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MICROBIOLOGICAL PROFILE OF ORAL INFECTIONS IN DIABETIC PATIENTS AND NON-DIABETIC CONTROLS IN SOUTHWEST, CAMEROON

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ABSTRACT

Background: Oral microbial flora is increasingly being incriminated in oral infections. There is paucity of information on the importance of aerobic oral flora in diabetes. The purpose of this study was to compare aerobic oral microbial flora in diabetics and non-diabetics and to relate these microbes with oral infections.

Materials and Methods: This study involved 154 diabetics and 111 non-diabetics aged 18 years and above. Oral washes were inoculated unto blood agar, chocolate agar, Mac Conkey agar and Sabouraud's agar and isolates were identified by standard biochemical tests. Oral exam was conducted by a Dentist to assess oral infections and oral health status of participants.

Results: Thirteen different genera of aerobic microbes were identified. The most prevalent microbes were *Streptococcus* sp (99.6 %), *Candida albicans* (17.0 %), *Serratia* Spp (7.2 %), other *Candida* Spp (6.8 %), Coagulase negative Staphylococci (CNS) (6.4 %) and *Klebsiella* Spp (5.7 %). *Candida* sp was more prevalent in diabetic patients than non-diabetics. Gram negative aerobic bacteria were significantly isolated from cases of dental caries.

Conclusion: The oral microbiological profile of diabetic patients was different from those of non-diabetics and aerobic Gram negative bacteria may play an important role in dental diseases in diabetic patients.

Keywords: Oral microbiological profile; oral infections; diabetes; Cameroon

PROFIL MICROBIOLOGIQUE DES INFECTIONS BUCCALES CHEZ LES PATIENTS DIABETIQUES ET TEMOINS NON DIABETIQUES DU SUD-OUEST, CAMEROUN

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RÉSUMÉ

Contexte: La flore microbienne orale est de plus en plus incriminée dans les infections buccales. Il existe peu d'informations sur l'importance de la flore buccale aérobie chez les diabétiques. Le but de cette étude était de comparer la flore microbienne aérobie orale chez les diabétiques et les non diabétiques et de déterminer le lien que ces germes ont avec les infections buccales.

Matériels et méthodes: Cette étude a porté sur 154 diabétiques et 111 non-diabétiques âgés de 18 ans et plus. Le liquide de lavage buccal a été inoculé sur des géloses au sang, au chocolat, de Mac Conkey et de Sabouraud respectivement, et les souches bactériennes ont été identifiées par des tests biochimiques standards. L'examen oral a été mené par un dentiste afin d'évaluer les infections buccales et l'état de santé bucco-dentaire des participants.

Résultats: Treize genre différents de microorganismes aérobies ont été identifiés. Les microbes les plus répandus étaient *Streptococcus sp* (99,6%), *Candida albicans* (17,0%), *Serratia spp* (7,2%), les autres espèces de *Candida* (6,8%), les staphylocoques à coagulase négative (SCN) (6,4%) et *Klebsiella spp* (5,7%). *Candida* spp était plus fréquent chez les patients diabétiques que chez les non-diabétiques. Les bactéries Gram négatives aérobies ont été considérablement isolées des cas de caries dentaires.

Conclusion: Le profil microbiologique oral des patients diabétiques était différent de ceux des non- diabétiques. Les bactéries Gram négatif aérobies peuvent jouer un rôle important dans les maladies dentaires chez les patients diabétiques.

