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Original Article

Microbiology Section

Detection of *gbpA* and *gbpB* in *Streptococcus mutans* Isolated from Patients with Oral Potentially Malignant Disorders: A Pilot Study

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ABSTRACT

Introduction: Glucan-binding proteins (*gbps*) in *Streptococcus mutans* (*S. mutans*) are considered vital virulence factors contributing to plaque formation and caries progression. These proteins also aid in maintaining biofilm formation on the tooth surface and further colonisation of *S. mutans*.

Aim: To phenotypically characterise *S. mutans* from clinical samples of patients with Oral Potentially Malignant Disorder (OPMD), healthy individuals with and without caries, and to assess the frequency of the *gbpA* and *gbpB* genes among the groups.

Materials and Methods: This pilot study was conducted for a period of two months from May to June 2023 in the Department of Microbiology at Saveetha Dental College and Hospitals, Chennai, Tamil Nadu, India. Saliva samples (N=60) were collected from 20 patients in each of the three different groups: Group 1 included OPMD cases, Group 2 comprised healthy individuals with caries, and Group 3 consisted of healthy individuals without caries (control). Demographic data including age, gender, geographical location, and any previous history of clinical illness were recorded. The samples were promptly transferred to the microbiology lab and cultured on sterile Mutans Sanguis (MS) agar, followed by incubation at 37°C for 48 hours. *S. mutans* were phenotypically characterised, and the frequency of the *gbpA* and *gbpB* genes was assessed using Polymerase Chain Reaction (PCR).

Results: The prevalence of *S. mutans* among the study population was found to be 9 (45%) in Group 1, 8 (40%) in Group 2, and 3 (15%) in Group 3. The study findings revealed the presence of *gbps* in *S. mutans* isolated from OPMD cases, patients with caries, and non-cariogenic healthy patients, with the frequency of *gbpA* as 8 (88%), 7 (87.5%), and 1 (33.3%), and *gbpB* as 9 (100%), 5 (62.5%), and 1 (33.3%), respectively.

Conclusion: The frequency of *gbpA* and *gbpB* from the clinical strains of *S. mutans* associated with caries and OPMD cases was observed in the present study. Periodic surveillance of such virulent determinants would aid in a theragnostic approach to alleviate the complications caused by *S. mutans* in OPMD cases.

Keywords: Caries, Pathogenesis, Oral health, Virulence

INTRODUCTION

Cancer is considered a major global health and economic burden in developing countries [1]. According to the International Agency for Research on Cancer (IARC), India's cancer incidence is expected to rise by over 1.7 million cases by 2035, along with an increase in the cancer-related death rate to 2 million cases [2]. Premalignant diseases are typically located on the floor of the mouth, tongue, gingivae, and buccal mucosa [3]. As stated by the World Health Organisation (WHO), *Candida* leukoplakia, lichen planus, oral submucous fibrosis, verrucous hyperplasia, keratoacanthoma, and erythroplakia are some of the Oral Potentially or Premalignant Malignant Disorders that often act as precursors to invasive squamous cell carcinoma of the oral cavity [4]. The most common risk factors in the development of OPMDs are tobacco chewing, smoking, and long-term alcohol consumption [5].

One widely researched area is the potential link between the altered oral microbiota of oral lesions, molecular alterations, and the development of cancer [6]. The oral cavity of healthy individuals is home to over 700 different types of bacteria, mostly commensals that are crucial for maintaining oral homeostasis by reducing inflammation and producing proinflammatory cytokines [7,8]. In association with OPMD lesions, the most commonly reported organisms include *Fusobacterium* spp., *Veillonella* spp., *Actinomyces* spp., *Clostridium* spp., *Haemophilus* spp., Enterobacteriaceae spp., *Prevotella* spp., *Porphyromonas gingivalis, Capnocytophaga gingivalis*, and Streptococci spp. Among these, the most prevalent gram-positive, facultative anaerobic coccus in the human oral cavity is *S. mutans*. Despite being a commensal of the oral cavity [9], *S. mutans* is a major bacterial organism responsible for the pathogenesis of dental caries, periodontal diseases, and other oral diseases. The production of extracellular polysaccharides, development of biofilm, protein alterations, and acid generation due to the role of virulent genes and proteins like Gtfs, Gbps, and Ftfs attribute to *S. mutans* pathogenesis, leading to caries [10]. Due to its significant presence in oral diseases, the presence of *S. mutans* was suspected in oral premalignant lesions.

