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Predominance of non-*Candida albicans* species oral colonization among patients on anticancer therapy: A call for improved fungi diagnosis in Tanzania

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ABSTRACT

Objectives: This study aimed to determine the oral carriage prevalence of *Candida* species. and identify factors associated with the carriage of *Candida* species among cancer patients on treatment.

Design: A hospital-based cross-sectional study

Setting: The study was conducted at a tertiary-level cancer hospital Ocean Road Cancer Institute (ORCI) in Dar es Salaam, Tanzania.

Participants: We enrolled 196 participants who consented to join the study. Oral swabs were collected from all participants and inoculated onto Sabouraud Dextrose Agar and chromogenic agar for phenotypic identification of *Candida* species.

Primary outcome: The study reported the high prevalence of oral carriage of *Candida* species among cancer patients on treatment at the tertiary-level cancer hospital in Dar es Salaam, Tanzania.

Results

A total of 196 participants were enrolled in the study. The overall oral carriage of *Candida* species was 37.8% (74/196). The prevalence was higher among patients undergoing both chemotherapy and radiotherapy (44.4%) than those in monotherapy (13.3% chemotherapy, 20% radiotherapy). *Candida krusei* was the commonest isolated species, 48.6% (36/74). Head and neck (aOR, 15.09, 95%CI 3.05-74.59, p=0.00), gastrointestinal (aOR, 14.14, 95%CI 2.25-88.63, p=0.00) malignancies and diabetes (aOR=3.18, 95% CI=1.03-9.77, p=0.04) were factors independently associated with oral carriage of *Candida* species.

Conclusion

Oral carriage of *Candida* species among cancer patients receiving treatment at ORCI is high, mainly due to *C.krusei* species. This is alarming since *C.krusei* has intrinsic resistance to fluconazole, a common antifungal agent used to manage fungal infections in adults. Therefore, efforts should be put into conducting regular check-ups for such opportunistic

pathogens as they can lead to subsequent infections. Furthermore, studies conducted to determine the antifungal profile of the causative agents is warranted since, different causative agents might have different profiles.

Strengths and limitation of the study

- This is the first study that reports the isolation of non *Candida albicans* species in the oral cavity among Tanzanian cancer patients receiving therapy in a national cancer institute.
- We could not perform antifungal susceptibility testing in the present study to show the antifungal profile among different Candida species for patent managment.

Keywords

Oral candidiasis, Cancer patients, Chemotherapy and radiotherapy, Gastrointestinal malignancy, Head and neck malignancy

BACKGROUND

Oral carriage of *Candida* species is the major predisposing factor to oral candidiasis in immune-compromised patients(1). Cancer is mentioned as an immune-compromising condition that accounts for great morbidity and mortality(2). Globally it is estimated that 1 in every 3 persons suffers from cancer by 75 years(3). The use ofradiation therapy, chemotherapy, and/or a combination of both is documented to compromise the patient's

immune status further and thus predisposes these patients to opportunistic infections like oral candidiasis(4–6). In addition, cancer therapy counteracts neutrophil's function and induces neutrophil depletion, predisposing the person to fungal infections like oral candidiasis(7). It is estimated that the rate of oral candidiasis among cancer patientsranges from 7 to 52%(8). Variation in the magnitude depends on the type of malignancy,whereby head and neck cancer have a higher prevalence, followed by haematological malignancies(9–11). Historically, *C. albicans* species have been the most common cause of oral candidiasis; however, recently,non-*Candida albicans*species are increasingly implicated as causative agents of candidiasis(12). The shift of species from *C. albicans* to non-*C. albicans* species can potentially cause treatment challenges, especially inresource-limited areas where treatment is usually empirical. In addition, studies have shown that *C. albicans* and non-*C. albicans* though closely related, differ in their antifungal susceptibility profiles(7,13). Therefore identifying a specific causative agent can help inpatient management.

There is limited data on the predominant *Candida* species colonizing cancer patients undergoing cancer treatment in our geographical area. Therefore, we conducted the present study to determine the current prevalence of oral carriage of *Candida* species among cancer patients receiving cancer treatment and evaluate the association between some factors and oral carriage.

MATERIALS AND METHODS

Study design and settings

A hospital-based cross-sectional study was conducted from July to August 2019 at Ocean Road Cancer Institute (ORCI) in Dar es Salaam, Tanzania. ORCI is located along the Indian Ocean inIlala district, Dar es Salaam, Tanzania. It is a public national referral hospital for cancer treatment in Tanzania. Currently, ORCI serves more than 50,000 patients, including

about 28,000 cancer patients, 10,000 cancer screening patients, and 12,000 non-cancer patients. In addition, ORCI attends to over 15,000 clients in the outreach programs in the Tanzania mainland regions.

Study population, sample size,and sampling procedure

Adult patients aged 18 years and above on anticancer therapy present at the clinic or ward on the day of data collection were eligible for inclusion in the study. Sample size estimation was done using the Kish Leslie formula at a 95% confidence interval(14). To avoid underestimating Candida oral carriage, we excluded cancer patients who had taken antifungal agents in the past four weeks.

Data collection

A well-structured questionnaire was used to collect socio-demographic information such as age, sex, education status, employment status, and clinical information, including the type of malignancy, type of anticancer treatment, stage of malignancy, inpatient and outpatient services and diabetes status.

Sample collection and laboratory procedures

Oral swabs were collected from each participantas per standard procedures. Briefly, a sterile cotton wool swab was used to collect the sample from the mouth of the patient. Then, the samples were transported toMuhimbili University of Health and Allied Sciences (MUHAS) and processed in a Microbiology laboratory.

The oral swabs were inoculated into Sabouraud dextrose agar (SDA) media (Oxoid, Basingstoke RG24 8PW, UK) and chromogenic candida agar (CHROMagar Candida Oxoid). All media were incubated aerobically at 37^0C for 24-48 hours for phenotypic identification of *Candida* species. *Candida* species were identified based on colour and colonial morphology on CHROM agar as per the manufacturer's instructions.

Quality control

All the reagents were prepared following the manufactures instructions. In addition, we performed sterility and performance tests to check for the quality of prepared media.

Variables

The independent variables were, age, sex, education status, employment status, type of malignancy, type of anticancer treatment, stage of malignancy, treatment services (inpatient or outpatient) and diabetes status. The dependent varible was detection of candida species by phenotypic method.

Statistical analysis

We used STATA version 15.1 software for statistical analysis. Continuous variables were summarized as the median and interquartile range (IQR), while proportions were used to describe categorical variables. Group differences were determined using Fisher's exact test for categorical variables. Binary logistic regression was performed to identify factors associated withoral colonization. In addition, multivariable logistic regression was performed to examine the associations between the outcome variable and independent variables after adjustment for other variables. At a 95% confidence level, factors with a *p-value <0.05* were considered statistically significant.

Patient and public involvement

This study was designed to investigate the prevalence of oral candida carriage and the causative agents among cancer patients to better plan infection prevention and control practices, thus improving patient care. Patients were not involved in designing this research, however, the proposed study was presented to the members of the department of Microbiology and immunology of Muhimbili University of Health and Allied Sciences

before the recruitment of participants began. Patinets who were colonized with candida species were notified as soon as the sample processing was complete and the final report was communicated to the to hospital management and the infection prevention and control team of Ocean Road Cancer Institute.

RESULTS

Socio-demographic and clinical characteristics

A total of 196 cancer patients with a mean age of 54 years, a standard deviation (SD) ± 14.2, were enrolled in the study. Of the 196 participants, 69.9% were female, and nearly half (87/196, 44.4%) had acquired primary education. The majority, 143/196 (73%) of the participants, were inpatients, and about three-quarters, 151/196 (77%), received both chemotherapy and radiotherapy treatment. Head and neck cancers were the most prevalent type of malignancies 100/196 (51%), whereas only a few participants had gastrointestinal cancer 7/196 (8.7%). Many participants were either in stage 2 (78/196;39.8%) or stage 3 (73/196;37.2%). Twenty-two participants (11.2%) had diabetes (Table 1).

