



## **The diversity of microbial flora in the mouths of patients with sickle cell anemia**

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### **Abstract**

Sickle cell disease is an autosomal recessive hemoglobinopathy predominant in the Middle East. SCA patients and are being categorized as a public health issue as it affects a significant percentage of the world's population. The aim of this study is to review the Oral Cavity Microbiome in Patients with Sickle cell disease and study the association of dental caries among sickle cell disease patients in relation to their oral microflora. The objectives of this study were also to identify the composition of oral microbiome in a group of patients with sickle cell anemia (SCA) in relation to their general health, age, and sex. Furthermore, several studies have shown that the oral microflora is related to systematic health, oral illness, and a co-relation with several diseases has been linked. The oral microbiome is dependable on multiple factors and circumstances such as, environmental and the host general health, for example hereditary sicknesses such as SCA plays a has a huge rule on the influence of the oral microflora of the host.

**Keywords:** Sickle Cell Disease, Microbiome, Oral Cavity, Dental illness

### **1. Introduction**

Sickle cell anemia (SCA) is a common hereditary disease. It is prevalent in many parts of the world because of that, it presents a worldwide health concern. In fact, many Reports have estimated that more than 10 million individuals in Africa, Arab countries, and India are sickle cell anemetic. SCA is prevalent in 2 major parts of Saudi Arabia: The Eastern and South- western provinces (19). In Sickle Cell Disease, there is an abnormality of the hemoglobin that carries

oxygen to cells throughout the body. The abnormal hemoglobin, identified as hemoglobin S, has a lower functional capacity and that results in multiple systemic complications. Hemoglobin S distorts the shape of the red blood cell into a sickle and sometimes a crescent shape, giving the disease its name. SCA is predominantly found within the Arab Indian group of the b-globin gene cluster in the Eastern area of Saudi Arabia and with the Benin haplotype in the Southwestern area of Saudi Arabia. In SCD patients, the life cycle of red blood cells are affected severely and lowered

from the usual 120 days to around 10 days (19). This is due to the abnormal hemoglobin presence and their sickle shaped blood cells. Further, the red blood cell breaks down prematurely in the spleen of SCD patient, which in turn, causes fewer red blood cells circulation in the body and leads to sickle cell anemia disease. Moreover, hemoglobin in the red blood cell is the main component that is responsible of delivering oxygen to the cells throughout the body, this results shortage results in multiple symptoms of oxygen deficiency, including fatigue, dizziness, lightheadedness, irritability, and breathing shortage. Further, the fast breaking down of the red blood cells, hemolytic anemia, can also result in the yellowing of the patient skins and eyes. Oral health illnesses of hemolytic anemia patients are generally characterized as, paleness of the oral mucosa and pain due to Vaso-occlusive problems within the blood circulation of the dental pulp. Oral manifestations of SCA disease are well recognized in SCA. Recently, several reports have also linked SCA patients with jaw changes. This link has been attributed to SCA being an illness that depends on multiple factors and system disease, including changes in the teeth that are caused by deficiency in the formation of dental and bone tissues with an associated prevalence of opacities in the teeth. In addition, several reports have demonstrated a relation between SCA and dental caries. Dental caries is also happens also in regards to multiple oral factors, the process is also promoted by several factors such as, the formation of microbial biofilms, diffusion process, saliva, and sugar that cause an imbalance between disease-causing and protective factors that influence the dynamic of demineralization and remineralization. As a result, the progressive dysbiosis is thought to cause demineralization of dental tissue.

The oral cavity contains one of the most diverse and unique communities of microbes in the human body, however those communities are relatively understudied as compared to the gut. For example, a PubMed search with “oral microbiome” resulted in 746 articles, as compared to 5605 with “gut microbiome”. A milliliter of saliva contains approximately 108 microbial cells

(2), also a wide range of studies have revealed up to 700 unique prokaryotic taxa, it is estimated that a typical healthy oral microflora compromise of a range of about 100 and up to 200 distinct bacterial communities. Likewise, recent developments in Next Generation Sequencing (NGS) applications has opened new doors for metagenomic studies in larger scale in diverse populations, this development is allowing characterization of the microbiome community in a certain niche and, in some areas, the functional roles of the microbiome and its implications on the health (3). Furthermore, it is estimated that only up to 57% of the oral bacterial species have been officially characterized, and only around 13% have been cultivated thus far, yet remain uncharacterized, and about 30% are uncultivated. Hence, why NGS techniques and application make analysing microbiomes in certain niches relatively quick and easier. Further, NGS have also vastly expanded our awareness of unculturable and rare microbiota communities. Thus, this study aims to shed new light into the oral microbiome of Sickle Cell anemia patients. The oral microflora is an exciting and expanding field of research. Oral microbiome is crucial to health as it can cause both oral and systemic diseases. It rests within biofilms throughout the oral cavity and forms an ecosystem that maintains health in a state of equilibrium. However, certain imbalances in this state of equilibrium allow pathogens to manifest and cause disease. Disruption of the oral microbiome leads to dysbiosis. Identifying the microbiome in health is the first step of human microbiome research, after which it is necessary to understand the role of the microbiome in the alteration of functional and metabolic pathways associated with the diseased states (4). The Human Oral Microbiome Database (HOMD) is based on 16S ribosomal RNA (rRNA) gene reference sequences and is a valuable resource. The expanded Human Oral Microbiome Database (eHOMD) covers numerous microbial species inhabiting the mouth and nose. However, annotation or classification is hampered by the high conservation of 16S sequences among closely related species. For this reason, an intermediate taxonomic “superspecies level” was added between genus and species, which

