Candida spp. Colonizing the Curious Lesions of Patients with Dental Carries: A Case Study from Mwanza Tanzania

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Abstract

Introduction: *Candida* spp. has the ability to utilize dentinal structures like collagen for growth due to their ability to producing acidogenic and aciduric conditions. This study investigated the prevalence and factors associated with *Candida* spp. colonization of curious lesions among patients with dental caries attending Bugando Medical Centre and Sekou Toure Regional Referral Hospital, in Mwanza, Tanzania.

Methods: A cross-sectional hospital based study was conducted between March and July 2017 among patients with dental carries attending a dental clinic at Bugando Medical Centre and Sekou Toure Regional Referral Hospital. Sample was taken from the curious lesions using a sterile wooden toothpick and processed to isolate *Candida* spp. Data were analyzed using STATA version 13.0 software following the objectives of the study. p value of less than 0.05 at 95% confidence interval was considered statistically significant.

Results: A total of 259 patients with a median age of 25 years were enrolled. The majority were from rural areas 61.4% (159/259) and 91.1% (236/259) reported cleaning their teeth at least once a day. *Candida* spp. were detected in 49 (18.9%) patients with the majority of them being *Candida albicans* 39 (79.6%). On univariate logistic regression analysis; increase in age (OR 1.02, 95% CI 1.00-1.04, p=0.018), not having the habit of cleaning teeth (OR 2.54, 95% CI 1.01-6.41, p=0.042) and having a history of being diagnosed with dental carries (OR 2.01, 95% CI 1.03-3.92, p=0.039) were found to be associated with *Candida* spp. colonization.

Conclusion: Patients suffering from dental caries with poor oral hygiene were significantly more found to be colonized by *Candida* spp. posing them at high risk of developing severe cariogenic lesions. More studies to evaluate the pathogenic potential of these *Candida albicans* are highly recommended.

Keywords: Candida albicans, Dental carries, Curious lesions

Background

Dental caries are an infectious disease which is a major concern for dentists [1]. It results from the demineralization of hard tissues (Enamel, Dentin and Cementum) leading to the destruction of organic matter of the tooth as a result of acid by oral microorganisms [2]. Streptococcus mutans were considered for a long time as the major causatives agents of dental caries [3]. Recently, other microorganisms that produce acidogenic and aciduric conditions have been considered in the onset and the development of cariogenic lesions [4]. Candida albicans is considered a commensal in the oral cavity, however, due to its ability to produce acidogenic and aciduric conditions it can potentially cause dental caries [5].

Candida spp. are an opportunist microorganism found in the oral cavities and may cause serious candidiasis and have been reported being a major cause of denture stomatitis [6]. A strong association has been found between the prevalence of Candida spp. and dental caries-particularly in children and young adults [7]. However, little has been done on the association of Candida spp. oral colonization and dental caries in adults. Due to the cariogenic potential of Candida albicans, it has been demonstrated to enhance in vitro colonization of dental biofilm by Streptococcus mutans and reported to possess a much greater ability to dissolve hydroxyapatite and even induce dental caries in rats experimentally than S. mutans [5].

Candida albicans colonization has been found to significantly be associated with dental caries due to the fact it possesses various virulence factors such as adherence, persistence, dimorphism and or germ tube formation, phenotypic switching, interference with host defense system, synergism with bacteria, and production of hydrolases or other metabolites [8]. In Africa, dental caries are one of the prevalent complaints among patients attending dental clinics. Similarly, the prevalence of oral candida colonization in adults is reported to range from 22.9%-33.9% [9,10]. However, the data on the magnitude of Candida spp. colonization in these curios lesions and factors associated are still limited. The current study was proposed to determine the prevalence and risk factors associated with Candida spp. colonizing the curios lesions of patients with dental caries at Bugando Medical Centre and Sekou Toure Regional Referral Hospital Mwanza Tanzania. The data will add potential knowledge regarding the role of Candida spp. and dental caries.

Methodology

The descriptive cross-sectional hospital based study was conducted from March 2017 to July 2017. The study was conducted at the dental clinics of the Bugando Medical Centre (BMC) and Sekou Toure Regional Referral Hospital (SRRH). The BMC is the tertiary hospital for the Lake Zone regions of Tanzania and a teaching hospital for the Catholic University of Health and Allied Sciences. The dental clinic of BMC serves an average of 5-10 patients per day. The SRRH is the regional hospital for the Mwanza region with a dental clinic that serves 15 to 25 patients per day.