Table 1:Distribution of socio-demographic and clinical characteristics among cancer patients (N = 196)

Variable	Total number(N)	Percentage (%)
Age group (Mean =54; SD± 14.2)		
<54	101	51.5
>54	95	48.5
Gender		
Male	59	30.1
Female	137	69.9
Educational level		
Primary	87	44.4
Secondary and above	80	40.8
Non formal	29	14.8
Smoking		
No	165	84.2
Yes	31	15.8
Patient care		

Inpatient	143	73.0
Outpatient	53	27.0
Treatment type		
Chemotherapy	30	15.3
Radiotherapy	15	7.7
Chemotherapy and Radiotherapy	151	77.0
Type of Malignancy		
Head and neck	100	51.0
Gastrointestinal	17	8.7
Breast,cervical&prostate	26	13.3
Other	53	27.0
Cancer stage		
1	29	14.8
2	78	39.8
3	73	37.2
4	16	8.2
Diabetes status		
No	174	88.8
Yes	22	11.2

Others:leukemia,lymphoma,liver, kaposi sarcoma

Prevalence of oral colonization of *Candida* species

The overall prevalence oforalcolonization of *Candida* species was 37.8% (74/196). A higher carriage rate of 44.4% (67/151) was observed in patients treated with both chemotherapy and radiotherapy compared to each treatment separately;13.3% (4/30) and 20% (3/15) for chemotherapy and radiotherapy respectively (*p=0.02*). Patients with head and neck malignancies had a higher oral carriage, 54% (54/100) of *Candida* species, than other types of malignancies (*p<0.0001*). Although not statistically significant, detection of *Candida* species was more prevalent among diabetic patients than non-diabetic; 54.5 % (12/22) vs. 35.6 % (62/174) (*p=0.08)*. There was no difference in the carriage rate of *Candida* species in other parameters such as age, gender, smoking habits, education level, and cancer stage (Table 2).

Table 2:Prevalence of oral *Candida* carriage among cancer patients by social-demographic and clinical factors

Variable	Total number	Candida colonization	*P-value*

		n (%)	
Overall	**196**	**74 (37.8)**	
Age group			
<54	101	41 (40.6)	0.46
>54	95	33 (34.7)	
Gender			
Male	59	18 (30.5)	0.20
Female	137	56 (40.9)	
Educational level			
Primary	87	38 (43.7)	
Secondary and above	80	29 (36.3)	0.26
Non formal	29	7 (24.1)	
Smoking			
No	165	62 (35.6)	0.90
Yes	31	12 (38.7)	
Patient care			
Inpatient	143	52 (36.4)	0.51
Outpatient	53	22 (41.5)	
Treatment type			
Chemotherapy	30	4 (13.3)	
Radiotherapy	15	3 (20.0)	**0.02**
Chemotherapy and Radiotherapy	151	67 (44.4)	
Type of Malignancy			
Head and neck	100	54 (54.0)	
Gastrointestinal	17	8 (47.1)	
Breast, cervical& Prostate	26	2 (7.7)	**< 0.01**
Other	53	10 (18.9)	
Cancer stage			
1	29	10 (34.5)	
2	78	29 (37.2)	0.85
3	73	30 (41.1)	
4	16	5 (31.3)	
Diabetes status			
No	174	62 (35.6)	0.08
Yes	22	12 (54.5)	

Others:leukemia,lymphoma,liver, kaposi sarcoma; In bold p-value of less than 0.05 that indicates statistically significant association(Fisher's exact test)

Candida species isolated from cancer patients

A total of 74 patients had one type of *Candida* spp. in their oral cavity, making 74 candida isolates. Of the 74 *Candida* spp isolated, 61(82.4%) were non-C. *albicans. Candida krusei* was the dominant species accounting for 48.6% (36/74), followed by *Candida tropicalis* (33.8%, 25/74) and lastly, *C. albicans* (17.6%, 13/74) (Figure 1).

Predictors of *Candida* species oral colonization

On bivariate analysis, participants receiving both chemotherapy and radiotherapy treatment were five times more likely to have oral carriage of *Candida* than other treatment types (cOR5.18, 95%CI 1.72-15.58, *p<0.0001*). Likewise, in comparison to breast, cervical and prostate malignancies, patients with head and neck malignancies (cOR,14.09, 95%CI 3.16-62.83, p<0.0001) and those with gastrointestinal cancer (cOR, 10.67, 95%CI 1.89-60.08, *p=0.01*) had an increased probability of having oral carriage of *Candida spp* (Table 3).

After adjusting the effect of confounding factors on multivariable analysis, some types of malignancies remained associated with the oral carriage of *Candida* species among cancer patients. Participants with head and neck malignancies were 15 more likely (aOR, 15.09, 95%CI 3.05-74.59, p<0.0001) to have oral carriage of *Candida* species, while those with gastrointestinal cancer were fourteen more likely (aOR, 14.14, 95%CI 2.25-88.63, p<0.0001) to have candidiasisas compared to those with breast, cervical and prostate malignancies. In addition, the probability of being colonized by *Candida* species was three times higher among diabetic patients than non-diabetic patients (aOR=3.18, 95% CI=1.03-9.77, p=0.04) (Table 3).

Table 3:Bivariate and Multivariate logistic regression for the factors associated with *Candida* oral carriage

Variable	Detection of *Candida*spp, n (%)	Univariate cOR	95% CI	p-value	Multivariate aOR	95% CI	*p*-value
Gender (p=0.2)							
Male	18 (30.5)	Ref			Ref		
Female	56 (40.9)	1.57	0.82-3.02	0.21	1.69	0.82-3.49	0.16
Treatment type (p=0.02)							
Chemotherapy	4 (13.3)	Ref			Ref		
Radiotherapy	3 (20.0)	1.63	0.31-8.43	0.56	1.97	0.31-12.55	0.47
Chemotherapy and Radiotherapy	67 (44.4)	5.18	1.72-15.58	**<0.01**	2.22	0.52-9.56	0.28

Type of Malignancy (p=0.00)							
Head and neck	54 (54.0)	14.09	3.16-62.83	**<0.01**	15.09	3.05-74.59	**<0.01**
Gastrointestinal	8 (47.1)	10.67	1.89-60.08	**0.01**	14.14	2.25-88.63	**<0.01**
Breast, cervical & Prostate	2 (7.7)	Ref			Ref		
Other	10 (18.9)	2.79	0.56-13.80	0.21	4.45	0.80-24.98	0.09
Diabetes status (p=0.08)							
No	62 (35.6)	Ref			Ref		
Yes	12 (54.8)	2.17	0.89-5.30	0.08	3.18	1.03-9.77	**0.04**

cOR *stands for crude odd ratio(Binary logistic regression),* ***aOR*** *stands for adjusted odd ratio (Log likelihood)and* ***Ref*** *stands for referenceassociation*

DISCUSSION

Oral candidiasis, which is usually preceded by colonization, is a problem among immunocompromised patients with cancer, especially in cytotoxic therapy. In the present study, we report a prevalence of oral *Candida* species colonization among cancer patients at ORCI undergoing chemotherapy and/or radiotherapy to be 37.8%. Our finding is slightly higher compared to 25%, which was reported in Nagasaki, Japan, by Kawashita, Y *et al.,* 2011(15), and 30.1% reported in France by Grigorov J *et al.,*2010(16). On the other hand, Al-Abeid *et al*., 2004 reported a much higher prevalence of *Candida* colonization, i.e., 72.6% in Jordanian cancer patients(14). The observed differences may be attributed to geographical location, population characteristics, and sampling protocol.

The role of cell-mediated host immunity (CMI) in controlling fungal infections is well known. Scientific evidence shows that cytotoxic chemotherapy and radiation used in treating malignancies compromises CMI, thus predisposing a person to fungal infections. In the current study, nearly all patients who had head and neck malignancy received radiotherapy and chemotherapy. Oral colonization was highest in this group (54.0%) among all patients.

