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THE CONSORTIUM OF CANDIDA SPECIES IN THE SUBGINGIVAL MICROBIOTA OF TYPE 2 DIABETICS WITH CHRONIC PERIODONTITIS - A CASE CONTROL STUDY

Periodontology

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ABSTRACT

Background: Type 2 Diabetics have an increased susceptibility to opportunistic infections, especially Candidiasis. *Candida* species have been found in periodontal pockets expressing virulence factors that may contribute for the pathogenesis of Chronic periodontitis (CP). We aimed to investigate the association if any of *C.albicans (CA)* and other *Non-Candida albicans Candida (NCAC)* species in CP with and without DM.

Material and Methods: Clinical parameters were recorded and subgingival plaque samples obtained from 45 subjects evenly distributed into 3 groups; Group 1 (Healthy), Group 2 (CP with Type 2 DM) and Group 3 (CP without DM) were cultured on Sabouraud Dextrose Agar, followed by Gram's staining and Germ tube test.

Results: Microbiological analyses revealed the presence of *Candida* colonies in the subgingival biofilm of Groups 2 and 3 and were positive for *NCAC* species only.

Conclusion: Candida co-infection is a plausible etiological factor for CP in Type 2 diabetics and non-diabetics.

KEYWORDS

Chronic Periodontitis, Diabetes Mellitus, *Candida albicans, Non Candida albicans Candida.*

INTRODUCTION:

Periodontal disease is an ecogenetic multifactorial disease, characterized by interaction between periodontopathogens and the host defense mechanism which eventually leads to tissue destruction.[1,2] Although bacteria have a major role to play in the pathogenesis of periodontal disease, the yeast *C. albicans (CA)* has also been isolated from periodontal pockets, with a prevalence ranging from 14 to 19%.[3,4] The prevalence of yeasts was 19.7% in individuals with periodontal pockets > 7 mm and 15.6% in individuals with pockets ≤ 7 mm.[5-7] These microorganisms can co-aggregate with bacteria in the subgingival biofilm and adhere to epithelial cells.[8-10] It has been suggested that *CA* invades the periodontal tissues, causing substantial damage by the production of metabolites.

Although *CA* is the species most commonly associated with oral lesions, other *Candida* species, including *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, and *C. dubliniensis* have also been isolated.[9, 11] The proposed virulence factors of *Candida* species include, adhesion, phenotypic diversity, hyphal formation, production of phospholipases, proteinases, or other metabolites, synergistic co-aggregation or competition with bacteria and mechanisms for adaptation in the host environment which might influence the inflammatory process and cause tissue destruction.[9,12]

Diabetes Mellitus (DM), a highly prevalent metabolic disorder, marked by elevated blood glucose levels is a risk factor for periodontitis. The oral manifestations in DM, are predominantly related to the degree of glycemic control. They include periodontal disease, angular cheilitis, taste alterations, lichen planus, mucosal conditions like burning mouth syndrome, altered wound healing and susceptibility to infection.[13-15] Candida species are commensals in healthy humans. Alterations in host immunity, bacterial flora or local environmental factors, are believed to determine *the* transition of *Candida* from a commensal to an opportunistic pathogen.[16]

Diabetics have a higher prevalence of *CA* in the oral cavity, and specifically the subgingival biofilm of periodontal pockets. This could indicate their co-participation in the progression of periodontal disease in these subjects.[3] One biological mechanism proposed to explain the increased incidence and severity of periodontal disease in individuals with Diabetes is the finding of elevated levels of inflammatory mediators in the gingival crevicular fluid from periodontal pockets of patients with diabetes and poor glycemic control compared to those with controlled diabetes or without diabetes. Those with poor glycemic control have considerable periodontal destruction with an equivalent bacterial challenge.[13]

Our study aimed to investigate the subgingival colonization if any by *Candida* species in chronic periodontitis (CP) with and without Diabetes Mellitus and also ascertain the *Candida* species isolated from the subgingival plaque sample as either *albicans (CA)* or *Non Candida albicans Candida (NCAC)*. To the best of our knowledge, this appears to be the first ever study to explore the role if any of *CA* and other *NCAC* species in CP with or without DM.