Mots-clés: Profil microbiologique orale; infections buccales; diabète; Cameroun

INTRODUCTION

The microbial flora of the human oral cavity is highly diverse, consisting of mainly bacteria and fungi (1). The normal flora of the human mouth is mainly made up of streptococci and anaerobic Gram negative bacteria (1, 2). These microorganisms play an important role in preventing colonisation by pathogenic microbes; thereby maintaining the health of the oral cavity. It is known that disruption of the normal flora can trigger or influence the course of oral diseases (2). Apart from influencing the onset or course of oral infections, oral microbial flora is also associated with some systemic diseases (3, 4). Staphylococci and aerobic Gram negative bacteria are not endogenous flora but are considered transient colonisers of the oral cavity; from which they cause infections like pneumonia (5). Systemic changes such as disease, pregnancy and puberty are known to alter the microenvironment of the oral cavity and consequently influence the proportion and type of oral flora (2, 6). Diabetes has been associated with oral infections such as periodontitis (7, 8), dental caries (9), gingivitis (10) and candidiasis (11). The high prevalence of oral infections in diabetes patients has also been liked to poor oral hygiene (12). However, whether oral hygiene influences oral microbial colonisation is not fully elucidated. The southwest region of Cameroon is a rainforest zone and majority of the population depends on agriculture and pastoral activities for their livelihoods. Alcohol consumption is a normal practice of most inhabitants. In Cameroon like in most African countries, oral health is seen as a very low priority where the limited resources available to the health sector are directed towards life-threatening conditions such as HIV/AIDS, tuberculosis, and malaria. The few dental clinics in the Region are located in the urban towns leaving the rural areas with little or no dental services. Data from unpublished sources revealed that the level of dental education in the population is low and self-medication for oral health problems is a common practice.

Several studies have documented the oral microbiota in diabetic patients as well as other patient populations (5, 13, 14). Sharma and co-workers (2010) reported that both Gram positive and Gram negative bacteria are fairly involved in dental diseases and that the prevalence of bacteria increases with severity of disease. In another related study, it was reported that periodontal pathogens were different in diabetic patients and non-diabetic controls as well as in aggressive and chronic periodontitis (15). Khovidhunkit and colleagues (2009) noted the predominant microbes isolated from saliva to be mutans streptococci, lactobacilli and *Candida* sp. Also, a high prevalence (48.0%) of Enterobacteriaceae in the oral cavities of the denture-wearing population compared with 16.4% in the normal population has been reported (16). Most of previous studies associating oral microbial flora with oral infections in diabetic patients were focused on anaerobic bacteria. The few studies on aerobic flora (17) in diabetes are focused on different anatomical sites other than the oral cavity and little is known of the role of aerobic oral flora in oral infections in diabetes patients. The aim of this study is to compare aerobic oral microbial flora in diabetic patients and non-diabetic controls and to relate these microbes with oral infections and oral hygiene.

MATERIALS AND METHODS

The study was a cross-sectional research involving 265 participants (154 diabetic patients and 111 non-diabetic controls). Participants aged 18 years and above were recruited either from diabetic clinics or from the general population. Diabetes was confirmed by fasting blood sugar levels ≥ 126mg/dl, the use of hypoglycemic drugs and a history of diabetes. (18). Written consent was obtained from all patients. The study protocol was approved by the Ethical Clearance Committee of the University of Buea, Cameroon. Participants were asked to complete questionnaires bearing information on their demographic and medical history. Three millilitres of blood and oral washes were collected from each participant. The blood was used to test for plasma blood sugar as previously reported (18). To obtain the oral washes, each subject was asked to rinse his/her mouth with about 10ml of sterile Phosphate Buffered Saline (PBS) for 1 minute. The suspension was dispensed into a sterile collection cup (Equator Medical Inc., UK), placed in a cool box and transported to the laboratory within 2 hours of collection. The concentrated oral rinse technique was employed to culture both bacteria and fungi and procedures were conducted as previously described (19). Each oral rinse was transferred under aseptic conditions into a 15ml falcon tube, centrifuged at 2500rpm for 5 minutes and pellet was re-suspended in 1ml of PBS. Ten microlitres of the suspension was used to inoculate the following culture media: Blood agar supplemented with Colimycin-Nalidixic (CNA) acid mixture, this medium is selective for Gram positive bacteria; Chocolate agar supplemented with Polyvitex and Vancomycin-Colimycin-Nystatine mixture, MacConkey agar and Sabouraud's agar (BioMerieux SA., France) for the isolation of fungi. Culture plates were incubated at 37^{o}C under aerobic conditions for 24-48 hours, after which plates were observed for microbial growth. Then plates were examined for significant bacteria growth and characteristic colonial morphology. The number of colonies on each plate was counted and the number of colony-forming units (CFU) per ml calculated to indicate microbial density. For each characteristic morphotype, 5 distinct colonies were randomly selected and sub-cultured on appropriate medium, for identification. Isolates were Gram stained as previously reported (20) and then identified by

