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Comparative Evaluation to Determine the Candida Species in Patients with Oral Squamous Cell Carcinoma and Leucoplakia: An Original Research Study

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KEYWORDS

Candida Albicans, Oral Squamous Cell Carcinoma, Leucoplakia, CHROMagar Candida, Sabouraud Dextrose Agar

ABSTRACT:

Aim: This study aims to determine the Candida species in patients with oral squamous cell carcinoma and leucoplakia

Materials and Methods: This study investigated oral health issues and the presence of Candida species in patients with mouth patches and ulcers. A total of 70 patients were initially examined, with 20 diagnosed with leukoplakia and another 20 with oral squamous cell carcinoma (OSCC). The final analysis focused on 60 patients aged 35 to 60 who had biopsy-confirmed invasive OSCC and either adjacent dysplasia or non-dysplastic leukoplakia. Patients with untreated primary lesions were included, while those with conditions like lichen planus or recent chemotherapy were excluded. Informed consent was obtained from all participants. The study categorized participants into three groups: 20 healthy individuals, 20 with leukoplakia, and 20 with OSCC. The main goal was to identify and characterize Candida species across these groups. Samples were collected using sterile swabs for microbiological analysis on Sabouraud Dextrose Agar (SDA), and Colony Forming Units (CFUs) were counted. Specific species identification was performed using CHROMagar Candida.

Statistical Analysis and Results: The study examined a total of 60 participants aged between 35 and 60, including 31 males and 29 females. The participants were grouped into three distinct categories: Group 1 consisted of 20 healthy individuals, Group 2 included 20 patients diagnosed with leukoplakia, and Group 3 comprised 20 patients suffering from oral squamous cell carcinoma. In Group 1, the analysis revealed that 16 out of 20 healthy participants were free from Candida species, indicating a relatively low prevalence in this cohort. Conversely, Group 2 showed a significant increase in Candida presence, with only 1 patient reporting no detection of the fungus. Notably, all patients in Group 3 had Candida present, indicating complete colonization among those with oral squamous cell carcinoma. The study reported on the growth of Candida on Sabouraud dextrose agar, which was found in 19 participants. Additionally, chrome agar results showed growth in 18 individuals. To assess the differences in Candida colonization between the groups, the researchers employed one-way ANOVA analysis, aiming to explore the relationship between oral health and the presence of Candida.

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Conclusion: The study indicated that OSCC patients had a significantly higher Candida carriage than those with leucoplakia, suggesting greater susceptibility to fungal infections. Leucoplakia patients also had more Candida than healthy individuals. Notably, non-albicans species like C. tropicalis, C. krusei, and C. glabrata were common and associated with oral pathologies. Increased colony-forming units (CFU/mL) in OSCC patients suggest greater virulence and potential for tissue invasion.