consisted of indistinguishable species sharing closely related sequences, reducing the error rate of short- and long-read 16S rRNA. However, the HOMD is derived from microbial culture data. Because of culture conditions and interactions of the microbiome with other factors, 20%–60% of oral microorganisms in the HOMD are unculturable. Sequencing the metagenomes of bacteria isolated from the oral cavity enables the recognition of some unculturable oral microbes.

## **2. Microbiome**

Microbial communities are defined as the collection of microorganisms living together. essentially, microbial communities are defined as a group of bacterial species in a niche, in which microbes' organisms interact with one another in the environment. Furthermore, in 1988, Whipps while working on the flora of rhizosphere microbes coined the first definition of the term microbiome. He defined the microbiome as a combination of the word's "micro" and "biome". In which, a microbiome is a "microbial community" in a "well-defined niche in which it has unique physical and chemical properties." This definition describes a microbial group with unique functions and properties, also, its relationship with its surrounding habitat, resulting in the formation of specific niches. The most cited and widely recognized definition is by Lederberg (5), he describes the microbiomes in an ecological context, as a microbial community of symbiotic, commensal, and pathogenic microorganisms within a an area of space or niche.

Microbiota comprises of all living microbial members that forms the microbiome. Bacteria, fungi, archaea and are all considered as a member of the microbiome. Most researchers agree with this definition of the microbiome(6). Furthermore, symbiotic relationships have a wide range of significance on the microbial survival and the microbiome population dynamics and their capacities within the microflora. These interactions and relations between the microbiome members can be between microbes of the very

same family species or between distinct species, of the same or different genera, genus, and domain. The interactive motifs within these interactions can be positive such as mutualism, synergism and commensalism, or negative interaction such as amensalism, predation, parasitism and antagonism, or neutral interaction where there is no effect on the relation between the species. Microbial life interaction can influence the result of those interactions. For instance, bacteria that are competitors for the same resources can also be beneficial for each other when also take part in contending for the same resource at different niches. Moreover, the stability of a microbiome ecosystem depends on interactions for the same minerals at different concentration levels.

The significance of the microbiomes plays and important role in the health of the host. microorganisms from unrelated ecosystems and hosts can highly interact and impact each other (5). These findings have coined the term "A healthy microbial environment promotes healthy human being". Furthermore, according to the World Health Organization, "One Health" is a new approach which help in designing and implementing programs, policies, and research studies in which researchers and other important sectors work together to improve and develop new approaches for a better public health result. Notably, the One Health expansion includes ecological health and its relation to human way of living, health and culture suggests that the lifestyle of the microbiome plays and important part in human health and should be considered in societal decisions and policy making (7). For instance, urbanization has been linked with higher rates of illnesses such as asthma, allergies, and other chronic diseases. Moreover, pollution is a measure contributor to the loss of microbial diversity that has been observed in urban areas, in which it has been associated with disease development. Recently it has been put forward that exchanges in the microflora occurring concurrently with industrialization might be the underlying issue that increases immune, metabolic, and cognitive illnesses, including diabetes, obesity, allergies, asthma, inflammatory

bowel disease, and in some severe cases autism (8). Thus, the loss of diversity in turn is linked with an increased rates in the emergence of microbes resistance against antibiotics giving the rise to antibiotic resistant microbes. Thus, indicating the need for implementing plans and policies to restore healthy microbial diversity in the environment. It is particularly important to understand and research the complex relations in-between microflora across different hosts and environments also, their role and importance in the health of humans, plants, and animals. It has huge potential for innovative and important approaches from diagnosis to treatments (5).