standard biochemical tests. The Germ tube test was used to distinguish *Candida albicans* from other *Candida* species (11). To assess oral infections, oral exam was performed by a dentist based on World Health Organisation standards (21) and participants were classified as having one of the following: dental caries, gingivitis or periodontitis. Oral health status was analysed using the simplified oral hygiene index OHI-S (22) and participants were grouped into Good, Fair and Poor oral hygiene. Data from this study were expressed as frequencies and statistical significances were assessed using the Chi-square test. All data were analysed using SPSS (version 17.0) at 95 % confidence level and P-values < 0.05 were considered statistically significant.

RESULTS

A. Distribution of Oral Isolates among Study Participants

Most of the study participants were in the age group 50 years and above and were more females (150, 56.6 %) in the study than males (115, 43.4 %). Of the 265 participants, 264 (99.62%) had at least one of the isolates. **Table 1** presents the prevalence of microbes from the oral cavity of study participants. Isolates belonging to 13 genera were identified including; *Candida, Klebsiella, Serratia, Stapylococcus, Streptococcus, Escherichia, Acinetobacter, Providencia, Flavimonas, Burkholderia, Kluyvera, Citrobacter* and *Enterobacter*. Generally, the most prevalent microbes amongst study participants were viridans *Streptococcus* (163, 99.6 %), *Candida albicans* (45, 17.0 %), *Serratia* Spp (19, 7.2 %), other *Candida* Spp (18, 6.8 %), CNS (17, 6.4 %) and *Klebsiella* Spp (15, 5.7 %). Gram positive bacteria (GPC) were the most frequently isolated (281, 68.2 %) from the oral cavity; followed by Gram negative bacteria (GNR) (68, 16.5 %), then yeasts (63, 15.3 %).

TABLE 1: DISTRIBUTION OF ORAL ISOLATES IN DIABETICS AND NON-DIABETICS

Mouth Isolates	Diabetics n (%)	Non-diabetics n (%)	Total n (%)
Candida albicans	30 (19.5)	15 (13.6)	45 (17.0)
Citrobacter Spp	1 (0.6)	0 (0.0)	4 (0.4)
GBS	1 (0.6)	0 (0.0)	1 (0.4)
Viridans Streptococcus	153 (99.4)	110 (100.0)	263 (99.6)
Acinetobacter Spp	0 (0.0)	1 (0.9)	1 (0.4)
Burkholderia	2 (1.3)	0 (0.0)	2 (0.8)
Other *Candida* Spp	12 (7.8)	6 (5.5)	18 (6.8)
E. coli	1 (0.6)	0 (0.0)	1 (0.4)
Enterobacter Spp	2 (0.1.3)	0 (0.0)	2 (0.8)
Flavimonas Spp	0 (0.0)	1 (0.9)	1 (0.4)
GNR*	8 (5.2)	16 (14.5)	24 (9.1)
Klebsiella Spp	8 (5.2)	7 (6.4)	15 (5.7)
CNS	14 (9.1)	3 (2.7)	17 (6.4)
Providencia Spp	0 (0.0)	1 (0.9)	1 (0.4)
Serratia Spp	10 (6.5)	9 (8.2)	19 (7.2)
Kluyvera Spp	1 (0.6)	0 (0.0)	1 (0.4)
Total **	154 (58.3)	110 (41.7)	264 (100)