The study involved all patients with dental carries attending a dental clinic at BMC and SRRH. To reduce the possibility of underestimating patients with *Candida* spp. colonization on curios lesions, the study excluded all patients with a history of antifungal use in the past two weeks before enrollment. The

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minimum sample size of 246 patients with dental caries was obtained using the Kish Leslie formula [11]. Patients were serially recruited until the sample size was reached. The sample was collected by the experienced dentist at the center of the lesion on the affected dentine using a sterilized wooden toothpick (Jinhuashi Yiwei Zhusuchang, China), taking care not to allow sample contamination from the adjacent and cervical enamel.

The curious lesion sample was placed in the 2 ml vacutainer with brain heart infusion broth (Oxoid, UK) and transported to microbiology laboratory where it was immediately incubated at 37°C for 24 hours before subculture. After 24 hrs of incubation, 10 µl of BHI was inoculated on Sabouraud's Dextrose Agar (SDA) supplemented with 50 µg/ml gentamicin and 50 µg/ml chloramphenicol (HiMedia-Mumbai, India) to isolate *Candida* spp. as previously described [9]. Colonial morphology, germ tube test, and reaction on the chromogenic agar (Brilliance *Candida* agar, Oxoid-UK) were used in the identification of *Candida* spp. Species confirmation was done using Matrix Assisted Desorption/Ionization Time of Flight Mass Spectrometer (MALDI TOF_MS), (Bruker Daltonics, Bremen, Germany) on extracted cells harvested from SDA as previously described [9-12].

Data were entered on an excel spreadsheet for consistent check and cleaning then transferred to STATA version 13 for analysis. Categorical data were summarized using proportions while continuous data were summarized using the median and interquartile range. Logistic regression analysis was done to determine predictors of *Candida* colonization. A p value of less than 0.05 at 95% CI was considered statistically significant.

The protocol of this study was approved by the joint CUHAS/BMC research ethics and review committee (CREC) with certificate number CREC 279/2017. Permission to conduct the study was requested from the Director General of the Bugando Medical Center and Medical Officer In-charge of Sekou Toure Regional Referral Hospital. Patients were requested to sign the written informed consent forms before they were enrolled in the study.

Result

A total of 259 patients with curious lesions were involved in the study. Their median age was 25 years [13-16]. The majority of involved patients were from rural areas 61.4% (159/259) and 91.1% (236/259) reported routinely clean their teeth at least once a day. A total of 194(74.9%) had never had a previous history of dental caries. A total of 23(8.9%) patients reported not having a habit of brushing their teeth, *(Table 1).*

A total of 49 (18.9%) curios lesion samples were culture positive for *Candida* spp. Positive culture growth of *Candida* spp. were significantly higher among patients who do not often brush (8, 34.8%) their teeth than patients who reported cleaning their teeth at least once every day (41, 17.4), p=0.021). *Candida albicans* 39 (79.6%) was the most predominant species detected. Non-*Candida albicans* species were detected in 7 (14.3%) of positive culture growth samples, *(Figure 1).* All patients with Non-*Candida albicans* species had a history of carries.

Table 1*. Characteristics of the studied participants.*

Participant Characteristics	Number/Median	Percent (%)
Age in years	25	IQR:19-32
Gender		
Female	166	64.09
Male	93	35.91
Resident		
Rural	159	61.39
Urban	100	38.61
Occupation		
Formal employment	50	19.31
Student	87	33.59
No formal employment	122	47.1
Teeth brushing		
Once a day	236	91.1
Not often	23	8.9
History of having dental caries		
No	194	74.9
Yes	65	25.1

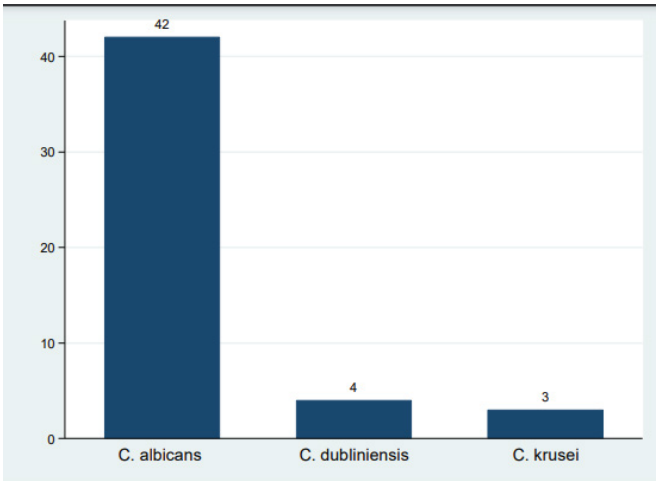

Figure 1. *Distribution of detected Candida species.*

On univariate logistic regression analysis; increase in age (OR 1.02, 95% CI 1.00-1.04, p=0.018), not having the habit of brushing teeth (OR 2.54, 95% CI 1.01-6.41, p=0.042) and having a history of being diagnosed with dental carries (OR 2.01, 95% CI 1.03-3.92, p=0.039) were found to be associated with *Candida* spp. Colonization *(Table 2).*