S. mutans produces a class of proteins called gbps that support dental biofilm maintenance and cell attachment to teeth, promoting the production of plaque and dental caries. There are four types of *gbp* genes-*gbpA, gbpB, gbpC*, and *gbpD*- that *S. mutans* produces and are responsible for biofilm formation. The glucosyltransferases (gtfs) genes encode glucan-binding enzymatic proteins whose ability to bind glucan keeps them cell-associated even in the absence of a cell wall anchor [11]. The *gbps* significantly contribute to the development of biofilms, sucrose-dependent adherence, and the preservation of a solid, symbiotic microbial community in the oral cavity [12]. The production of *gbps* is often influenced by pH [12]. The *GbpA* protein encoded by *gbpA* is known to facilitate cellular adhesion to the surface [13], and the *GbpB* protein encoded by *gbpB* plays a vital role in peptidoglycan formation, maintaining cell integrity [14]. With this literature background, it is evident that *gbp* are important virulence factors of *S. mutans* in dental

caries pathogenesis, yet a gap exists regarding their frequency in cases with OPMDs. This highlights the need for a novel study to understand the frequency of *gbps* among *S. mutans* strains from different groups of individuals and to compare the frequency with healthy individuals. This study aimed to phenotypically characterise *S. mutans* from clinical samples of patients with OPMD, healthy individuals with and without caries, and to assess the frequency of *gbpA* and *gbpB* in the clinical strains of *S. mutans*.

MATERIALS AND METHODS

This pilot study was conducted at Saveetha Dental College, Chennai, Tamil Nadu, India for a period of two months from May to June 2023. The investigation was designed as a pilot study, so power analysis was not performed, and the study was conducted with a minimum sample size of 20 in each group. Institutional human ethical clearance and consents were obtained prior to the collection of the samples (Ref: SRB/SDC/UG-2141/23/MICRO/125, IHEC/SDC/UG-2141/23/MICRO/275). Saliva samples were collected in sterile specimen containers and immediately transferred to the Microbiology laboratory for processing. The samples were streaked onto sterile Mitis Salivarius (MS) agar supplemented with sucrose and incubated at 37°C for 48 hours. After incubation, the colonies were phenotypically characterised by Gram staining and a negative catalase test [15].

Sampling and isolation of *S. mutans*: The study population included three groups of patients (N=60), with 20 individuals (n) in each group: OPMD patients (group 1), healthy individuals with caries (group 2), and healthy individuals without caries (group 3). As a comparative evaluation of the case-control groups, healthy individuals with and without caries were included.

Inclusion criteria: Patients with specific clinical manifestations of oral premalignant disorders such as leukoplakia, lichen planus, oral submucous fibrosis, verrucous hyperplasia, keratoacanthoma, and erythroplakia were included in group 1. Since *S. mutans* is a potent cariogenic pathogen, healthy patients with caries were included in group 2. As *S. mutans* is a normal oral commensal, a third group was included comprising healthy individuals without caries, acting as the control group.

Exclusion criteria: Patients with other manifestations of wounds or accidental lesions and patients under antibiotic therapy were excluded from the study.

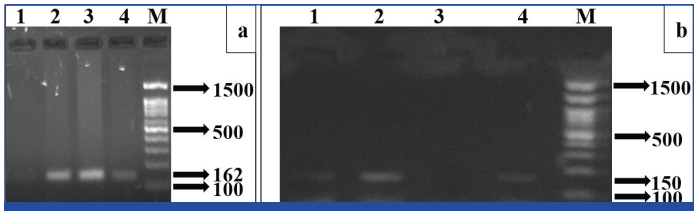
Molecular detection of *gbpA* and *gbpB*: Following the manufacturer's recommendations, genomic DNA was isolated from fresh cultures of *S. mutans* using a Qiagen extraction kit, and it was then stored at -20°C until future need. A 15 µL amplification reaction mixture was prepared using 5.6 µL of double-distilled water and 7.8 µL of 2× Master Mix from Takara, Japan. Specific primers for *gbpA* and *gbpB* were added [Table/Fig-1], and the PCR conditions were set for 36 cycles in a thermocycler (Eppendorf Mastercycler, Germany). The PCR amplicons were visualised on a 1% agarose gel electrophoresis and confirmed using a 100-bp DNA ladder [Table/Fig-2].

