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SALIVA AS A PROSPECTIVE DIAGNOSTIC MEDIUM FOR BACTERIAL BIOFILM PATHOGENICITY IN DIABETIC PATIENTS

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ABSTRACT

Diabetic patients are at an increased risk of oral health problems, such as periodontal disease, caries, and candidiasis, due to their impaired immune response and changes in the composition of saliva. Studies have shown that saliva from diabetic patients has a different composition compared to saliva from healthy individuals, with its high levels of glucose, proteins and low level of antimicrobial peptides. These changes can create an environment that is more favourable for the growth of oral biofilm forming bacteria, such as *Streptococcus mutans* and *Porphyromonas gingivalis*. Analysis of saliva can provide important information about the presence of biomarkers for oral health problems, such as cytokines, enzymes, and growth factors. These biomarkers can be used to monitor the progression of oral infections and to evaluate the effectiveness of treatment. Saliva can be a useful tool in assessing oral health in diabetic patients, and has the potential to provide valuable information for the prevention and management of oral infections.

Keywords:, Biofilm, Biomarkers, Diabetic, Infection, Saliva

1. INTRODUCTION

Diabetes mellitus (DM) is one of the most common non-communicable diseases and its complications are a major cause of morbidity and mortality across the globe contributing substantially to health care costs **[1]**. According to the International Diabetes Federation **[2]**, 75% of diabetic individuals live in low and middle-income countries, and it affects 1 in 11 people globally. Its global incidence projections for

2030 and 2045 are approximately 643 and 783 million respectively **[2]**. India has the second-highest burden of diabetes in the world with an estimated 77 million cases in 2019 which is expected to rise to over 134 million by 2045 **[3]**.

Rapidly emerging scientific evidence demonstrates the close association between hyperglycaemia and poor oral health in diabetic patients **[4, 5]**. People with diabetes face a higher-than-normal risk of developing oral infections, including gum disease, tooth decay, thrush, dry mouth, slow healing and burning mouth syndrome **[6]**. Disruption in the oral cavity environment and deviations in microbial ecosystem with augmented cariogenic bacterial load were found in diabetic patients **[7]**. Diabetes facilitates oral bacterial infections by impairing the body's immune response and altering the environment in the mouth providing a favourable condition for bacterial growth, particularly higher levels of salivary glucose **[8, 9]**. Diabetes mellitus has been reported to have an association with significant changes in the subgingival and salivary microbiome **[10]**, *Porphyromonas gingivalis, Tannerella forsythia, Streptococcus mutans, Actinomyces species* and *treponema denticola* are a few clinically reported pathogens **[11]**. Decreased salivary flow rate, high blood and salivary glucose levels and weakened immune system combinely contributes to an environment that favours the biofilm formation and persistent infections in oral cavity of diabetic patients **[12, 13, 14]**.

Complications associated with oral infection in diabetes can become unmanageable due to the establishment of biofilms by these infectious bacteria **[15]**. Biofilms are recalcitrant to antibiotic treatment and a major cause of persistent and recurrent infections **[16]**. These biofilms can cause inflammation and tissue damage in the mouth making it more challenging to manage and prevent oral health problems in diabetes **[16, 17, 18]**. Oral Biofilm operates by allowing microorganisms to work together to form a protective layer that can resist mechanical removal, host defence and antimicrobial agents **[19, 20]**. These organisms generally adhere to the surface of teeth, gums, tongue and other oral tissues and form a matrix which protects them from antimicrobial agents and the immune system **[21, 22]**. This can lead to the development of various oral manifestations including periodontal disease and dental caries **[13, 22]**. Microorganisms in the biofilm exchange nutrients and release acids that can erode tooth enamel leading to the formation of cavities **[23]**. Under healthy conditions the salivary enzymes and antimicrobial agents resist the advancement of biofilm to establish oral infections **[24]**. The significant alternations in salivary composition, flow rate and pH induced by

diabetes contribute to an increased risk of oral biofilm formation and subsequent oral complications in diabetes **[25]**.

Saliva has been emerging as a prospective diagnostic medium for systemic diseases such as diabetes, cancer, autoimmune diseases etc., **[26]**. Salivary biomarkers such as proteins, nucleic acids, metabolites, enzymes and microorganisms that are associated with these diseases were also reported by earlier works **[27, 28]**. Some of these biomarkers have exhibited potential towards early disease diagnosis, monitoring of disease prognosis and to assess treatment efficacy **[26, 29]**. This review focuses on the recent advances in salivary research in oral health and diabetes and addresses the prospective role of saliva in reflecting oral infections that are associated with biofilm-forming bacteria.