MATERIALS AND METHODS

Source of the data

The study was conducted in the department of Periodontics, K.L.E. Society's Institute of Dental Sciences, Bengaluru, India. The research proposed was approved by the institutional review board and institute ethics committee. Subjects between the age group of 24 and 65 years of both genders were recruited for the study. 45 subjects were evenly distributed into 3 groups; Group1- Healthy controls (absence of proximal attachment loss, and pockets >3 mm at any site in the mouth, less than 15% of sites with bleeding on probing or redness), Group 2- CP with Type 2 DM, Group 3- CP without DM. Chronic periodontitis was defined as subjects having at least 20 teeth with a minimum of eight sites with pocket depths of >4 mm, and six sites with clinical attachment loss of >3 mm.[17]

A written informed consent was obtained from all the patients prior to their enrollment for the study. The glycosylated hemoglobin (HbA1c) levels were estimated for all subjects in Group 2. Chronic smokers, alcoholics, smokeless tobacco users, subjects with aggressive periodontitis, pregnant women, lactating mothers, denture wearers, subjects with any medical condition that could affect the periodontal tissues and presence of yeasts, such as HIV, chronic pulmonary disease treated by inhaled corticoids, non-steroidal anti-inflammatory drugs or antibiotic therapy and subjects having received periodontal therapy 6 months prior to the commencement of study were excluded from the study.

Clinical parameters including Plaque index (PI)-Silness and Loe (1964)[18], Gingival index (GI)-Loe and Silness (1963)[18], Probing pocket

depth (PPD) and Clinical attachment level (CAL)[19] were recorded for all the subjects recruited for the study.

SAMPLE COLLECTION FOR MICROBIOLOGY:

After careful removal of supragingival plaque and saliva with a sterile gauze and relative isolation with cotton rolls, the teeth were dried and subgingival biofilm/plaque samples were obtained using size 40 sterile paper-points. From each subject, samples were collected from three deepest or most diseased sites after leaving the sterile paper points for 20 seconds in each pocket.[20]

YEAST CULTURE AND QUALITATIVE ANALYSES:

To determine the number of yeast positive patients, the subgingival plaque samples were cultured immediately after sample collection. Each paper-point was immediately rolled on sterile plates containing Sabouraud Dextrose agar and streaked for isolation. The plates were incubated at 37°C, for 2–4 days and checked daily for growth. Once the growth was found, the yeast colonies were recovered and Gram's staining and germ tube test was performed to differentiate *CA* from *NCAC* species. [20](Figure 1)

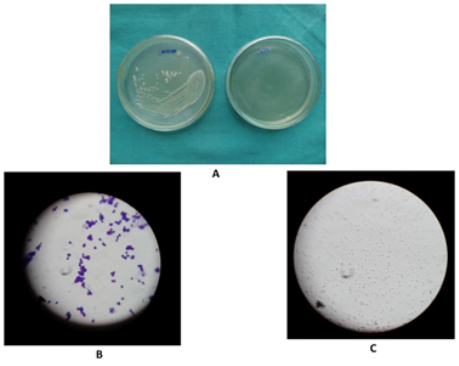

Figure 1: Macroscopic and Microscopic morphology of *Candida* isolates.

A. Sabouraud Dextrose Agar showing *Candida* colonies.
B. Gram positive oval budding yeast cells (GPOBYC) (100X magnification)
C. Germ tube test (40X magnification)

RESULTS:

The results are presented in mean and standard deviation for continuous data. The normality assumption was tested using Shapiro-Wilks test. Comparison of mean values between the groups were analysed using Student 't' test and One way ANOVA. p value less than 0.05 was considered statistically significant.

In our study DM was found to be more prevalent in age group of 40 to 49 years with 46.7% positive cases. Males (n=12; 80%) had higher predilection for DM than females (n=3; 20%) in Group 2.Mean age of diabetic was 46.2± 8.678 (35-62) and in non-diabetic was 48.3± 11.57 (25-64)

Microbiological analyses revealed that 6 subjects of 16 subjects (40%) in Group 2 presented *Candida* colonies in the subgingival biofilm while only 4 of 15 subjects (25%) in Group 3 were positive for these microorganisms. Patients who were positive for *Candida* in both the groups presented only *NCAC* (Figure 2).

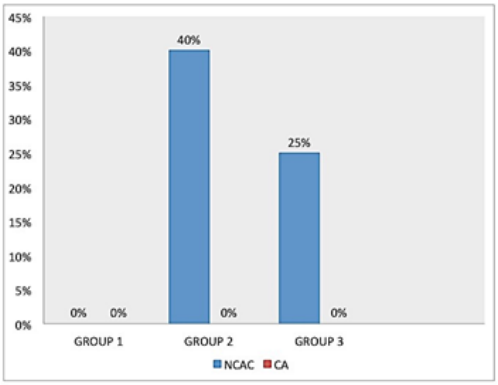

Figure 2: Percentage of samples with *Candida* species in the three study groups

This is the first ever study to report the role of *NCAC* in patients with Chronic periodontitis with or without Diabetes Mellitus. Qualitative assessment of the *NCAC* species was not performed in the study.