Introduction

Oral Squamous Cell Carcinoma (OSCC) is recognized as the most prevalent and formidable type of oral cancer, arising from the squamous epithelial cells that line the complex structure of the oral cavity. This aggressive malignancy accounts for a significant proportion of oral cancer cases globally, with its onset closely linked to a variety of high-risk factors. Among these, the dual perils of tobacco and alcohol consumption are particularly prominent, contributing greatly to the disease's prevalence.[1,2] Additionally, the traditional practice of using betel quid, a stimulant steeped in cultural significance, introduces its own array of health hazards, further complicating the landscape of oral cancer. In its initial stages, OSCC may present through potentially malignant disorders, such as leucoplakia, which manifests as white patches on the mucosal surfaces, and erythroplakia, characterised by red lesions that may signal a risk for malignant transformation.[3,4] These lesions often remain asymptomatic for prolonged periods, cleverly masking their potential to evolve into more severe and life-threatening forms of cancer. Initial clinical signs of OSCC may include persistent mouth sores or ulcers that resist healing, localized pain that can become utterly debilitating, and complications such as dysphagia (difficulty swallowing) and masticatory dysfunction (trouble chewing). Patients may also report sensations of numbness or altered sensations in the affected oral tissues, adding to the complexity of diagnosis. Due to the wide variability in symptoms experienced by individuals, timely detection of OSCC becomes essential for enhancing prognosis and improving overall treatment outcomes.[5,6] Managing Oral Squamous Cell Carcinoma (OSCC) often requires a comprehensive and multidisciplinary approach that addresses the complexity of the disease. This treatment strategy may begin with the surgical excision of tumors to effectively remove cancerous tissues and prevent further spread. To complement this surgical intervention, radiation therapy is carefully administered, precisely targeting any residual cancer cells that may remain, thereby minimizing the risk of recurrence and ensuring a more favourable prognosis.[7,8] In addition to localized treatments, systemic chemotherapy is frequently utilized as a proactive measure, aiming to counteract the potential dissemination of cancer throughout the body. The effectiveness of these treatments can be significantly influenced by lifestyle choices; specifically, the detrimental effects of tobacco and alcohol use are well-documented, as they play a crucial role in both the development and progression of OSCC. Cultural practices, such as the chewing of betel quid, further complicate this landscape and contribute to increased risks. Moreover, certain infections, particularly those caused by high-risk strains of human papillomavirus (HPV), have been linked to the onset of OSCC. Other significant contributing factors include poor oral hygiene, nutritional deficiencies that undermine the body's defences and genetic predispositions that affect DNA repair mechanisms, collectively heightening the likelihood of developing this aggressive form of cancer.[9.10]A notable clinical manifestation of OSCC is leukoplakia, which appears as distinctive, non-scrapable white lesions within the oral cavity. These lesions typically result from chronic irritation due to harmful substances such as tobacco and alcohol, serving as alarming indicators of potential malignant transformation. Understanding the multifaceted nature of OSCC is essential for implementing effective preventive and treatment strategies. The rate of malignant transformation for leukoplakia is reported to fall between 0.1% and 17.5%, underscoring an urgent need for vigilant monitoring. Accurate diagnosis often requires a biopsy to assess cellular changes that may indicate dysplasia or malignancy. Management strategies for leukoplakia extend beyond merely addressing risk factors; they demand ongoing vigilance and follow-up care. In

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instances where dysplastic lesions are identified, surgical excision or laser ablation may be recommended as proactive measures to mitigate the risk of progression to OSCC.[11,12] Recent research has illuminated a fascinating connection between certain species of the Candida genus, particularly Candida albicans and Candida tropicalis, and the development of higher grades of dysplasia as well as OSCC. This association highlights the critical importance of maintaining a balanced and healthy oral microbiome as part of comprehensive cancer prevention strategies. Innovations in diagnostic methodologies now include advanced techniques such as oral swabs, saliva samples, and microbiological culture methods. Furthermore, molecular techniques like PCR-RFLP are increasingly utilized to accurately identify specific Candida species in patients diagnosed with OSCC and leukoplakia.[13] These advancements aim not only to facilitate early detection but also to enable more targeted and effective interventions, ultimately striving for improved clinical outcomes.