This result is comparable to the studies done by Lone M S*et al.,*who found oral candidiasis was highest in head & neck cancer patients compared to other types of malignancies(11).

In the present study higher colonization rate (44.4%) was seen in patients receiving chemotherapy and radiotherapy together than in patients receiving monotherapy (either chemotherapy or radiation therapy). Similar results were obtained in a study conducted by Manish Jain *et al.,*2016 which observed a significant increase in oral carriage of *Candida* species in patients taking both radiation and chemotherapy(1). This observation may be explained by the fact that cytotoxic drugs given during chemotherapy cause dryness of oral mucosa facilitating infections by various pathogens, including fungi, and at the same time, radiation causes mucositis and changes in salivary glands, which lead to quantitative and qualitative changes in saliva, whereby thick saliva makes the oral environment conducive for fungal colonization(17). Hence, taken together, these factors increase the chances of fungal colonization.

Other researchers have identified *Candida albicans* as the most common species causing oral colonization(10,18). However, this was not the case in this study; we report *Candida krusei* as the predominant species detected in our study setting. In addition, the predominance of *Candida krusei* colonizing patients on cancer therapy in the area where fluconazole is the main therapy is alarming. This is because *Candida krusei* has intrinsic resistance to fluconazole(12). We also report the detection of *Candida tropicalis* which has been associated witha high predictive value for invasive fungal infection(19).

These results are worrisome as colonization is a risk factor for infection, putting colonized patients at risk of subsequent infection. Therefore, detection of non- *C. albicans* species, especially *Candida krusei,* empathizes the need for specie identification and drug susceptibility testing of the infecting *Candida* species in cancer patients before starting empirical therapy.

This study had a limitation; we could not perform antifungal susceptibility testing in the present study to show the antifungal profile among different *Candida* species.

CONCLUSION

Oral non-*Candida* species colonization is high among cancer patients at ORCI. Patients with head and neck malignancies are at high risk of colonization, a risk factor for subsequent infections. There is therefore, a need for prompt identification of causative agents of candidiasis among cancer patients and fungal susceptibility testing for better management of patients as resistance pattern differs between *C. albicans* and non-*C. albicans* species.

LIST OF ABBREVIATIONS

AIDS- Acquired Immunodeficiency Syndrome, **HIV-** Human Immunodeficiency Virus**,**

OC- Oral Candidiasis, **ORCI-** Ocean Road Cancer Institute

DECLARATIONS

Ethics approval and consent to participate

Ethical clearance was obtained from the Senate of Research and Publications Committee of the Muhimbili University of Health and Allied Sciences (MUHAS). Permission to conduct the study was obtained from the ORCI administration. Before enrolling in the study, written informed consent was obtained from each participant. Confidentiality of the study participants was ensured using codes instead of participants'names.

Consent for Publication

Not applicable

Availability of data and materials

All relevant data generated and analyzedduring this study are included in this manuscript.

Competing Interests

The authors declare that they have no competing interests.

Funding

No funding was received for this study.

Authors' contributions

UK and DK were involved in the study's conceptualization and performed data collection and laboratory work. UK, DK, and AMM performed all the statistical analyses. UK, DK, AMM, MFM, and MM were involved in drafting the manuscript. JM and MM were involved in a critical review of the manuscript.

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REFERENCES

1. Jain M, Shah R, Chandolia B, Mathur A, Chauhan Y, Chawda J, et al. The Oral Carriage of Candida in Oral Cancer Patients of Indian Origin Undergoing Radiotherapy and/or Chemotherapy. J Clin Diagn Res 2016 Feb 1
2. Jemal A, Siegel R, Xu J, Ward E. Cancer Statistics, 2010. CA Cancer J Clin 2010;60:277–300.
3. Johnson N. Tobacco Use and Oral Cancer: A Global Perspective. J Dent Educ 2001 Apr 1
4. Abid A, Maatouk F, Berrezouga L, Azodo C, Uti O, El-Shamy H, et al. Prevalence and Severity of Oral Diseases in the Africa and Middle East Region. Adv Dent Res.

2015;27(1):10–7.

5. Faini D, Maokola W, Furrer H, Hatz C, Battegay M, Tanner M, et al. Burden of serious fungal infections in Tanzania. Mycoses. 2015;58:70–9.

6. Bongomin F, Gago S, Oladele RO, Denning DW. Global and multi-national prevalence of fungal diseases—estimate precision. J Fungi. 2017;3(4).

7. Jayachandran AL, Katragadda R, Thyagarajan R, Vajravelu L, Manikesi S, Kaliappan S, et al. Oral Candidiasis among Cancer Patients Attending a Tertiary Care Hospital in Chennai, South India: An Evaluation of Clinicomycological Association and Antifungal Susceptibility Pattern. Can J Infect Dis Med Microbiol = J Can des Mal Infect la Microbiol Médicale 2016

8. Bashir Sher-i-Kashmir G, Ahmad GMC Baramulla J. Oral Candida colonization and infection in cancer patients and their antifungal susceptibility in a tertiary care hospital MICROBIOLOGICAL PROFILE OF URINARY TRACT INFECTIONS AND IT ANTIBIOTIC SUSCEPTIBILITY PROFILE IN A TERTIARY CARE HOSPITAL View project Mohd Suhail Government Medical College Srinagar. Artic Int J Adv Res 2014

9. Epstein JB, Thariat J, Bensadoun R-J, Barasch A, Murphy BA, Kolnick L, et al. Oral complications of cancer and cancer therapy. CA Cancer J Clin. 2012;62(6):400–22.

10. Schelenz S, Abdallah S, Gray G, Stubbings H, Gow I, Baker P, et al. Epidemiology of oral yeast colonization and infection in patients with hematological malignancies, head neck and solid tumors. J Oral Pathol Med. 2011;40(1):83–9.

11. Suhail Lone D, Bashir D, Bali D, Sajad S, Bashir H, Ahmad D, et al. Oral Candida colonization and infection in cancer patients and their antifungal susceptibility in a tertiary care hospital. Int J Adv Res Journalwww.journalijar.com Int J Adv Res. 2014;2(5):541–50.

12. Mushi MF, Mtemisika CI, Bader O, Bii C, Mirambo MM, Groß U, et al. High Oral Carriage of Non-albicans Candida spp. among HIV-infected individuals. Int J Infect Dis 2016 Aug 1

13. Mushi MF, Bader O, Bii C, Groß U, Mshana SE. Virulence and susceptibility patterns of clinical Candida spp. isolates from a tertiary hospital, Tanzania. Med Mycol 2018 Jul 1

14. Al-Abeid HM, Abu-Elteen KH, Elkarmi AZ, Hamad MA. Isolation and characterization of Candida spp. in Jordanian cancer patients: Prevalence, pathogenic determinants, and antifungal sensitivity. Jpn J Infect Dis. 2004;57(6):279–84.