2. Changes in salivary composition in diabetes and its influence on oral health

The composition of saliva can influence the microbial environment in the oral cavity and the ability of bacteria to colonize and cause disease **[30]**. Saliva contains a variety of antimicrobial proteins and peptides, including lysozyme, lactoferrin and defensins **[26, 31]**. Additionally, saliva has enzymes like amylase and lipase that can break down the carbohydrates and lipids required for bacterial growth, thus reducing the survival of bacteria in the oral cavity **[32, 33]**. The buffering capacity of saliva, its flow rate and subsequent swallowing encourage the elimination of a large number of bacteria, which is crucial for maintaining the balance of the oral microbiome **[34, 35]**. This pathology contributes to reduced salivary flow rate, its composition and pH (reduction in pH due to metabolic changes) leading to a decrease in buffering capacity and elevated glucose levels in the oral cavity **[13]**. This can promote the growth of acidophilic bacteria such as *Streptococcus mutans*, which can colonise and form biofilms on the tooth surface, further exhibiting a risk of dental caries and oral infection **[36]**. Individuals with diabetes also experience changes in selected mineral and electrolyte levels in their saliva **[37]**. Studies have shown that individuals with diabetes may have reduced levels of calcium and potassium **[38, 39]**. Reduced levels of these minerals can lead to demineralization of teeth, making them more susceptible to decay **[38, 39]**. Studies also directs the association between diabetes and altered levels of antimicrobial peptides in saliva, such as defensins and cathelicidins **[40]**. These are considered as essential components of the innate immune system which can impart their ability to fight oral infection **[39, 41]**. Their suboptimal occurance can lead to an increased risk of developing periodontal disease, a chronic inflammatory condition that affects the

gums and bones that support the teeth **[42]**. Elevated blood glucose associated with diabetes can impede the immune system's ability to combat infections by decreasing the effectiveness of WBC **[43]**. This can make it difficult for individuals with diabetes to combat oral infections, leading to further complications in oral health.

3. Oral cavity changes in diabetes favouring infections

Patients with diabetes exhibit a substantial propensity for the development of oral diseases, with peridontitis having the highest prevalence rate at 67.8%. Diabetes-related xerostomia, dental caries, gingivitis, periodontal disease, increased susceptibility to oral infections, burning mouth, taste alteration, and slow wound healing are some of the significant oral symptoms and sequelae **[44]**. **Table 1** is a compilataion of diverse ways by which diabetes can affect the oral cavity and lead to infections.

Table 1: Oral manifestation favoured by diabetes

Oral Manifestation	Description
Infection Related	
Periodontitis	**Peridontitis and diabetes have a bidirectional relationship. It is characterised by microbially-associated, host-mediated inflammation (i.e., by loss of marginal periodontal ligament fibres, apical migration of the junctional epithelium, and apical spread of the bacterial biofilm along the root surface of teeth) [45, 46].**
Gum Disease	**People with diabetes are at a higher risk of developing gum disease, an infection of the tissues surrounding the teeth [46, 47].**
Thrush	**Individuals with uncontrolled diabetes are more susceptible to fungal infections due to their reduced immunity (eg: candidiasis) [48].**
Dental caries	**This occurs as a result of the bacteria in dental plaque on the teeth producing acid, which damages tooth structures (such as enamel), causing tooth decay. Excessive levels of dental plaque and xerostomia make it common among diabetic individuals [49].**
Halitosis	**A person's poor breath is a sign of halitosis. It may be brought on by poor dental hygiene, cavities, or periodontitis. Due to the frequent occurrence of these disorders in diabetic patients, patients with DM are susceptible to halitosis [50].**
Delayed Healing	**People with diabetes may have a slower healing process in their mouth, which can increase the risk of infections and other oral health problems [46, 47].**
Other Changes	
Taste perception alterations	**Patients with DM express changes in taste perception, most notably hypogeusia [51].**
Dry Mouth	**High blood sugar levels can lead to decreased saliva production, which can cause a dry mouth [46, 47].**

4. Oral biofilm and its correlation with diabetes

Oral biofilms are highly organized microbial communities that develop on the surface of teeth and other oral structures. This harbors polymicrobial communities (bacteria, fungi, viruses, and other microbes) and appears as sticky and yellowish coating on structures like teeth **[52, 53]**. The composition of an oral biofilm may vary based on the surface where it grows and the oral health and hygiene of the individual **[53]**. Efforts towards understanding biofilm origin and development revealed the initial attachment

of free-living bacteria (planktonic cells) on a surface as the key step Following the attachment they form a matrix-enclosed microbial community, creating its own microenvironment, which varies depending on the host or other natural niches **[54, 55]**. The correlation between oral biofilm and diabetes has grown progressively more evident. Individuals with diabetes are more likely to experience oral biofilm and associated dental issues like teeth decay and gum disease **[56, 57]**. Elevated blood glucose level in diabetic patients facilitate the expansion of microbes with biofilm forming tendency eventually resulting in the development of dental plaque **[57]**. Additionally, the weak immune system often associated with diabetic patients make them more vulnerable to illnesses, including oral infections. This makes teeth issues and the oral biofilm concern more severe **[58, 59]**.

