forms were obtained. Ethical clearance was obtained from the Institution Research Ethics Committee. A number of 102 subjects were screened consisted of 45 healthy individuals at hospital campus with no history of the prosthesis or had a systemic illness. The dental prosthesis patients group (P group) had 42 patients with complete dentures or removable partial dentures with no signs and/or symptoms of stomatitis. The third group (C-P group) consisted of 15 patients with diagnosed oral cancer wearing dental prosthesis attending outpatient Department of Oral Medicine & Oral Radiology. Among that Nine patients had carcinoma involving gingivobuccal sulcus, whereas the rest of the patients had carcinoma involving tongue, retro-molar area, and soft and hard palate. Demography and brief history were collected.

2.2. Isolation and identification of S mutans

Swab was collected from the oral cavities of normal subjects, dental prosthesis wearing patients (P group) and diagnosed cancer patients wearing a dental prosthesis (C-P group). Collected samples were thoroughly washed using phosphate buffer saline and used for culture procedure. Colonies grown on blood agar plates were identified and characterized following the standard microbiological lab protocol. *S. mutans* was identified based on (according to the information in Bergeys Manual of Determinative Bacteriology 9th ed., 1994) colonial morphology on selective agar, Gram staining, specific growth characteristics, and fermented different carbohydrates sources (mannitol, sorbitol, sucrose, and inulin) were determined to follow the method described by Fingold and Baron (1986) [9]. Additionally, *S. mutans* was identified by the commercial biochemical test system API 20 strep.

Twenty strains of *S. mutans* grown from normal healthy subjects, fifteen of prosthesis patients (P group), and eleven from cancer patients with a prosthesis (C-P group). The virulence factors, such as the ability to adhere to the oral epithelial cells, production of glycan and the production of lactic acid of these strains were examined. Few of representative isolates of each group were used to see their activity on oral cancer cell line using cell cytotoxicity assay.

2.3. Estimation of glycan

To quantify the glycan, bacterial culture were harvested and the supernatant of each bacterial culture was collected in a similar manner. The presence of glycans involves in the periondate oxidation of vicinal hydroxyl groups which forms Schiff base with amine- or hydrazide-based substrate [10]. The chroma of the schiff base, the concentration

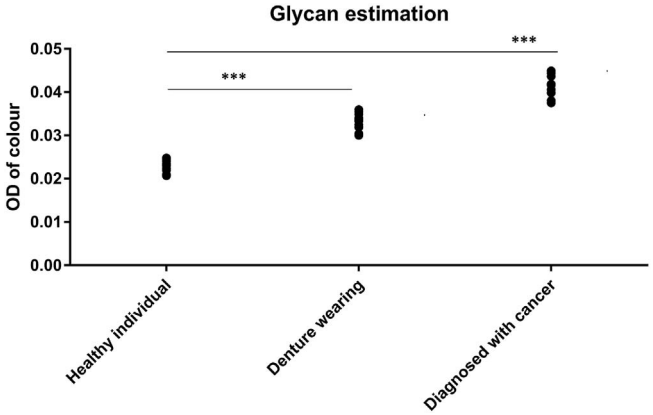

Fig. 1. Glycan production in different groups.

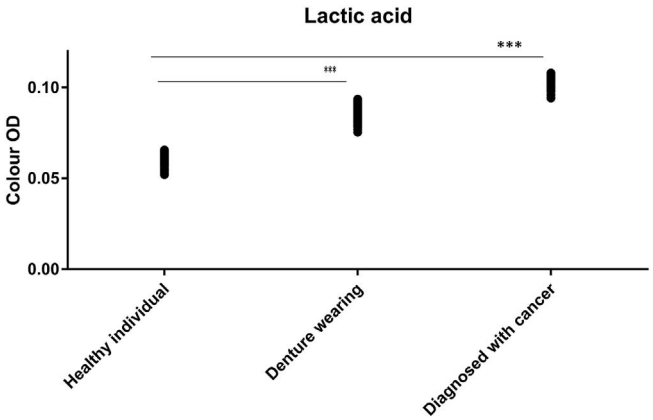

Fig. 2. Lactic acid production in different groups.

were positively correlated and the colour change was recorded through changes in the optical density at 450 with ELISA reader.

2.4. Estimation of lactic acid production

The quantity of lactic acid produced in broth during metabolism determined by transferring 5 ml of culture broth of each isolates into 10 ml tube. One ml of phenolphthalein indicator (0.5% in 5% alcohol) was added drop wise into tube followed by titration with 0.25 M NaOH for the appearance of pink colour. The titratable acidity was calculated as the quantity of lactic acid % W/V where each millilitre of 1 N NaOH is equivalent to 90.08 mg of lactic acid.

2.5. Adherence assay

To perform the assay Buccal epithelial cells (BECs) were collected by gently rubbing the oral mucosal surface of the cheek with a sterile swab, from the mouth of a healthy volunteer with no history of illness and negative culture growth of sample from the mouth, the floor of the mouth and gingivobuccal sulcus. Adherence assays were performed using a previously described protocol [11] with minor modification. Isolates of *S. mutans* from each group were selected based on their glycan and lactic acid production. In P group and C-P group those isolates with high glycan and lactic acid were used for the test. Representative isolates were grown for overnight in Todd-Hewitt broth and incubated at 37 °C while shaking at 100 rpm. Bacterial cells were harvested by centrifugation (6000 rpm, 15 min), washed three times with PBS water by repeated centrifugation, and finally, a suspension was prepared of bacterial cells adjusted to the concentration of 10^6 cells/mL using McFarland control. The bacterial culture supernatant was obtained after the growth density was reached to OD 1.0