### 3. Oral Microbiome:

Diverse microorganisms colonise the oral cavity and unique to this niche as they have evolved specifically for oral colonization (9). Within the oral cavity, there are a unique environment such as the surfaces of the teeth and the surfaces of the gum membranes. These surfaces are constantly exposed to oral fluids such as saliva. The microbial communities that grow on these surfaces are also unique, for instance any one site in the oral can contain up to ~50 species a subset of ~1,000 species that are capable of oral colonization. Moreover, feature of many successful oral colonizers, is providing resistance to the mechanical forces of fluid flow. The main colonizers of oral surfaces are predominantly facultative anaerobes such as *Streptococci* and *Actinomyces* species. The area of the subgingival tissues has reduced oxygen levels which favour population shifts with increasing abundance of strict anaerobes such as *Bacteroidaceae spp.* and *Spirochaetes*. Additionally, microbial composition the spatial and structural organization of natural microbial communities is being increasingly recognized as essential for physical and metabolic interspecies interactions that can be antagonistic or cooperative. Microorganisms inhabiting teeth surfaces form multispecies biofilm communities that are often embedded in a matrix of extracellular substances. On the other hand, epithelial surfaces necessitate a specialized colonization tool.

Although organisms do form biofilms on these surfaces, there is less time for biofilm to reach maturation than with teeth surfaces. Additionally, microorganisms penetrate and form within epithelial tissues and in some cases intracellularly. Further, a homeostatic balance exists between the host and microbial communities. The residing microbiome is thought to compete with and exclude pathogens as a component of the area stability, as well as, contributing to tissue and immune system development (10). Prolonged accumulations of biofilms also known as plaque on tooth surfaces is caused by gingivitis. Gingivitis is a controlled immune inflammatory state that does not permanently damage the integrity of the tissues supporting the teeth. Furthermore, saliva also plays an important role in the oral ecosystem stability by acting as a buffering agent in the oral environment. It provides the necessary nutrition to the oral microflora and possessing antimicrobial properties that are antagonistic to external microbes. The microbes that inhabiting and living throughout the areas of the oral cavity is fairly predictable, making it easily identifiable from the other microbiomes of human body habitats (11). However, while the niches in the oral cavity are largely inhabited by same microbes, some may be present in different areas and concentrations. Further, a study combining samples from 10 different niches in the gastrointestinal tract in over 200 subjects from the United States, placed these niches into four groups based on the similarity and overall composition (12). Furthermore, the oral microbiome is one of the most important and complex microbial communities in the human body. It is also one of the five research priorities (oral cavity, nasal cavity, vagina, intestine, skin) of the human microbiome project (HMP). The understanding of oral microbes has become very important in further understanding the role of oral microorganisms in periodontal diseases, caries, and other oral diseases. The inflammation of periodontitis leads to the loss of connective tissues and bones (13).

## 4. Sickle Cell Anaemia:

### 4.1. Sickle Cell Anaemia Disease:

Sickle cell disease (SCD) is an inherited disorder of the hemoglobin. It is defined by the generation of long bonds of hemoglobin when deoxygenated form sickle shaped red blood cells, that generally progressive into multiple organ damage, and increased mortality. It is estimated that around 310,000 infants are born annually worldwide with SCA. Most of these individuals with SCD are born in the Mediterranean, India, sub-Saharan Africa, and Middle East. SCD abnormality can cause small blood vessels to get stuck, which lead to slow blood and oxygen flow throughout the body. Moreover, red blood cells are flexible and round, moving easily through blood vessels. However, in patients with sickle cell anemia, the red blood cells become rigid. Normally, red blood cells can live up to 120 days before the body needs to replace them, while sickle cells last only 10 to 20 days causing anemia because of red blood cells disorder.

Haemoglobin (Hb) is a tetrameric protein, it is formed by a combinations of different globin subunits, each globin subunit is associated with the cofactor hemo, which can carry a molecule of oxygen. Hb is expressed by red blood cells, both immature red blood cells mature red blood cells. Further, several genes are responsible for encoding different types of globin proteins, and their various tetrameric variations which generate multiple types of Hb. In which, are normally expressed at different stages of life — embryonic, fetal, and adult. Hb A (HbA) is the most abundant (>90%) form of adult Hb, comprises two  $\alpha$ -globin subunits (encoded by the duplicated HBA1 and HBA2 genes) and two  $\beta$ -globin subunits. A single nucleotide substitution in HBB results in the sickle Hb (HbS) allele S; the mutated protein generated from the S allele is the sickle  $\beta$ -globin and has a substitution of amino acid. Conditions of deoxygenation, when the Hb is not bound to oxygen, Hb tetramers, includes two of these mutant sickle  $\beta$ -globin subunits that is, HbS can polymerize and cause the erythrocytes to form a

sickle or crescent shaped from which the disease takes its name. Sickle erythrocytes generally lead to Vasocclusive episodes that dominantly effect SCD patients. SCD has many symptoms, including severe anemia, episodes of pain, in addition to a range of complications, most dominantly, acute chest syndrome (ACS) that causes chest pain, dizziness, chronic kidney disease (CKD), heart failure, splenomegaly, Immunodeficiency pulmonary hypertension, gallstones, Joint damage, stroke and in severe cases paralysis and delayed puberty.