15. Kawashita Y, Funahara M, Yoshimatsu M, Nakao N, Soutome S, Saito T, et al. A retrospective study of factors associated with the development of oral candidiasis in patients receiving radiotherapy for head and neck cancer. 2017;

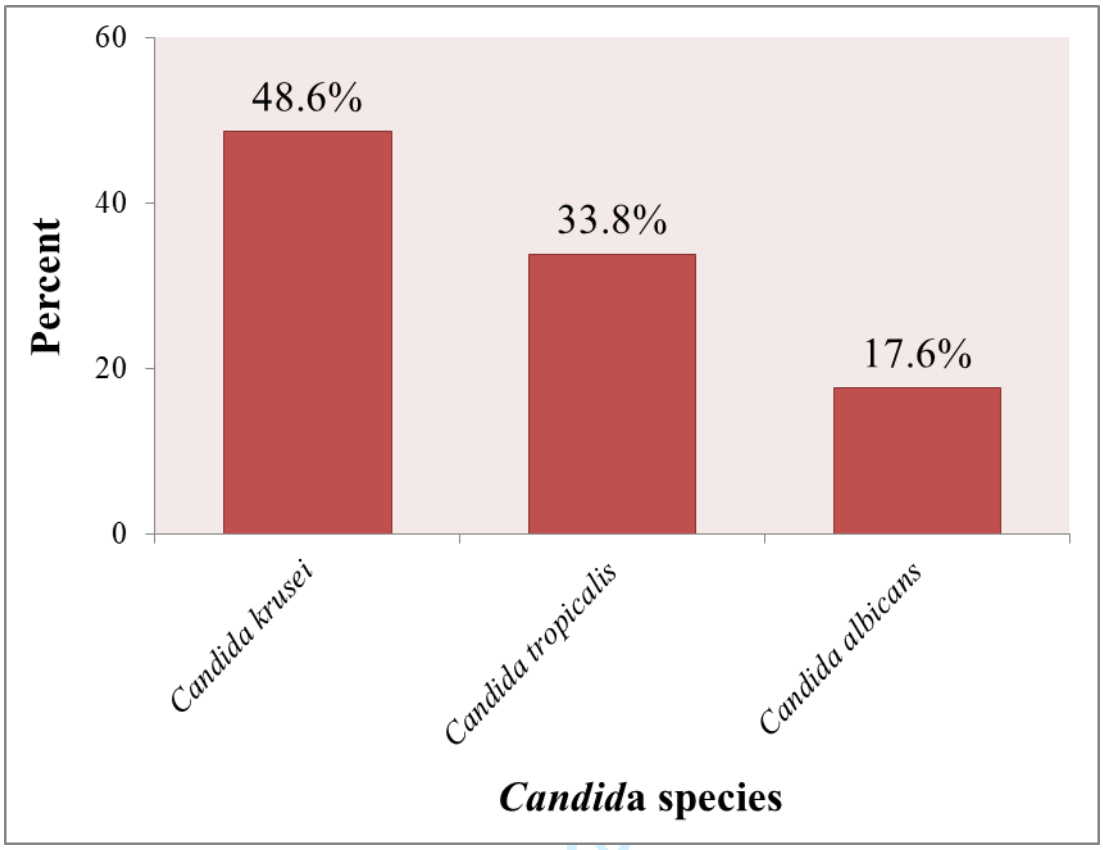
16. Gligorov J, Bastit L, Gervais H, Henni M, Kahila W, Lepille D, et al. Prevalence and treatment management of oropharyngeal candidiasis in cancer patients: Results of the French candidoscope study. Int J Radiat Oncol Biol Phys. 2011;80(2):532–9.

17. Main BE, Calman KC, Ferguson MM, Kaye SB, MacFarlane TW, Mairs RJ, et al. The effect of cytotoxic therapy on saliva and oral flora. Oral Surg Oral Med Oral Pathol 1984

18. Bensadoun RJ, Patton LL, Lalla R V., Epstein JB. Oropharyngeal candidiasis in head and neck cancer patients treated with radiation: update 2011. Support Care Cancer 2011 Jun

19. Lalla R V., Latortue MC, Hong CH, Ariyawardana A, D'Amato-Palumbo S, Fischer DJ, et al. A systematic review of oral fungal infections in patients receiving cancer therapy. Support Care Cancer 2010 Aug

Figure 1: Distribution of *Candida* species isolates from cancer patients



The figure illustrates the distribution of specific *Candida* species isolates that were obtained from 196 cancer patients at Ocean road Cancer Institute (ORCI).

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Page 1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Pages 2 and 3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Page 4
Objectives	3	State specific objectives, including any prespecified hypotheses	Page 4
Methods			
Study design	4	Present key elements of study design early in the paper	Page 5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page 5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	Page 5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Page 6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Page 6
Bias	9	Describe any efforts to address potential sources of bias	Page 5
Study size	10	Explain how the study size was arrived at	Page 5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Pages 6 and 7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Pages 6 and 7
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	NA
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	NA
		(*e*) Describe any sensitivity analyses	NA
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	NA
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Pages 7 and 8
		(b) Indicate number of participants with missing data for each variable of interest	NA
Outcome data	15*	Report numbers of outcome events or summary measures	Pages 8 and 9
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Page 10
		(*b*) Report category boundaries when continuous variables were categorized	NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	Page 11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Page 12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	NA
Generalisability	21	Discuss the generalisability (external validity) of the study results	Pages 11 and 12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	NA

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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The predominance of non-Candida albicans species oral colonization among patients on anticancer therapy: Findings from a cross-sectional study in Tanzania

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The predominance of non-*Candida albicans* species oral colonization among patients on anticancer therapy: Findings from a cross-sectional study in Tanzania

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ABSTRACT

Objectives: This study aimed to determine the oral carriage prevalence of *Candida* species. and identify factors associated with the carriage of *Candida* species among cancer patients on treatment.

Design: A hospital-based cross-sectional study

Setting: The study was conducted at a tertiary-level cancer hospital Ocean Road Cancer Institute (ORCI) in Dar es Salaam, Tanzania.

Participants: We enrolled 196 participants who consented to join the study. Oral swabs were collected from all participants and inoculated onto Sabouraud Dextrose Agar supplemented with 50mg/ml gentamicin and 50 mg/ml chloramphenicol, and chromogenic agar for phenotypic identification of *Candida* species.

Primary outcome: The study reported the high prevalence of oral carriage of *Candida* species among cancer patients on treatment at the tertiary-level cancer hospital in Dar es Salaam, Tanzania.

Results

A total of 196 participants were enrolled in the study. The overall oral carriage of *Candida* species was 37.8% (74/196). The prevalence was higher among patients undergoing both chemotherapy and radiotherapy (44.4%) than those in monotherapy (13.3% chemotherapy, 20% radiotherapy). *Candida krusei* was the commonest isolated species, 48.6% (36/74). Head and neck (aOR, 15.09, 95%CI 3.05-74.59, p=0.00), gastrointestinal (aOR, 14.14, 95%CI 2.25-88.63, p=0.00) malignancies and diabetes (aOR=3.18, 95% CI=1.03-9.77, p=0.04) were factors independently associated with oral carriage of *Candida* species.

Conclusion

Oral carriage of *Candida* species among cancer patients receiving treatment at ORCI is high, mainly due to *C.krusei* species. This is alarming since *C.krusei* has intrinsic resistance to fluconazole, a common antifungal agent used to manage fungal infections in adults.

Therefore, efforts should be put into conducting regular check-ups for such opportunistic pathogens as they can lead to subsequent infections. Furthermore, studies conducted to determine the antifungal profile of the causative agents is warranted since, different causative agents might have different profiles.

Strengths and limitation of the study

- We have highlated presence non *Candida albicans* species among cancer patients
- Unable to confirm species using biochemical and molecular tests
- Failure to perfom antifungal susceptibility testing for patient management

Keywords

Candida carriage, Cancer patients, Chemotherapy and radiotherapy, Gastrointestinal malignancy, Head and neck malignancy

BACKGROUND

Oral carriage of *Candida* species is the major predisposing factor to oral candidiasis in immune-compromised patients(1). Cancer is mentioned as an immune-compromising condition that accounts for great morbidity and mortality(2). Globally it is estimated that 1 in every 3 persons suffers from cancer by 75 years(3). The use ofradiation therapy, chemotherapy, and/or a combination of both is documented to compromise the patient's

immune status further and thus predisposes these patients to opportunistic infections like oral candidiasis(4–6). In addition, cancer therapy counteracts neutrophil's function and induces neutrophil depletion, predisposing the person to fungal infections like oral candidiasis(7). It is estimated that the rate of oral candidiasis among cancer patientsranges from 7 to 52%(8). Variation in the magnitude depends on the type of malignancy,whereby head and neck cancer have a higher prevalence, followed by haematological malignancies(9–11). Historically, *C. albicans* species have been the most common cause of oral candidiasis; however, recently, non-*Candida albicans* species are increasingly implicated as causative agents of candidiasis(12). The shift of species from *C. albicans* to non-*C. albicans* species can potentially cause treatment challenges, especially inresource-limited areas where treatment is usually empirical. In addition, studies have shown that *C. albicans* and non-*C. albicans* though closely related, differ in their antifungal susceptibility profiles(7,13). Therefore identifying a specific causative agent can help inpatient management.