Materials and Methods

This study was designed to investigate oral health issues and the potential presence of Candida species in patients experiencing discomfort associated with patches and ulcers in the oral cavity. Initially, the research included a cohort of 70 patients who reported such symptoms, undergoing an extensive series of clinical investigations and diagnostic assessments. From this group, 20 patients were identified and diagnosed with leukoplakiaa condition characterized by white patches in the mouth while another 20 patients were confirmed to have oral squamous cell carcinoma (OSCC), a malignant tumor of the oral cavity. After refining the sample, the study ultimately focused on a total of 60 patients, comprising a diverse population of both males and females, aged between 35 and 60 years. The inclusion criteria for participants in this study were strictly defined. Each patient needed to have a biopsy-confirmed diagnosis of invasive OSCC. Moreover, they had to present with either adjacent or synchronous oral epithelial dysplasia (OED) or non-dysplastic leukoplakia. In addition, participants were required to demonstrate characteristics of leukoplakia that were either homogeneous or non-homogeneous, such as speckled or verrucous forms. Importantly, only patients with untreated primary lesions or those suffering from recurrent or persistent conditions were considered eligible for this study. In contrast, individuals showing conditions that could mimic leukoplakia but do not fall under the category of potentially malignant disorders like lichen planus, frictional keratosis, white sponge nevus, or oral candidiasiswere systematically excluded. Furthermore, patients with severe systemic illnesses, including uncontrolled diabetes, renal disease, or immune disorders, as well as those who had recently undergone chemotherapy or radiotherapy, which could potentially obscure the primary lesion, were also excluded. In addition, those with distant metastasis or a second primary malignancy outside the oral cavity were not permitted to participate in the study. All participants provided informed consent, ensuring they understood the study's purpose and procedures. The study was structured into three well-defined groups for comparative analysis. Group 1 consisted of 20 healthy individuals, whose oral health was assessed to evaluate the presence of Candida species. Group 2 comprised 20 patients who had received a clinical diagnosis of leukoplakia, while Group 3 featured 20 patients diagnosed with oral squamous cell carcinoma. The central aim of the research was to systematically identify and characterize the presence of Candida species across all three groups, considering the potential implications for oral health and pathology. Sample collection was conducted using a sterile cotton swab, which was employed to rub firmly over the lesions of interest in the study participants. This method facilitated the retrieval of samples for analysis. Microbiological investigations were performed by isolating these samples and inoculating them onto Sabouraud Dextrose Agar (SDA), which was enriched with chloramphenicol to suppress bacterial growth and allow for the growth of fungi. The inoculated samples were then incubated at a controlled temperature of 37°C for a duration ranging from 24 to 48 hours, with an allowance for up to 7 days for the growth of slower-growing fungal species. Fungal load was quantified by counting the Colony Forming Units (CFUs) per milliliter (mL) in each sample to provide a measure of the prevalence of fungal organisms within the oral lesions. For the identification of specific Candida species, the study utilized CHROMagar Candida, a specialized differential medium that enables researchers to distinguish between various Candida species based on their distinct color characteristics. For example, Candida albicans appears

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green, Candida tropicalis manifests as blue, and Candida krusei presents as pink or purple on this medium. Ultimately, the objectives of this study were to elucidate both the presence and the types of Candida species in patients diagnosed with oral squamous cell carcinoma and leukoplakia, contributing valuable insights into the potential role of these fungi in oral carcinogenesis and their relationship with these conditions.

Statistical Analysis and Results

In this study, we conducted all statistical analyses using SPSS software version 29.0, which is specifically designed for statistical computing and data analysis in the social sciences. To assess the significance of our findings, we employed the chi-square test, a method well-suited for comparing proportions across different groups. This approach enabled us to carry out a thorough and rigorous examination of categorical data, ensuring that our results accurately represent the underlying trends and relationships present in the dataset.

Results

This study encompassed a total of 60 participants, comprising both male and female individuals aged between 35 and 60 years. A detailed statistical description of the participants based on age and gender is presented in Table 1, which includes demographic data indicating that out of the 60 patients, 31 were male and 29 were female. This demographic breakdown is further illustrated in Graph 1, showcasing the distribution of patients alongside additional associated details. The participants were categorized into three distinct groups for the purpose of the study. Group 1 consisted of 20 healthy individuals, whose oral health was thoroughly assessed to evaluate the presence of Candida species in their oral cavities. Group 2 included 20 patients who had received a clinical diagnosis of leukoplakia, a condition characterized by white patches on the mucous membranes of the oral cavity. Meanwhile, Group 3 comprised 20 patients diagnosed with oral squamous cell carcinoma, a type of cancer affecting the tissues of the mouth. Table 2 provides a comprehensive analysis of Group 1 (n=20), focusing on both the quantitative and qualitative differences in Candida colonization among these healthy individuals. To determine the significance of the findings, the Pearson Chi-Square test was utilized. Notably, the results indicated that Candida species were absent in 16 of the 20 healthy participants, highlighting a significant absence of fungal colonization in this group. Table 3 focuses on Group 2 (n=20), which includes patients with a confirmed diagnosis of leukoplakia. Similar analyses were conducted to explore both the quantitative and qualitative aspects of Candida colonization. The findings again employed the Pearson Chi-Square test, revealing that only 1 out of the 20 patients diagnosed with leukoplakia exhibited an absence of Candida species, indicating a higher prevalence in this patient group compared to the healthy cohort. In Table 4, the focus shifts to Group 3 (n=20), consisting of patients diagnosed with oral squamous cell carcinoma. This group underwent an analysis of Candida colonization that sought to evaluate both quantitative and qualitative differences. The Pearson Chi-Square test results showed that none of the patients in this group were free of Candida species, indicating a complete presence of fungal colonization among individuals with this serious condition. Table 5 presents the results of assessing Candida species growth using Sabouraud dextrose agar media across the different study groups, with findings indicating that Candida growth was observed in 19 out of the total participants. This suggests a considerable presence of these organisms across the studied cohorts. Table 6 details the findings from the use of chrome agar media for assessing Candida growth among the various groups, where growth was recorded in 18 individuals. This method demonstrated a slightly lower prevalence when compared to the Sabouraud dextrose agar results. Finally, Table 7 offers an estimation of data across all studied groups utilizing one-way ANOVA, allowing for statistical comparisons and insights into the differences in Candida colonization among the groups. This comprehensive approach aims to elucidate the relationship between oral health status and the presence of Candida species in a clinical context.