Target gene	Primer sequence	Annealing temperature (°C)	Amplicon size (bp)
gbpA	F: GGTGGTTCTGTGCCTGATGA	55	162
	R: TTGCCAGCCTGATACACGTT		
gbpB	F: AGCAACAGAAGCACAACCATCA	55	150
	R: CCACCATTACCCCAGTAGTTTCC		

[Table/Fig-1]: Primer details and the PCR conditions for the detection of *gbpA* and *gbpB*.

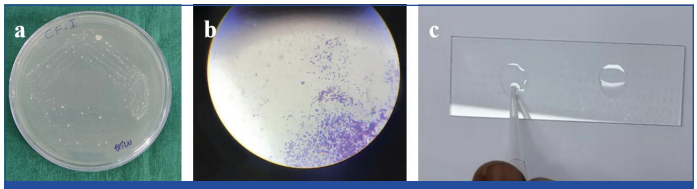
RESULTS

S. mutans formed smooth, raised, adhesive colonies with a characteristic frosted glass appearance on the MS agar. Gram staining showed gram-positive cocci in short chains. The negative catalase test excluded the possibility of Staphylococci spp. being present, further confirming the presence of Streptococci spp. [Table/Fig-3].



[Table/Fig-2]: Agarose gel electrophoretogram with the amplification of (a) *gbpA* (amplicon size 162 bp) and (b) *gbpB* genes (amplicon size 150 bp) with standard DNA ladder (Lane M = 100 bp DNA marker).

Frequency of *S. mutans* and detection of *gbpA* and *gbpB*: [Table/Fig-4] displays the frequency of *S. mutans, gbpA and gbpB*



[Table/Fig-3]: a) Colony morphology of *S. mutans* on MS agar plate; b) Gram staining showing gram-positive cocci in short chains; and c) Negative catalase test.

Groups	OPMD (Group 1) n (%)	Healthy individuals with caries (Group 2) n (%)	Healthy individuals without caries (Group 3) n (%)
S. mutans	9 (45)	8 (40)	3 (15)
gbpA	8 (88)	7 (87.5)	1 (33.3)
gbpB	9 (100)	5 (62.5)	1 (33.3)

[Table/Fig-4]: Frequency of *gbpA* and *gbpB* genes among the clinical strains of *Streptococcus mutans* isolated from the three different groups under study.

DISCUSSION

The OPMD are a group of lesions and conditions that may precede the development of Oral Squamous Cell Carcinoma (OSCC). They usually form due to prolonged mechanical and chemical irritation of the oral mucosa in association with various risk factors. Such abnormal OPMD lesions have an altered microbiome compared to the healthy oral mucosa, contributing to the progression of the lesion to tumorous tissues. *S. mutans* is known to be attributed to this progression with its potent virulence factors. Three key characteristics of *S. mutans*, such as the production of large amounts of glucan extracellular polymers from sucrose, acidogenicity, and the ability to thrive in harsh environments, make it a potent pathogen in the oral cavity [16]. However, the role of *S. mutans* and its mechanisms in OPMD conditions are still unclear.

Alterations in the oral microbiome transform Streptococci into a major potential biomarker, as documented in a study where oral swabs from OSCC lesions showed differences in the *Streptococcus* spp. count between the case and the control [17]. In this context, in the present investigation, it was found that the frequency of *S. mutans* in the saliva of patients with OPMDs was the highest (45%), slightly higher than in healthy individuals with caries (40%), and lowest (15%) in the group with healthy individuals. The property of bacterial adherence to mucosal lesions and surfaces suggests that *S. mutans* adheres to OPMD lesions and plays an important role in colonisation and the spread of infection in such cases [18,19].