Clinical data analyses presented no statistically significant differences (p >0.05) for any of the clinical parameters (PI, GI, PPD, CAL) compared between CP patients who were *Candida*-positive with DM and *Candida*-positive without DM. (Table 1).

Table 1: Comparison of Clinical parameters between *Candida*-positive and negative subjects in CP with and without Diabetes [Group 2 and group 3]:

Group			N	Mean	SD	'p' value
CP With Diabetes	Hba1c	Pos.	6	8.417	1.3963	0.116
		Neg.	9	7.422	.9038	
	Plaque Index	Pos.	6	1.5817	.22999	0.920
		Neg.	9	1.6089	.61746	
	Gingival Index	Pos.	6	1.897	.4607	0.381
		Neg.	9	1.696	.3936	
	Probing Pocket Depth	Pos.	6	4.09	.495	0.212
		Neg.	9	3.81	.342	
	Clinical Attachment Level	Pos.	6	4.715	.4842	0.641
		Neg.	9	4.602	.4237	
CP Without Diabetes	Plaque Index	Pos.	4	1.6550	.35828	0.304
		Neg.	12	1.9417	.49077	
	Gingival Index	Pos.	4	2.038	.2887	0.640
		Neg.	12	1.932	.4056	
	Probing Pocket Depth	Pos.	4	4.29	.282	0.362
		Neg.	12	4.06	.445	
	Clinical Attachment Level	Pos.	4	4.928	.4779	0.819

Among the *Candida*-positive group, the mean values of clinical parameters (PI, GI and PPD) did not show any statistically significant difference (p >0.05) between groups 2 and 3. However, the mean clinical parameters of the *Candida*-negative samples of groups 2 and 3 showed a statistical significance (p< 0.05). Mean CAL values were statistically not significant for both the *Candida*-positive and *Candida*-negative subjects of group 2 and 3 (p >0.05) (Table 2).

Table 2: Comparison of Clinical parameters between the groups in Candida positive and Candida negative subjects:

Candida			N	Mean	SD	'p' value
Pos.	Plaque Index	CP With Diabetes	6	1.5817	.22999	0.701
		CP Without Diabetes	4	1.6550	.35828	
	Gingival Index	CP With Diabetes	6	1.897	.4607	0.605
		CP Without Diabetes	4	2.038	.2887	
	Probing Pocket Depth	CP With Diabetes	6	4.09	.495	0.495
		CP Without Diabetes	4	4.29	.282	
	Clinical Attachment Level	CP With Diabetes	6	4.715	.4842	0.514
		CP Without Diabetes	4	4.928	.4779	
Neg.	Plaque Index	Healthy	15	.6553	.28053	<0.001
		CP With Diabetes	9	1.6089	.61746	
		CP Without Diabetes	12	1.9417	.49077	
	Gingival Index	Healthy	15	.795	.3202	<0.001
		CP With Diabetes	9	1.696	.3936	
		CP Without Diabetes	12	1.932	.4056	
	Probing Pocket Depth	Healthy	15	1.40	.654	<0.001
		CP With Diabetes	9	3.81	.342	
		CP Without Diabetes	12	4.06	.445	
	Clinical Attachment Level	CP With Diabetes	9	4.602	.4237	0.251
		CP Without Diabetes	12	5.062	1.1002	

The mean HbA1c levels (7.8%) of group 2 subjects were well within the excellent range of 7-8%. The correlation of Glycosylated haemoglobin (HbA1c) with the clinical parameters (PI, GI, PPD) in Group 2 showed a positive correlation (r = 0.311, 0.188 and 0.457 respectively) with no statistical significance (p >0.05). A negative correlation was seen between Clinical attachment level (CAL) and the mean HbA1c levels in Group 2 (r = -0.004) with no statistical

significance (p >0.05). The duration of DM was not considered during the analysis of results. Paradoxically, no statistical significance association could be established between the severity of CP and HbA1c levels in the present study (p >0.05).

DISCUSSION:

Despite the strong association displayed by the "Red Complex" periodontopathogens with severe periodontitis, periodontal pockets can harbor a great variety of microorganisms, including yeasts. [16,20-22] Although the role of yeasts in chronic periodontitis is largely unclear it has been reported that the proportion of yeasts in periodontal pockets is similar to that of some bacterial periodontopathogens, which indicate a plausible role for *Candida* species in the pathogenesis of Periodontitis. Studies have suggested that changes in cellular and humoral immune responses may allow *Candida* species to colonize the subgingival environment.[23,24] The results of subgingival *Candida* species in the previous studies range from 17% to 69.2% of isolates.[25,26] Some authors also indicate the transient presence of yeasts in the subgingival area.[27]