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Table 1: Age & gender based statistical description of contributing patients

Age Group (Yrs)	Male	Female	Total	P value
35-40	6	5	11	0.01
41-45	7	5	12	0.20
46-50	8	6	14	0.06
51-55	6	8	14	0.50
56-60	6	5	11	0.40
Total	31	29	60	*Significant
*p<0.05 significant				

Graph 1: Patients demographic distribution and associated details

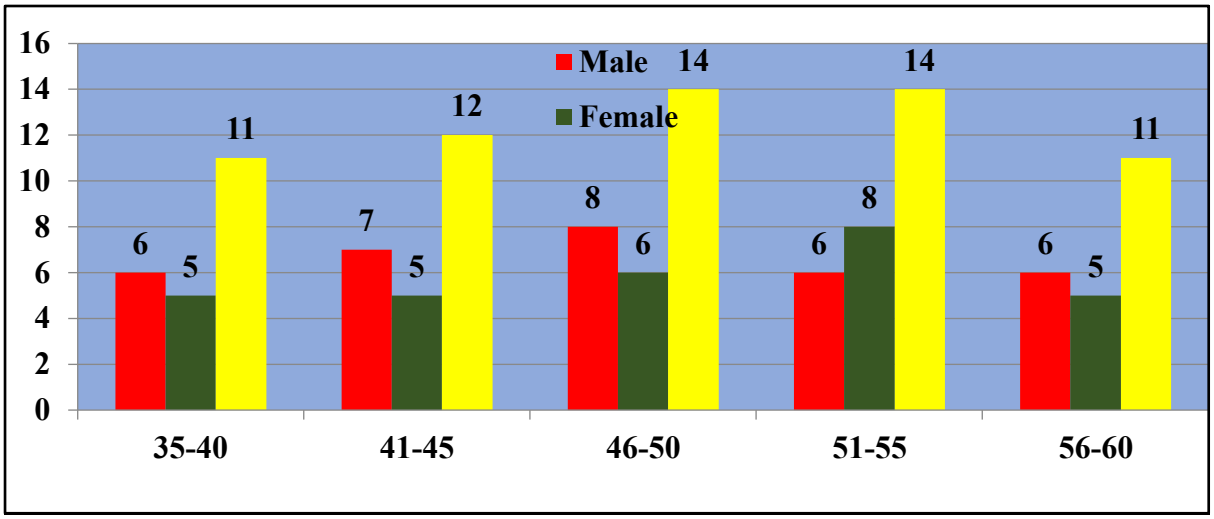

Table 2: Group 1 (n=20) healthy patients, analysing both the quantitative and qualitative differences in Candida colonisation among individuals, and to assess the significance of the findings, the Pearson Chi-Square test was employed

Evaluation	N	Mean	Std. Dev.	Std. Error	95% CI	Pearson Chi-Square Value	df	p value
Prevalence of Candida species	2	1.11	1.08	1.01	1.03	1.06	1.11	0.01*
Non-albicans candida (NAC)	0	-	-	-	-	-	-	-
Fungal load (CFU)	1	1.04	1.02	1.04	1.02	1.03	1.06	0.06
Invasion	1	1.04	1.02	1.04	1.02	1.03	1.06	0.06
Absence of Candida	16	4.12	3.19	3.04	3.02	2.96	2.11	0.70