There is limited data on the predominant *Candida* species colonizing cancer patients undergoing cancer treatment in our geographical area. Therefore, we conducted the present study to determine the current prevalence of oral carriage of *Candida* species among cancer patients receiving cancer treatment and evaluate the association between some factors and oral carriage.

MATERIALS AND METHODS

Study design and settings

A hospital-based cross-sectional study was conducted from July to August 2019 at Ocean Road Cancer Institute (ORCI) in Dar es Salaam, Tanzania. ORCI is located along the Indian Ocean inIlala district, Dar es Salaam, Tanzania. It is a public national referral hospital for cancer treatment in Tanzania. Currently, ORCI serves more than 50,000 patients, including

about 28,000 cancer patients, 10,000 cancer screening patients, and 12,000 non-cancer patients. In addition, ORCI attends to over 15,000 clients in the outreach programs in the Tanzania mainland regions.

Study population, sample size,and sampling procedure

Adult patients aged 18 years and above on anticancer therapy present at the clinic or ward on the day of data collection were eligible for inclusion in the study. Participants were randomly selected and those who conseted were included in the study until the sample size was reached. Sample size estimation was done using Kish Leslie formula for cross-sectional study (N =z2pq/ε2); considering the prevalance of 15%(12), 95% CI and ε at 0.05, the estimated sample size was 196. To avoid underestimating candida oral carriage, we excluded cancer patients who had taken antifungal agents in the past four weeks. Patients with signs and symptoms of dry mouth were also excluded to avoid overestimation of oral candida carriage. These included: mouth sores, thristness, cracks and cuts on lips, difficulty in swallowing and loss of sense of taste.

Data collection

Data collection was conducted by two research asistants who were medical doctors trained on the study protocol. A well-structured questionnaire was used to collect socio-demographic information such as age, sex, education status, employment status, and clinical information, including the type of malignancy, type of anticancer treatment, stage of malignancy, salivation status, inpatient and outpatient services and diabetes status.

Sample collection and laboratory procedures

Oral swabs were collected from each participantas per standard procedures. Briefly, a sterile cotton wool swab was used to collect the sample from the mouth of the patient. Swabs were not taken from oral lesions to avoid overestimating oral acrriage. After collection, the

samples were transported to Muhimbili University of Health and Allied Sciences (MUHAS) and processed in a Microbiology laboratory.

The oral swabs were inoculated into Sabouraud dextrose agar (SDA) media supplemented with 50 mg/ml gentamicin and 50 mg/ml chloramphenicol (Oxoid, Basingstoke RG24 8PW, UK) and chromogenic candida agar (CHROMagar Candida Oxoid). All media were incubated aerobically at 37^0C for 24-48 hours for phenotypic identification of *Candida* species. *Candida* species were identified based on colour and colonial morphology on CHROMagar as per the manufacturer's instructions. *C.albicans* isolates were further confirmed by germ tube test. Growth of colonies in less than three quadrants of the plate and absence of pseudohyphae in Gram stain indicated oral candida carriage.

Quality control

All the reagents were prepared following the manufactures instructions. In addition, we performed sterility and performance tests to check for the quality of prepared media.

Variables

The independent variables were, age, sex, education status, employment status, type of malignancy, type of anticancer treatment, stage of malignancy, treatment services (inpatient or outpatient) and diabetes status. The dependent varible was detection of candida species by phenotypic method.

Statistical analysis

We used STATA version 15.1 software for statistical analysis. Continuous variables were summarized as the median and interquartile range (IQR), while proportions were used to

describe categorical variables. Group differences were determined using Fisher's exact test for categorical variables. Binary logistic regression was performed to identify factors associated withoral colonization. In addition, multivariable logistic regression was performed to examine the associations between the outcome variable and independent variables after adjustment for other variables. At a 95% confidence level, factors with a *p-value <0.05* were considered statistically significant.

Patient and public involvement

This study was designed to investigate the prevalence of oral candida carriage and the causative agents among cancer patients to better plan infection prevention and control practices, thus improving patient care. Patients were not involved in designing this research, however, the proposed study was presented to the members of the department of Microbiology and immunology of Muhimbili University of Health and Allied Sciences before the recruitment of participants began. Patients who were colonized with *Candida* species were notified as soon as the sample processing was complete and the final report was communicated to the to hospital management and the infection prevention and control team of Ocean Road Cancer Institute.

RESULTS

Socio-demographic and clinical characteristics

A total of 196 cancer patients with a mean age of 54 years, a standard deviation (SD) ± 14.2, were enrolled in the study. Of the 196 participants, 69.9% were female, and nearly half (87/196, 44.4%) had acquired primary education. The majority, 143/196 (73%) of the participants, were inpatients, and about three-quarters, 151/196 (77%), received both chemotherapy and radiotherapy treatment. Head and neck cancers were the most prevalent type of malignancies 100/196 (51%), whereas only a few participants had gastrointestinal

177 cancer 7/196 (8.7%). Many participants were either in stage 2 (78/196;39.8%) or stage 3
178 (73/196;37.2%). Twenty-two participants (11.2%) had diabetes (Table 1).
179

180 **Table 1:Distribution of socio-demographic and clinical characteristics among cancer**
181 **patients (N = 196)**

Variable	Total number(N)	Percentage (%)
Age group (Mean =54; SD± 14.2)		
<54	101	51.5
>54	95	48.5
Gender		
Male	59	30.1
Female	137	69.9
Educational level		
Primary	87	44.4
Secondary and above	80	40.8
Non formal	29	14.8
Smoking		
No	165	84.2
Yes	31	15.8
Oral hygiene practices		
Frequency of tooth brushing		
1 time a day	4	2
2 times a day	192	98
Tooth cleaning material		
Mouth wash	0	0
Toothpaste	196	100
Type of tooth brush		
Plastic	196	100
Chewing stick	0	0
Denture		
No	196	100
Yes	0	0
Patient care		
Inpatient	143	73.0
Outpatient	53	27.0
Treatment type		
Chemotherapy	30	15.3
Radiotherapy	15	7.7
Chemotherapy and Radiotherapy	151	77.0
Type of Malignancy		
Head and neck*	100	51.0
Gastrointestinal	17	8.7
Breast,cervical&prostate	26	13.3
Other	53	27.0
Cancer stage		
1	29	14.8
2	78	39.8
3	73	37.2
4	16	8.2

Diabetes status		
No	174	88.8
Yes	22	11.2

*Others:leukemia,lymphoma,liver, kaposi sarcoma, * also includes oropharyngeal cancer and oral squamous cell carcinoma*

Prevalence of oral colonization of *Candida* species

All 196 cultured plates showed growth in less than three quadrants making the overall prevalence of oral colonization of *Candida* species to be 37.8% (74/196). A higher carriage rate of 44.4% (67/151) was observed in patients treated with both chemotherapy and radiotherapy compared to each treatment separately;13.3% (4/30) and 20% (3/15) for chemotherapy and radiotherapy respectively (*p=0.02*). Patients with head and neck malignancies had a higher oral carriage, 54% (54/100) of *Candida* species, than other types of malignancies (*p<0.0001*). Although not statistically significant, detection of *Candida* species was more prevalent among diabetic patients than non-diabetic; 54.5 % (12/22) vs. 35.6 % (62/174) (*p=0.08)*. There was no difference in the carriage rate of *Candida* species in other parameters such as age, gender, smoking habits, education level, and cancer stage (Table 2).