*** GNR represents gram-negative rods which were isolated but unable to type by available biochemical tests.**
****Percentage based on the number of respondents**

B. Comparison of Oral Isolates between Diabetics and Non-Diabetics

Figure 1 demonstrates the distribution of oral microbes among diabetics and non-diabetics. There was a significant difference in the rate of isolation of yeasts (*Candida* spp) from the oral cavity between diabetics and non-diabetics (**χ2-test: P < 0.001**). Yeasts were frequently isolated from the oral cavity of diabetic patients (42, 66.7 %) than non-diabetics (21, 33.3 %). Also, more Gram positive bacteria were isolated from diabetics than non-diabetics (**168, 59.8** % versus **113, 40.2** %) but this difference was not statistically significant. Similarly, the distribution of Gram negative bacteria among diabetics and non-diabetics did not show any significant difference (**χ2-test: P = 0.732**).

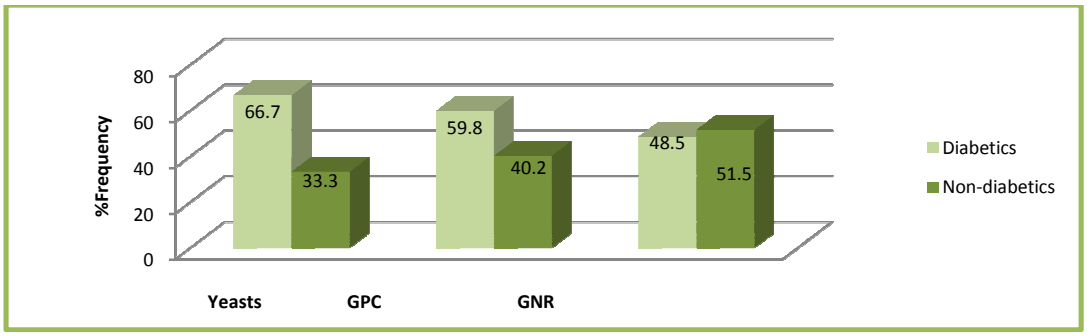

FIGURE 1: FREQUENCY OF ISOLATION OF ORAL MICROBES IN DIABETICS AND NON-DIABETICS

TABLE 2: DISTRIBUTION OF ORAL ISOLATES WITH RESPECT TO ORAL HYGIENE STATUS OF STUDY PARTICIPANTS

Oral Isolates	Oral hygiene status			Chi Square Test
	Good n (%)	Fair n (%)	Poor n (%)	
Candida spp	17 (19.3)	30 (24.6)	14 (42.4)	$\chi 2 = 6.849$; P = 0.033
Gram positive bacteria	88(100)	122 (100)	33 (100)	-
Gram negative bacteria	21 (23.9)	32 (26.2)	10 (30.3)	$\chi 2 = 0.530$; P = 0.767
Total	88	122	33	

C. Distribution of Microbes with respect to Oral Infections in Diabetes Patients

Table 3 shows the isolation of microbes with respect to oral infections in diabetics. Thirty eight diabetics were diagnosed with gingivitis, 39 with periodontitis and 45 with dental caries. Gram positive bacteria were isolated from all cases of oral infection. Gram negative bacteria were significantly isolated from cases of dental caries (**$\chi 2$ Test: P= 0.021**). Although more *Candida* sp was isolated from cases of dental caries, the distribution of *Candida* with respect to oral disease was not significant (**$\chi 2$ Test: P > 0.05**).