In recent years, there could be a drastic change in the prevalence and frequencies, may be due to the host and environmental factors. Being a polymicrobial conglomeration, the carious scrapings in the present study would have shown the presence of *S. mutans* in only eight cases and not in the other 12 cases and this is a unique finding if this study. Similar

findings were found in a study conducted by Salman HA et al., [20].

The increased prevalence of *S. mutans* in OPMD patients could indicate that the ability to form biofilms and adhere to the lesions is potent among these clinical strains. Among various virulence determinants, gbps are a group of virulent proteins responsible for their ability to bind glucan and assist *S. mutans* colonies in colonising the oral mucosa even in the absence of cell wall membrane proteins [11]. *gbpA* and *gbpB* proteins encoded by the respective genes are known for their roles in cellular adhesion and peptidoglycan formation, respectively, underlying caries pathogenesis. Glucans are glucose homopolymers that make up glycans and play a vital role in pathogenesis by their ability to attach to the oral mucosal epithelium [21]. The high frequency of *gbpA* and *gbpB* in this study may be linked to the *S. mutans'* ability to adhere more strongly and form robust biofilms. When compared to its frequency among individuals with caries, its role may be correlated with increased colonisation and progression of the lesions in OPMD cases. Similar observations were documented in an earlier study where higher synthesis of glycans and lactic acid was observed from the clinical strains of *S. mutans* derived from prosthesis patients and patients with cancer [22].

Another observation made in this study is the low percentage (62.5%) of *gbpB* in healthy individuals with caries, while it was a striking 100% in OPMD patients. This contrasts with an earlier study on the *gpbB* genes, which showed nearly 85% in comparison with the gpbA gene, with a lesser prevalence of 80% in the subjects studied [23]. This difference could be due to the unclear function of *gbpB* in the pathogenesis of caries and the fact that caries pathogenesis is mainly due to the property of adherence of *S. mutans* to the tooth structure, which is facilitated by GbpA and GbpC proteins [24]. The frequency of *gbpA* and *gbpB* was lower in the control group, supporting less virulence among the commensal traits in the oral cavity. Looking towards future prospects, the information provided in this study could be beneficial in developing novel drugs that target these specific genes *gbpA* and *gbpB*, leading to reduced complications in cancerous lesions and other orodental disease manifestations. The frequency of various other virulent genes should also be assessed using a larger sample size. This is crucial because the clinical significance of *gpbA* and *gpbB* is closely associated with bacterial adherence to the tooth surface and is critical in the formation of the plaque matrix. Additionally, with the prevalence of *S. mutans* and drug-resistant traits being high in dental settings [25,26], it is crucial to regularly monitor their occurrence in all dental clinics. Predicting putative vaccine candidates for priority pathogens is common through computational approaches [27,28], and *gbps* may be considered as a novel target in designing an anti-caries vaccine.

Limitation(s)

The limitation of the study was that, being relatively new, the available data for comparison ranges from minimal to none. Although the potential for result distortion exists due to the small sample size, statistical analysis could not be conducted. The limited sample size could also hinder the ability to predict results on a larger scale. While *S. mutans* is a significant microorganism, this study only assessed the frequency of *gbp* types A and B in *S. mutans* present in OPMDs and did not explore the association of other types of virulence factors.

CONCLUSION(S)

The prevalence of *S. mutans* was comparatively higher in OPMD patients than in healthy individuals with and without caries. The findings of the study document the frequency of *gbpA* and *gbpB* among the clinical strains of *S. mutans* in association with OPMD cases and caries. *gbpA* and *gbpB* being found to be frequent among the test strains, the findings of the study may be used to design novel drugs targeting these genes both for the diagnosis and treatment of oral lesions in OPMD cases.

Authors contribution: IST contributed for the literature search, data collection analysis and manuscript drafting. ASSG contributed for the conceptualisation, design, data verification, manuscript review. JVP contributed for the final verification and review of the manuscript.

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