Table 2:Prevalence of oral candida carriage among cancer patients by social-demographic and clinical factors

Variable	Total number	Candida colonization n (%)	*P-value*
Overall	**196**	**74 (37.8)**	
Age group			
<54	101	41 (40.6)	0.46
>54	95	33 (34.7)	
Gender			
Male	59	18 (30.5)	0.20
Female	137	56 (40.9)	
Educational level			
Primary	87	38 (43.7)	
Secondary and above	80	29 (36.3)	0.26

Non formal	29	7 (24.1)	
Smoking			
No	165	62 (35.6)	0.90
Yes	31	12 (38.7)	
Patient care			
Inpatient	143	52 (36.4)	0.51
Outpatient	53	22 (41.5)	
Treatment type			
Chemotherapy	30	4 (13.3)	
Radiotherapy	15	3 (20.0)	**0.02**
Chemotherapy and Radiotherapy	151	67 (44.4)	
Type of Malignancy			
Head and neck*	100	54 (54.0)	
Gastrointestinal	17	8 (47.1)	
Breast, cervical& Prostate	26	2 (7.7)	**< 0.01**
Other	53	10 (18.9)	
Cancer stage			
1	29	10 (34.5)	
2	78	29 (37.2)	0.85
3	73	30 (41.1)	
4	16	5 (31.3)	
Diabetes status			
No	174	62 (35.6)	0.08
Yes	22	12 (54.5)	

*Others:leukemia,lymphoma,liver, kaposi sarcoma; In bold p-value of less than 0.05 that indicates statistically significant association(Fisher's exact test), * also includes oropharyngeal cancer and oral squamous cell carcinoma*

Candida species isolated from cancer patients

A total of 74 patients had one type of *Candida* spp. in their oral cavity, making 74 candida isolates. Of the 74 *Candida* spp isolated, 61(82.4%) were non-C. *albicans. Candida krusei* was the dominant species accounting for 48.6% (36/74), followed by *Candida tropicalis* (33.8%, 25/74) and lastly, *C. albicans* (17.6%, 13/74) (Figure 1).

Predictors of *Candida* species oral colonization

On bivariate analysis, participants receiving both chemotherapy and radiotherapy treatment were five times more likely to have oral carriage of candida than other treatment types (cOR5.18, 95%CI 1.72-15.58, *p<0.0001*). Likewise, in comparison to breast, cervical and

prostate malignancies, patients with head and neck malignancies (cOR,14.09, 95%CI 3.16-62.83, p<0.0001) and those with gastrointestinal cancer (cOR, 10.67, 95%CI 1.89-60.08, *p=0.01*) had an increased probability of having oral carriage of *Candida spp* (Table 3).

After adjusting the effect of confounding factors on multivariable analysis, some types of malignancies remained associated with the oral carriage of *Candida* species among cancer patients. Participants with head and neck malignancies were 15 more likely (aOR, 15.09, 95%CI 3.05-74.59, p<0.0001) to have oral carriage of *Candida* species, while those with gastrointestinal cancer were fourteen more likely (aOR, 14.14, 95%CI 2.25-88.63, p<0.0001) to have candidiasisas compared to those with breast, cervical and prostate malignancies. In addition, the probability of being colonized by *Candida* species was three times higher among diabetic patients than non-diabetic patients (aOR=3.18, 95% CI=1.03-9.77, p=0.04) (Table 3).

Table 3:Bivariate and Multivariate logistic regression for the factors associated with candida oral carriage

Variable	Detection of *Candida*spp, n (%)	Univariate cOR	95% CI	p-value	Multivariate aOR	95% CI	*p*-value
Gender (p=0.2)							
Male	18 (30.5)	Ref			Ref		
Female	56 (40.9)	1.57	0.82-3.02	0.21	1.69	0.82-3.49	0.16
Treatment type (p=0.02)							
Chemotherapy	4 (13.3)	Ref			Ref		
Radiotherapy	3 (20.0)	1.63	0.31-8.43	0.56	1.97	0.31-12.55	0.47
Chemotherapy and Radiotherapy	67 (44.4)	5.18	1.72-15.58	**<0.01**	2.22	0.52-9.56	0.28
Type of Malignancy (p=0.00)							
Head and neck*	54 (54.0)	14.09	3.16-62.83	**<0.01**	15.09	3.05-74.59	**<0.01**
Gastrointestinal	8 (47.1)	10.67	1.89-60.08	**0.01**	14.14	2.25-88.63	**<0.01**
Breast, cervical & Prostate	2 (7.7)	Ref			Ref		
Other	10 (18.9)	2.79	0.56-13.80	0.21	4.45	0.80-24.98	0.09
Diabetes status							

(p=0.08)							
No	62 (35.6)	Ref			Ref		
Yes	12 (54.8)	2.17	0.89-5.30	0.08	3.18	1.03-9.77	**0.04**

cOR *stands for crude odd ratio(Binary logistic regression),* ***aOR*** *stands for adjusted odd ratio (Log likelihood)and* ***Ref*** *stands for referenceassociation,* also includes oropharyngeal cancer and oral squamous cell carcinoma*

DISCUSSION

Oral candidiasis, which is usually preceded by colonization, is a problem among immunocompromised patients with cancer, especially in cytotoxic therapy. In the present study, we report a prevalence of oral *Candida* species colonization among cancer patients at ORCI undergoing chemotherapy and/or radiotherapy to be 37.8%. Our finding is slightly higher compared to 25%, which was reported in Nagasaki, Japan, by Kawashita, Y *et al.,* 2011(14), and 30.1% reported in France by Grigorov J *et al.,*2010(15). The high prevalence reported here might be attributed by the high number of patients who had advanced stage (2 and 3) of cancer. In the present study majority of participants were either in stage 2 (37.2%) or stage 3 (41.1%) of cancer making them more prone to oral candida carriage. On the other hand, Al-Abeid *et al*., 2004 reported a much higher prevalence of candida colonization, i.e., 72.6% in Jordanian cancer patients(16). The observed differences may be attributed to geographical location, population characteristics, and sampling protocol. There is limited literature on oral candida carriage in the study settings, both locally and nearby geographical area. However, the prevalence of oral candida carriage has been reported to be 10.3% among people living with HIV in Mwanza, 10.3% while that of the control group in the same study was reported to be4.5% (12). Different methodological aooproaches might be a contributing factor for the observed difference, whereby in a study conducted in Mwanza used more sensitive test (Matrix-assisted laser desorption ionization-time of flight mass

spectrometry) for confirmation of candida isolates versus the use of CHROMagar in the present study which might have overestrimated the reported prevalance. Furthermore, our study participants were on either chemotherapy and/or radiotherapy which is a risk factor for oral candida colonization compared to the population used in Mwanza who were not in such therapy.

The role of cell-mediated host immunity (CMI) in controlling fungal infections is well known. Scientific evidence shows that cytotoxic chemotherapy and radiation used in treating malignancies compromises CMI, thus predisposing a person to fungal infections. In the current study, nearly all patients who had head and neck malignancy received radiotherapy and chemotherapy. Oral colonization was highest in this group (54.0%) among all patients. This result is comparable to the studies done by Lone M S*et al.,*who found oral candidiasis was highest in head & neck cancer patients compared to other types of malignancies(11).

In the present study higher colonization rate (44.4%) was seen in patients receiving chemotherapy and radiotherapy together than in patients receiving monotherapy (either chemotherapy or radiation therapy). Similar results were obtained in a study conducted by Manish Jain *et al.,*2016 which observed a significant increase in oral carriage of *Candida* species in patients taking both radiation and chemotherapy(1). This observation may be explained by the fact that cytotoxic drugs given during chemotherapy cause dryness of oral mucosa facilitating infections by various pathogens, including fungi, and at the same time, radiation causes mucositis and changes in salivary glands, which lead to quantitative and qualitative changes in saliva, whereby thick saliva makes the oral environment conducive for fungal colonization(17). Hence, taken together, these factors increase the chances of fungal colonization.