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species								
*p<0.05 significant								

Table 3: Group 2 (n=20) Confirmed diagnosed leucoplakia patients were analysed for both quantitative and qualitative differences in Candida colonisation among individuals, and to assess the significance of the findings, the Pearson Chi-Square test was employed

Evaluation	N	Mean	Std. Dev.	Std. Error	95% CI	Pearson Chi-Square Value	df	p value
Prevalence of Candida species	4	1.17	1.08	1.02	1.45	2.12	2.02	0.2*
Non-albicans candida (NAC)	5	1.19	1.09	1.12	1.47	2.22	2.03	0.07
Fungal load (CFU)	5	1.19	1.09	1.12	1.47	2.22	2.03	0.07
Invasion	5	1.19	1.09	1.12	1.47	2.22	2.03	0.07
Absence of Candida species	1	1.04	1.02	1.04	1.02	1.03	1.06	0.06
*p<0.05 significant								

Table 4: Group 3 (n=20) Confirmed diagnosed oral squamous cell carcinoma patients were analysed for both quantitative and qualitative differences in Candida colonisation among individuals, and to assess the significance of the findings, the Pearson Chi-Square test was employed

Evaluation	N	Mean	Std. Dev.	Std. Error	95% CI	Pearson Chi-Square Value	df	p value
Prevalence of Candida species	5	1.19	1.09	1.12	1.47	2.22	2.03	0.07
Non-albicans candida (NAC)	6	1.20	1.11	1.17	1.50	2.24	2.05	0.08
Fungal load (CFU)	5	1.19	1.09	1.12	1.47	2.22	2.03	0.07
Invasion	4	1.17	1.08	1.02	1.45	2.12	2.02	0.2*
Absence of Candida species	0	-	-	-	-	-	-	-
*p<0.05 significant								

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Table 5: An assessment of the growth of Candida species using Sabouraud dextrose agar media across different study groups

Case Groups	Present	Absent
Healthy individuals	7(25)	13(75)
Leucoplakia	12(51)	8(49)
Oral squamous cell carcinoma	19(80)	1(20)

Table 6: An assessment of the growth of Candida species using chrome agar media across different study groups

Case Groups	Present	Absent
Healthy individuals	4(19)	16(81)
Leucoplakia	13(41)	7(59)
Oral squamous cell carcinoma	18(70)	2(30)

Table 7: Estimation amongst all studied groups using one-way ANOVA

Variables	Degree of Freedom	Sum of Squares ∑	Mean Sum of Squares m∑	F	Level of Sig. (p)
Between Groups	7	1.253	2.452	1.2	0.01*
Within Groups	20	2.281	1.241	_	
Cumulative	125.10	16.271	*p<0.05 significant		

Discussion

Evans M et al reviewed in their study that oral cavity squamous cell carcinoma (OCSCC) represents a formidable global health challenge, marked by alarmingly high mortality rates and a tendency for frequent recurrences, alongside the potential for metastasis to distant sites. Despite noteworthy advancements in treatment protocols, the overall survival rate for patients diagnosed with locally advanced OCSCC lingers precariously around 50%. This statistic underscores the pressing need for innovative therapeutic strategies and enhanced early detection methods that could significantly improve patient outcomes.[14,15]Jagadeesan D et al showed in their study that effectively managing OCSCC necessitates a comprehensive, multidisciplinary approach, often involving a combination of surgical interventions such as tumour excision and neck dissection procedures that require meticulous precision to achieve optimal results. In conjunction with surgical options, pharmacological therapies, including chemotherapy and radiotherapy, play a crucial role in the treatment regimen.[16] Zhou J et al included in their study that the design of these treatment strategies is highly individualised, tailored to consider critical factors such as the specific stage and location of the tumour, as well as the patient's overall health status and personal treatment preferences. This holistic approach aims not only to eradicate cancer but also to preserve the quality of life for those affected. Histologically, OCSCC presents a diverse array of variants recognised by the World Health Organisation,