TABLE 3: DISTRIBUTION OF ORAL ISOLATES WITH RESPECT TO ORAL DISEASE IN DIABETES PATIENTS

Oral Isolates	Oral disease					
	Gingivitis		Periodontitis		Dental caries	
	n (%)	$\chi 2$ Test	n (%)	$\chi 2$ Test	n (%)	$\chi 2$ Test
Candida spp	11(28.9)	P=0.75	14 (35.9)	P=0.393	7 (37.8)	P=0.087
Gram positive bacteria	38 (100)	-	39 (100)	-	45(100)	-
Gram negative bacteria	12 (31.6)	P= 0.17	12 (30.8)	P = 0.61	14(31.1)	P=0.02
Total	38		39		45	

DISCUSSION

In the present study, the distribution of microorganisms in the oral cavity was analysed and the prevalence was compared between diabetics and non-diabetics in order to determine the role of diabetes on oral microbial colonization by aerobic microbes. The most commonly isolated microbes from the oral cavity of participants were *Streptococcus* sp (263, 99.6%), *Candida albicans* (45, 17.0%), *Serratia* (19, 7.2%), other *Candida* sp (18, 6.8%), CNS (17, 6.4%) and *Klebsiella* (15, 5.7%), (**Table 1**). These results are concurrent with reports from other studies which showed that streptococci, staphylococci and *Serratia* were among the

predominant oral isolates in both diabetics and non-diabetics (13). Mutans Streptococcus, Lactobacilli and *Candida* spp have been identified as the predominant microbes from saliva of diabetes patients (14). Oral Gram negative aerobic bacteria have been shown to play a role in infections in diabetic patients (23). Isolation of oral isolates was compared between diabetics and non-diabetics (**Figure 1**). It was revealed that yeasts (*Candida*) were significantly isolated from diabetics than non-diabetics (P<0.001). Yeasts were isolated from 66.7 % of diabetic patients and 33.3 % of non-diabetic controls. However, no significant difference in the isolation of aerobic bacteria was observed in the two groups. These results corroborated with that of other studies (11, 24). Abu-Elteen and co-workers (2006) reported oral Candida in 58.3% of diabetics compared with 30% in healthy controls. The high prevalence of oral candidal colonization has been attributed to the effect of hyperglycaemia (11). Results from this study revealed that diabetes mellitus might enhance oral candidal colonization and proliferation (11, 25). On the contrary, Pinducciu and colleagues (1997) did not notice any significant difference in the isolation of both anaerobic and aerobic microbial flora in 31 diabetics and 20 non-diabetics. This difference might be due to discrepancy in the number of subject as our study was based on a higher study population (154 diabetics and 111 non-diabetics).

Recently, socioeconomic status has been associated with poor oral hygiene and consequently, oral infections. In the present study, oral hygiene was significantly associated with microbial (yeast) colonization (**Table 2**). In a recent study, it was noted that diabetes was an added risk for oral disease in the low-income population of Northern Manhattan (12). The authors suggested that oral disease prevention and treatment programs may need to be part of the standards of continuing care for patients with diabetes. Apart from hyperglycemia and smoking, denture-wearing has also been reported to influence oral candidal colonization (24). The mechanism for this is not fully understood but it is probably due to the effect of denture wearing on oral hygiene. The association of microbes and oral infections is increasingly being reported (27, 28). Sharma and colleagues (2011) reported both gram positive and gram negative organisms to be fairly involved in dental diseases in diabetic patients. In another related study, streptococci and enteric bacteria were frequently isolated from dental root canal in clinically asymptomatic cases of periapical pathosis (29). In the present study, we noticed a significant association between Gram negative aerobic bacteria and dental caries (**Table 3**). Our result is in line with that of Sharma and co-workers (2011) in which both aerobic and anaerobic gram negative bacteria were associated with periodontitis, dental caries and gingivitis.

From the present study, it can be concluded that diabetics and non-diabetics may harbour different oral microorganisms which may alter their oral health and that aerobic Gram negative bacteria may play an important role in dental diseases in diabetic patients.

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Author's Contributions

MEAB – Conception, sample collection and analysis, data analysis and compilation of results

PNF – Verification of data and results, supervision and guidance

KFHL – Substantial review of the manuscript for final publication

TNA – Conception, verification of data and results, supervision and guidance

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