Other researchers have identified *Candida albicans* as the most common species causing oral colonization(10,18). However, this was not the case in this study; we report *Candida krusei*

as the predominant species detected in our study setting. In addition, the predominance of *Candida krusei* colonizing patients on cancer therapy in the area where fluconazole is the main therapy is alarming. This is because *Candida krusei* has intrinsic resistance to fluconazole(12). We also report the detection of *Candida tropicalis* which has been associated witha high predictive value for invasive fungal infection(19).

These results are worrisome as colonization is a risk factor for infection, putting colonized patients at risk of subsequent infection. Therefore, detection of non- *C. albicans* species, especially *Candida krusei,* empathizes the need for specie identification and drug susceptibility testing of the infecting *Candida* species in cancer patients before starting empirical therapy.

This study had some limitations; we did not collect information about some variables which could affect oral candida carriage. These variables includes; duration of cancer treatment, prolonged use of antibiotics, history of dental caries and periodontal diaseases. Furthermore we did not perform biochemical tests and molecular tests to further confirm/differentiate *Candida* species. Also antifungal susceptibility testing was not preformed in the present study to show the antifungal profile among different *Candida* species. Nonetheless, the study has shown the contribution of non-*C.albicans* in the oral cavity of cancer patients which could potentially lead to subsequent infections which might be difficult to treat due to their instric resistance to conventional antifungal agents.

CONCLUSION

Oral non-*Candida* species colonization is high among cancer patients at ORCI. Patients with head and neck malignancies are at high risk of colonization, a risk factor for subsequent infections. There is therefore, a need for prompt identification of causative agents of candidiasis among cancer patients and fungal susceptibility testing for better management of patients as resistance pattern differs between *C. albicans* and non-*C. albican*s species.

LIST OF ABBREVIATIONS

AIDS- Acquired Immunodeficiency Syndrome, **HIV-** Human Immunodeficiency Virus**,** **OC-** Oral Candidiasis, **ORCI-** Ocean Road Cancer Institute

DECLARATIONS

Ethics approval and consent to participate

Ethical clearance was obtained from the Senate of Research and Publications Committee of the Muhimbili University of Health and Allied Sciences (MUHAS), Ref.No. DA.25/111/01C. Permission to conduct the study was obtained from the ORCI administration. Before enrolling in the study, written informed consent was obtained from each participant. Confidentiality of the study participants was ensured using codes instead of participants'names.

Consent for Publication

Not applicable

Availability of data and materials

All relevant data generated and analyzedduring this study are included in this manuscript.

Competing Interests

The authors declare that they have no competing interests.

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No funding was received for this study.

Authors' contributions

UK and DK were involved in the study's conceptualization and performed data collection and laboratory work. UK, DK, and AMM performed all the statistical analyses. UK, DK, AMM, MFM, and MM were involved in drafting the manuscript. JM and MM were involved in a critical review of the manuscript.

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REFERENCES

1. Jain M, Shah R, Chandolia B, Mathur A, Chauhan Y, Chawda J, et al. The Oral Carriage of Candida in Oral Cancer Patients of Indian Origin Undergoing Radiotherapy and/or Chemotherapy. J Clin Diagn Res
2. Jemal A, Siegel R, Xu J, Ward E. Cancer Statistics, 2010. CA Cancer J Clin [Internet]. 2010;60:277–300. http://cajournal.org
3. Johnson N. Tobacco Use and Oral Cancer: A Global Perspective. J Dent Educ https://onlinelibrary.wiley.com/doi/full/10.1002/j.0022-0337.2001.65.4.tb03403.x
4. Abid A, Maatouk F, Berrezouga L, Azodo C, Uti O, El-Shamy H, et al. Prevalence and Severity of Oral Diseases in the Africa and Middle East Region. Adv Dent Res. 2015;27(1):10–7.
5. Faini D, Maokola W, Furrer H, Hatz C, Battegay M, Tanner M, et al. Burden of serious fungal infections in Tanzania. Mycoses. 2015;58:70–9.
6. Bongomin F, Gago S, Oladele RO, Denning DW. Global and multi-national prevalence of fungal diseases—estimate precision. J Fungi. 2017;3(4).
7. Jayachandran AL, Katragadda R, Thyagarajan R, Vajravelu L, Manikesi S, Kaliappan S, et al. Oral Candidiasis among Cancer Patients Attending a Tertiary Care Hospital in

Chennai, South India: An Evaluation of Clinicomycological Association and Antifungal Susceptibility Pattern. Can J Infect Dis Med Microbiol = J Can des Mal Infect la Microbiol Médicale http://pmc/articles/PMC4923570/

8. Bashir Sher-i-Kashmir G, Ahmad GMC Baramulla J. Oral Candida colonization and infection in cancer patients and their antifungal susceptibility in a tertiary care hospital MICROBIOLOGICAL PROFILE OF URINARY TRACT INFECTIONS AND IT ANTIBIOTIC SUSCEPTIBILITY PROFILE IN A TERTIARY CARE HOSPITAL View project Mohd Suhail Government Medical College Srinagar. Artic Int J Adv Res https://www.researchgate.net/publication/262919798

9. Epstein JB, Thariat J, Bensadoun R-J, Barasch A, Murphy BA, Kolnick L, et al. Oral complications of cancer and cancer therapy. CA Cancer J Clin. 2012;62(6):400–22.

10. Schelenz S, Abdallah S, Gray G, Stubbings H, Gow I, Baker P, et al. Epidemiology of oral yeast colonization and infection in patients with hematological malignancies, head neck and solid tumors. J Oral Pathol Med. 2011;40(1):83–9.

11. Suhail Lone D, Bashir D, Bali D, Sajad S, Bashir H, Ahmad D, et al. Oral Candida colonization and infection in cancer patients and their antifungal susceptibility in a tertiary care hospital. Int J Adv Res Journalwww.journalijar.com Int J Adv Res. 2014;2(5):541–50.

12. Mushi MF, Mtemisika CI, Bader O, Bii C, Mirambo MM, Groß U, et al. High Oral Carriage of Non-albicans Candida spp. among HIV-infected individuals. Int J Infect Dis https://pubmed.ncbi.nlm.nih.gov/27401585/

13. Mushi MF, Bader O, Bii C, Groß U, Mshana SE. Virulence and susceptibility patterns of clinical Candida spp. isolates from a tertiary hospital, Tanzania. Med Mycol https://pubmed.ncbi.nlm.nih.gov/30380077/

14. Kawashita Y, Funahara M, Yoshimatsu M, Nakao N, Soutome S, Saito T, et al. A

retrospective study of factors associated with the development of oral candidiasis in patients receiving radiotherapy for head and neck cancer. 2017;

15. Gligorov J, Bastit L, Gervais H, Henni M, Kahila W, Lepille D, et al. Prevalence and treatment management of oropharyngeal candidiasis in cancer patients: Results of the French candidoscope study. Int J Radiat Oncol Biol Phys. 2011;80(2):532–9.