Diabetes is associated with an increased risk of oral biofilm infections, which can lead to various oral diseases **[60]**. People with diabetes and periodontitis have different oral microbiomes than those who do not have these diseases. Studies have shown that individuals with diabetes and periodontitis have higher concentrations of specific dental bacteria like *Porphyromonas gingivalis, Treponema denticola, and Tannerella forsuthia* in their mouth **[61, 62]**. Several factors contribute to the increased vulnerability of people with diabetes to oral infections due to biofilm-forming bacteria. Furthermore, changes in the composition and flow of saliva in individuals with diabetes can lead to reduced antibacterial activity and impaired clearance of oral biofilm **[62, 63]**. Diabetes also causes alteration in the oral microbiome, leading to changes in the types of organisms that dominate in the oral biofilm, potentially resulting in a shift towards a pathogenic microbial community **[63]**. Several salivary biomarkers have been associated with oral biofilm infection. Changes in the level of these biomarkers can indicate the presence and severity of biofilm infections and potentially useful for the diagnosis and management of these conditions **[64]**. A few such biomarkers gathered from accumulated literature in this background are discussed in **Table 2**.

Table 2: Salivary biomarkers associated with biofilm pathogenisity in oral cavity

Category	Description
Enzymes	**Matrix metalloproteinase 8 (MMP-8) produced by neutrophils and other cells and response to bacterial infection. Elevated levels of MMP-8 in saliva have been associated with the severity of periodontal diseases and the presence of biofilms. The level of proteases and phospholipases produced by oral bacteria can be used to assess the presence and severity of periodontitis [65].**
Metabolic by-products	**Lactic acid and hydrogen peroxide as markers for oral biofilm infections [64].**
Antibacterial proteins	**Interleukin-1-beta, a pro-inflammatory cytokine, and lactoferrin, an iron-binding protein with antibacterial properties, are two proteins that indicate the presence and severity of periodontitis [66].**

Peptides	**The levels of fimbrillin and collagen-binding proteins produced by oral bacteria can be used to assess the presence and severity of periodontitis [66].**
Antibodies	**The presence of antibodies in saliva can indicate the presence of an oral biofilm infection and can be used to monitor the response to treatment [66].**
Receptor activator of nuclear factor kappa- β ligand	**RANKL is a protein that plays a role in bone resorption in periodontitis. Elevated levels of RANKL in saliva or GCF can indicate the presence and severity of periodontitis [67].**

The relationship between oral biofilms and diabetes is complex and requires a multi-disciplinary approach to prevent, diagnose, and treat oral infections in individuals with these disorders. Current research in this field is focused on understanding the composition and behaviour of oral biofilms, as well as developing new strategies for preventing and treating oral diseases associated with biofilm formation.

5. Salivary bacterial biofilm pathogens and its probable connect with diabetic oral health

The contribution of diabetes mellitus towards periodontitis and other oral complications has stimulated steadily increasing amount of research that has illuminated saliva's promising potential as a medium to reflect bacterial biofilm pathogenicity and oral health in diabetic patients **[68]**. In addition to their existance in planktonic form, microorganisms often exhibit the multicellular aggregation tendency in biological fluids such as saliva **[69, 70]**. Considering the two-way association between salivary compositional changes and biofilm pathogenicity of oral microbiome, studies in the recent past has even assessed the biofilm formation tendency of such aggregates using individual single-species laboratory strains **[71, 72]**. Taking assistance of advaced tools such as next-generation sequencing and bioinformatics, researchers are able to analyze the oral microbiome in deapth **[73]**. Detailed studies comparing the salivary microbiomes of diabetic individuals with that of healthy controls may shed more light on crucial bacterial species and functional pathways that is involved in the oral health complications of diabetic patients **[73, 74]**. The current diagnostic practices such as clinical examination and measurement of clinical factors like pocket depth and attachment loss might not be precise enough to detect early-stage disease **[75]**. Saliva based new non-invasive diagnostic procedures that concentrate on biofilm forming bacterial species or specific functional pathways in the oral microbiome is the need of the hour for early detection and improved management of oral health problems concomitant with diabetes **[76]**.

6. CONCLUSION

Saliva is an intricate fluid that can reveal crucial information concerning the condition of the oral cavity and the prevalence of oral biofilm infections. In

diabetic patients, saliva has been found to play a crucial role in the development and progression of oral diseases, such as periodontitis and caries, due to the effect of elevated glucose levels on oral bacteria and oral tissues. Research has shown that diabetic patients have a higher risk of developing oral biofilm infections due to changes in the composition and quantity of saliva, including a decrease in the levels of certain antibacterial peptides. This leads to a shift in the oral microbiome, which can promote the growth of harmful bacteria and increase the risk of oral infections. Researchers are investigating the use of saliva as a diagnostic tool to detect oral biofilm infections and oral health in diabetic patients to solve this problem. Saliva analysis can reveal the existence and abundance of particular oral microbes as well as indicators of oxidative stress and inflammation, which are linked to oral diseases. Include the non invasive of salive , predictions and use in future point of care tool. Additionally, some studies have investigated the use of saliva as a non-invasive tool for monitoring glucose levels and the progression of diabetic complications in the oral cavity. Overall, the use of saliva as an indicator of oral biofilm infections and oral health in diabetic patients holds great promise for improving the early detection and management of oral diseases in this population.

Conflicts of Interest: The authors declare no conflict of interest.

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