It has also been found that diabetics show higher percentage of colony forming unit (CFU) scores as compared to non-diabetics and thus are more susceptible to oral candidiasis than non-diabetics.[28] Strain diversity testing demonstrated increased colonization of *Candida albicans* being the most prevalent species and non-albicans species in diabetics. Also, a decreased anti-fungal susceptibility was found in non-albicans isolates obtained from diabetic subjects.[29] Among immunosuppressed patients, non-albicans candidaemia occurred more frequently in those with exposure to broad spectrum antibiotics, exposure to fluconazole or DM.[30]

Periodontal disease and DM are closely associated and are highly prevalent chronic diseases with many similarities in pathobiology. A latest review suggested that the patients with uncontrolled diabetes are at an increasing risk of developing periodontal disease and show a tendency towards higher gingivitis scores. A careful evaluation of glycemic control, including the patient's diet, HbA1c and postprandial glucose levels, is critical in determining the risk assessment for progression to the oral complications, especially periodontitis.[31] The frequent occurrence of Candidal infections in patients with DM has been recognized for many years and oral Candidiasis in particular is thought to be more prevalent among these individuals. Chronic hyperglycemia contributes to the risk of oral Candidal infection by increasing salivary glucose levels, which promotes overgrowth of *Candida*.[32] When changes in the host environment cause an imbalance in the flora or a decrease in resistance, *Candida* becomes an opportunistic pathogen. A study on subgingival Candidal carriage assessment revealed statistically significant differences (p <0.05) between the Diabetic and control groups and a significant correlation between the subgingival Candidal carriage rate and severity of Periodontitis.[13]

Our study found statistically significant presence of *Candida* species in 40% cases in Group 2 and 25% cases in Group 3 (p <0.05). Studies with similar results suggested that the presence of severe CP increased the odds of having subgingival yeasts.[20, 26] However inter group comparison between groups 2 and 3 did not show any significant association for Candidal isolates similar to reports by a previous study.[33] This could be attributed to the fact that only qualitative and not quantitative microbiological analysis was attempted in our study.

The Candidal isolates in both Groups 2 and 3 showed the presence of *NCAC* and absence of *CA.* Few studies suggested the presence of *Candida* species other than *Candida albicans* in increasing numbers in the subgingival environment [34-36] whereas some contrasted by suggesting a common finding of *C.albicans* in the subgingival pocket.[37] In addition, a great variety of yeast species, such as *NCAC* have been identified in the subgingival sites of patients with yeast-positive severe CP, suggesting that the advanced form of CP was associated with a more complex yeast community residing in deeper pockets.[20] A clinician should suspect *NCAC* if treatment with commonly used antifungals fail, DM being a risk factor for this.[35,38] The pathogenesis of *NCAC* species is still incompletely understood. New insights into the morphogenesis of *NCAC* species are needed.

Age and gender are globally identified risk factors for DM. We found a higher prevalence of DM in the age group of 40-49 years. Age and gender differences could be attributed to genetic, environmental and other confounding factors.

Studies reveal that level of glycemic control as assessed by HbA1c, appears to be an important determinant in the relationship of Diabetes mellitus and Periodontitis.[39-43] The mean HbA1c level of 7.8 for Group 2 was distributed well within the excellent glycemic control range. In our study there was no linear correlation between HbA1c and the severity of periodontitis as assessed by the various clinical parameters. This is in contrary to other studies that showed a positive correlation[44-47] and negative correlation.[48] This could be attributed to the smaller sample size and also the duration of diabetes mellitus not being considered.

Although intragroup observations of clinical parameters showed higher values of PPD and CAL in subjects of groups 2 and 3, there was no significant difference (p >0.05) in PD and CAL between these groups which was concurrent by the findings of past studies.[5,26,49-50] Although these results were comparable to previous studies[51], the study design, severity of disease, number of sites assessed may contribute to the differences in results obtained by clinical examination.

CONCLUSION:

Yeasts are frequent isolates of the subgingival consortium of microorganisms in CP with or without DM and may have a role to play along with key pathogens in the etiopathogenesis of CP. The isolation of NCAC in all the yeast positive samples suggests that these species are more likely to be present in the subgingival samples compared to *Candida albicans* and could contribute to the pathogenesis of Chronic Periodontitis. Future research should be directed towards exploring the unearthed facts about NCAC species, their virulence factors and the mechanisms of virulence, drug resistance in subgingival biofilm and eventually the development of a specific treatment for periodontal disease caused by coinfection by these species. Although the specific role of yeasts as co-infecting agents remains to be established, the premise that they are innocent bystanders of the complex subgingival consortium of microbiota cannot be accepted either.

CONFLICT OF INTEREST: Nil

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