Discussion

Candida albicans is one of the microorganisms documented to have a high capacity of tolerating an acidic environment in the carious lesion and its ability to produce acid gives it the conducive environment to colonize and later cause dental carries [17]. The current study was conducted to determine the prevalence and distribution of *Candida* spp. in curious lesions of patients with dental caries. The prevalence of *Candida* spp. colonization in curious lesions in the current study is in agreement with the previous report of 18.3% and 19% reported in India [5,18]. The ability of the *Candida* spp. to colonize the hard surface of teeth and the production of microbial biofilm which has also been reported in previous studies could explain the findings [5,19]. It has been hypothesized that *Candida* spp. has a crucial role in the progress of curios lesions of patients

Table 2. *Factors associated with Candida colonization on curios lesion.*

Variable	Culture positive	No Fungal Growth	OR (95%CI)	p-value
Age in years*	25 (IQR:19-32)		1.023 (1.004-1.043)	0.018
Gender				
Male (93)	20 (21.5)	73 (28.5)	1	0.427
Female (166)	29 (17.5)	137 (82.5)	0.772 (0.408-1.46)	
Resident				
Urban (100)	24 (24.0)	76 (76.0)	1	0.1
Rural (159)	25 (15.7)	134 (84.3)	0.59 (0.31-1.105)	
History of carries				
No (194)	31 (15.9)	163 (84.0)	1	0.037
Yes (65)	18 (27.7)	47 (72.3)	2.01	
Teeth brushing				
Once a day (236)	41 (17.4)	195 (82.6)	1	0.042
Not often (23)	8 (34.8)	15 (65.2)	2.54 (1.01-6.37)	

with dental caries due to its virulence capability [19].

Nevertheless, the reported prevalence in this study is significantly lower than what was reported in systemic review and meta-analysis among children with carries which reported the prevalence of *Candida albicans* to range from 24% to 100% [20]. The difference in the age of the population studied can partly explain the difference observed. *Candida* spp. in the curious lesion is reported to increase with the decrease in age [21]. In the current study, the median age of the studied population was 25 years while the previous study has commonly reported curios lesion in children with a median age of 5-7 years [17,20].

Candida albicans was a predominantly detected species in this study and has also been documented as the only yeast species associated with curious lesions from different studies conducted previously [19-22]. The ability of *Candida albicans* to survive in a wide range of host environment, including acidic environment, explain the dominance of this specie in this study and other studies among patients with dental caries [13, 14].

Like other species, *Candida albicans* have been documented to have the ability to create and co-exist with other microorganisms in curios lesions due to the presence of acidic pH [20]. Furthermore, the evidence of *Candida albicans* ability to dissolve hydroxyapatite and advance caries lesion *in vivo* due to its cariogenic and acidogenic potential has been documented by Klinke, et al. [22].

Contrary to other previous studies the current study has also documented the presence of other non-*albicans Candida* spp. in curios lesions of patients with dental caries [19-22]. *Candida dubliniensis* which was detected in the current study has been documented to have the ability to hydrolyze hydroxyapatite just as *Candida albicans* and thus gives it the potential of advancing the carries lesion [15]. Furthermore, *Candida dubliniensis* and *Candida albicans* share the majority of phenotypic properties which may lead to misidentification of these species if not properly delineated [16]. Another non-*albicans Candida* spp. detected as *Candida krusei*. The detection of non-*albicans Candida* spp. in the current study is believed to have been enhanced by the use of Matrix Assisted Desorption/Ionization Time of Flight Mass Spectrometer (MALDI TOF_MS) which is a more sensitive and specific method used in *Candida* spp. identification [19-22].

In conclusion; Patients suffering from dental caries with poor oral hygiene were significantly more found to be colonized by *Candida* spp. posing them at high risk of developing cariogenic lesions. More studies to evaluate the pathogenic potential of these *C. albicans* are highly recommended.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Competing interests and Funding

None.

Authors' Contributions

MM, BO and MFM designed the work. MM and MFM recruited patients, VS, BO and MM performed laboratory investigations and results interpretations. MFM and SEM analyzed and interpreted the data. MFM wrote the first draft of the manuscript which was critically reviewed by SEM. All authors read and approved the final version of the manuscript.

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Ethics Approval and Consent to Participate

The protocol of this study was ethically approved by the joint CUHAS/BMC Research Ethics and review Committee (CREC) with certificate number CREC 279/2017. Permission to conduct the study was requested from the Director General of the Bugando Medical center and Medical Officer In-charge

of Sekou Toure Regional Referral Hospital. Patients were requested to sign the written informed consent forms before they were enrolled in the study.

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