### 4.2. Sickle Cell Disease Statistics:

SCD affects all body organs with many complications such as, acute chest syndrome (ACS), gallstones, stroke, and many other illnesses. Among all hemoglobinopathies, SCD has the highest health concern. It is the most common hemoglobin disease worldwide and one of the most important single-gene diseases of humanity. Worldwide, SCD carriers are about 7%. it is estimated that around 300,000–400,000 babies born yearly with severe forms of SCD (14). SCA is scattered all over sub-Saharan Africa, the Middle East, and parts of India. In Africa, around 200,000 of newly born babies are born yearly with SCD. SCD is the third main cause of hospital admissions and mortality among children during hospital stay in Africa.

In Saudi Arabia, SCD is a prevalent hemoglobinopathy disorder. In Saudi Arabia about 4.2% of population carries the sickle-cell trait and 0.26% has sickle-cell disease The highest prevalence is in the Eastern province, where approximately 17% of the population carries the gene and 1.2% has sickle-cell disease. Furthermore, according to the Saudi Premarital Screening Program (SPSP) report its prevalence to be 5/1000 for sickle cell trait (SCT) and 0.38/1000 for sickle cell disease (SCD) (14). Moreover, newborn screening estimated the prevalence of 21% for SCT and 2.6% for SCD. One of the main factors that are continuing the occurrence of SCT and increases the risk of sickle

cell patients is the increased frequency of consanguinity. In Saudi Arabia, premarital screening is the main prevention method to prevent the inheritance of hemoglobinopathies including SCD. SCD is the most common hemoglobinopathy globally. In Saudi Arabia. It is most prevalent in Jazan region after the eastern region. SCD was the most common cause of admissions. More than one-fifth of all patients' admissions for 1 month were of sickle cell patients. Similar results were also reported in Macca, Saudi Arabia, in 2013, which sickle cell patients had the largest portion of patients' admissions among anemic patients admitted to Al-Noor specialist hospital with a percentage of 38.38%.

## 5. Discussion

Dental caries is a multifactor illness that depends on a lot of different circumstances and processes that results from an imbalance between disease-causing and protective factors that influence demineralization and remineralization. Complications of dental caries in patients with SCA often include pulpitis, acute pain, and periapical periodontitis, are often linked to a poor quality of life. Furthermore, metanalysis reports has indicated that there is increased level of dental caries in patients with SCA in comparison to children without SCA (15). Several reports have indicated that there is an association between SCA and periodontal diseases and dental caries (19,1,16). The increase in SCA patient with dental illnesses has been attributed to the SCA-mediated impairment of blood flow to dental tissue that induces pulpitis, periapical periodontitis, with deficiency in the formation of dental and bone tissues and, consequently, an increased risk of infections. These conditions of restricted blood flow favour the colonization of facultative anaerobic bacteria such as *Streptococci* and *Actinomycin* species. For example, *Streptococci sanguinis*, which is a normal inhibitor of the oral microflora in humans, was present in our patients with SCA and could be the prime source of the cariogenic biofilm, but their presence alone is not

sufficient for the formation of the cariogenic biofilm. *Streptococci sanguinis* is known to produce H<sub>2</sub>O<sub>2</sub>, which inhibits the growth of many other microorganisms. Patients with SCA have more abundant bacteria from the *Proteobacteria* phylum and *firmicutes* than their counterparts. These results are similar to a study conducted in children from China in which the percentage of *Proteobacteria* was increased in children with dental caries compared to children with no dental caries and in children from Myanmar where *Proteobacteria* bacteria were the most abundant oral microbiota in children with dental caries (1,17,18).

SCA in Saudi (19) patients have a higher predominance of pathogenic bacteria in the oral cavity. This leads to a lot of dental health issues and tooth cavity and decay. This could also lead to more complicated oral health complications. Furthermore. Patients with SCA are immunocompromised due to the dysfunctional innate and adaptive immune system. This research further highlights the importance of upkeeping routine oral hygiene visits for patients with SCA. as we show here, patients with SCA a greater abundance of oral microbiota species that favor dental caries. Nevertheless, a well-designed longitudinal study with a large sample size evaluating the oral microbiota association with dental caries in patients with SCA is needed. We also recommend sitting up dental awareness among SCD patients for frequent dental visits and examination which includes, preventive dental care and promoting oral hygiene practices with toothbrushes, toothpaste, mouth floss and mouthwash are recommended.

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