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with six primary subtypes identified.[17] Oral cavity squamous cell carcinoma (OCSCC) presents in various forms, each with distinct characteristics and implications for prognosis. One type is verrucous carcinoma, often linked to tobacco chewing, characterized by wart-like lesions and generally regarded as less aggressive. Singh S et al reviwed in their study that in contrast, squamous basaloid cell carcinoma is a more aggressive variant frequently found on the tongue, known for early lymphatic spread and a poor prognosis. Spindle cell carcinoma is noted for its rapid growth and invasive nature, complicating treatment options. Adeno-squamous carcinoma combines features of both adenocarcinomas and squamous cells, making diagnosis more challenging. Lastly, adenoid squamous carcinoma typically appears in sun-exposed areas and the oral cavity, exhibiting unique histopathological traits. Additionally, several lifestyle factors contribute significantly to the development of OCSCC, highlighting the importance of awareness and preventive measures.[18,19] Marinho MFP et al showed in their study that tobacco use introduces carcinogens that damage oral tissues, while alcohol consumption, especially in conjunction with tobacco, increases cancer risk. Additionally, betel quid chewing is linked to oral submucosal fibrosis, further elevating risk. Viral infections, notably human papillomavirus (HPV), are also increasingly recognized as crucial in OCSCC development. Oral leukoplakia, often seen in patients at risk for OCSCC, manifests as white patches in the mouth and may feel rough or sensitive.[20,21] Qie Y, et al included in their study that the primary treatment aim is to eliminate harmful habits like tobacco and alcohol use and surgically remove lesions when necessary, particularly if dysplastic changes are detected. Given the high recurrence rates associated with OCSCC, long-term follow-up is essential, and some studies suggest antioxidant therapy may aid in enhancing remission and reducing recurrence. Diagnosing superimposed infections, such as candidiasis in patients with oral cavity squamous cell carcinoma (OCSCC) and leucoplakia, requires a comprehensive approach that employs a variety of methods. Microbiological cultures are commonly utilized to grow and identify yeast and fungal pathogens, establishing a definitive diagnosis. Advanced species identification techniques, such as CHROMagar, offer a visual differentiation of Candida species based on their unique colony characteristics, allowing for more targeted treatment strategies.[22,23] Lodi G et al reviewed in their study that furthermore, molecular methods like polymerase chain reaction (PCR) provide highly sensitive and specific detection of Candida DNA, enabling precise identification of the pathogen present, even in low quantities. This is particularly valuable in complex cases where traditional cultures may fail to yield results.[24] Roy SK et al showed in their study that histopathological techniques also play a critical role in diagnosing candidiasis. For instance, Periodic Acid-Schiff (PAS) staining is employed to enhance the visibility of fungal elements in tissue samples, allowing pathologists to confirm the presence of Candida species and assess their impact on the surrounding tissue architecture. This detailed multi-faceted diagnostic approach ensures accurate identification and effective management of superimposed infections in these vulnerable patient populations.[25]

Conclusion

Within the limitations of the study, the authors meticulously investigated the prevalence of different Candida species among patients diagnosed with oral squamous cell carcinoma (OSCC) and those with leucoplakia. The findings revealed a significant increase in Candida carriage among individuals with OSCC compared to those with leucoplakia, indicating that patients with OSCC are particularly susceptible to higher fungal loads. Additionally, the study noted that the incidence of Candida was greater in leucoplakia patients than in healthy individuals, thereby highlighting the potential risk associated with these conditions. Among the various species identified, a noteworthy prevalence of non-albicans Candida (NAC) was observed, specifically strains such as C. tropicalis, C. krusei, and C. glabrata. These particular species are increasingly recognised for their role in oral pathologies. The analysis also demonstrated that higher concentrations of colony-forming units per millilitre (CFU/mL) were found in OSCC patients, indicating heightened virulence and increased potential for tissue invasion compared to those with leucoplakia. A comprehensive understanding of the different Candida subtypes, along with the associated risk factors and diagnostic methods linked to OSCC, is essential for improving patient outcomes. This knowledge is pivotal

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in the development of more effective management and treatment strategies tailored for those affected by these oral conditions.

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