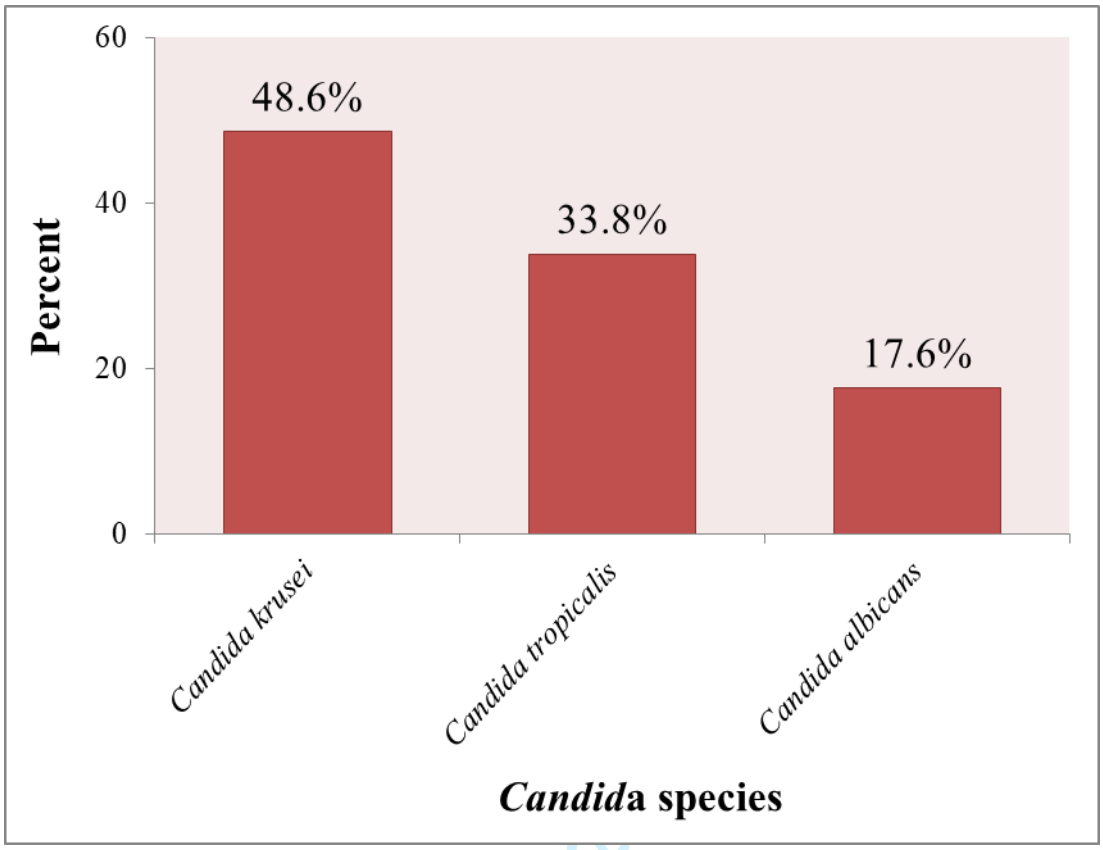
16. Al-Abeid HM, Abu-Elteen KH, Elkarmi AZ, Hamad MA. Isolation and characterization of Candida spp. in Jordanian cancer patients: Prevalence, pathogenic determinants, and antifungal sensitivity. Jpn J Infect Dis. 2004;57(6):279–84.

17. Main BE, Calman KC, Ferguson MM, Kaye SB, MacFarlane TW, Mairs RJ, et al. The effect of cytotoxic therapy on saliva and oral flora. Oral Surg Oral Med Oral Pathol https://pubmed.ncbi.nlm.nih.gov/6440071/

18. Bensadoun RJ, Patton LL, Lalla R V., Epstein JB. Oropharyngeal candidiasis in head and neck cancer patients treated with radiation: update 2011. Support Care Cancer https://pubmed.ncbi.nlm.nih.gov/21479787/

19. Lalla R V., Latortue MC, Hong CH, Ariyawardana A, D'Amato-Palumbo S, Fischer DJ, et al. A systematic review of oral fungal infections in patients receiving cancer therapy. Support Care Cancer /pmc/articles/PMC2914797/

Figure 1: Distribution of *Candida* species isolates from cancer patients



The figure illustrates the distribution of specific *Candida* species isolates that were obtained from 196 cancer patients at Ocean road Cancer Institute (ORCI).

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Page 1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Pages 2 and 3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Page 4
Objectives	3	State specific objectives, including any prespecified hypotheses	Page 4
Methods			
Study design	4	Present key elements of study design early in the paper	Page 5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page 5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	Page 5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Page 6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Page 6
Bias	9	Describe any efforts to address potential sources of bias	Page 5
Study size	10	Explain how the study size was arrived at	Page 5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Pages 6 and 7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Pages 6 and 7
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	NA
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	NA
		(*e*) Describe any sensitivity analyses	NA
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	NA
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Pages 7 and 8
		(b) Indicate number of participants with missing data for each variable of interest	NA
Outcome data	15*	Report numbers of outcome events or summary measures	Pages 8 and 9
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Page 10
		(*b*) Report category boundaries when continuous variables were categorized	NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	Page 11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Page 12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	NA
Generalisability	21	Discuss the generalisability (external validity) of the study results	Pages 11 and 12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	NA

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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The predominance of non-*Candida albicans* species oral colonisation among patients on anticancer therapy: Findings from a cross-sectional study in Tanzania

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ABSTRACT

Objectives: This study aimed to determine the oral carriage prevalence of *Candida* species and identify factors associated with the carriage of *Candida* species among cancer patients on treatment.

Design: A hospital-based cross-sectional study

Setting: The study was conducted at a tertiary-level cancer hospital Ocean Road Cancer Institute (ORCI), in Dar es Salaam, Tanzania.

Participants: We enrolled 196 participants who consented to join the study. Oral swabs were collected from all participants and inoculated onto Sabouraud Dextrose Agar supplemented with 50mg/ml gentamicin and 50 mg/ml chloramphenicol, and chromogenic agar for phenotypic identification of *Candida* species.

Primary outcome: The study reported the high prevalence of oral carriage of *Candida* species among cancer patients on treatment at the tertiary-level cancer hospital in Dar es Salaam, Tanzania.

Results: A total of 196 participants were enrolled in the study. The overall oral carriage of *Candida* species was 37.8% (74/196). The prevalence was higher among patients undergoing chemotherapy and radiotherapy (44.4%) than those in monotherapy (13.3% chemotherapy, 20% radiotherapy). *Candida krusei* was the commonest isolated species, 48.6% (36/74). Head and neck (aOR, 15.09, 95%CI 3.05-74.59, p=0.00), gastrointestinal (aOR, 14.14, 95%CI 2.25-88.63, p=0.00) malignancies and diabetes (aOR=3.18, 95% CI=1.03-9.77, p=0.04) were factors independently associated with oral carriage of *Candida* species.

Conclusion

The oral carriage of *Candida* species among cancer patients receiving treatment at ORCI is high, mainly due to *C.krusei* species. This is alarming since *C.krusei* has intrinsic resistance

to fluconazole, a common antifungal agent used to manage adult fungal infections. Therefore, efforts should be put into conducting regular check-ups for such opportunistic pathogens as they can lead to subsequent infections. Furthermore, studies conducted to determine the antifungal profile of the causative agents are warranted since different causative agents might have different profiles.

Strengths and limitations of the study

- We used chromogenic media for candida speciation, which can be adopted in other resource-limited settings to provide preliminary identification of Candida species for proper patient management
- Unable to confirm species using biochemical and molecular tests
- Failure to perform antifungal susceptibility testing for patient management

Keywords

Candida carriage, Cancer patients, Chemotherapy and radiotherapy, Gastrointestinal malignancy, Head and neck malignancy

BACKGROUND

Oral carriage of *Candida* species is the major predisposing factor to oral candidiasis in immune-compromised patients(1). Cancer is mentioned as an immune-compromising condition that accounts for significant morbidity and mortality(2). Globally it is estimated that 1 in every 3 persons will get cancer by the age of 75 years(3). The use of radiation therapy, chemotherapy, and a combination of both is documented to compromise the patient's immune status further and thus predisposes these patients to opportunistic infections like oral candidiasis(4–6). In addition, cancer therapy counteracts neutrophil function and induces neutrophil depletion, predisposing the person to fungal infections like oral candidiasis(7). The rate of oral candidiasis among cancer patients is estimated to be 7 to 52%(8).

Variation in the magnitude depends on the type of malignancy, whereby head and neck cancer have a higher prevalence, followed by haematological malignancies(9–11). Historically, *C. albicans* species have been the most common cause of oral candidiasis; however, recently, non-*Candida albicans* species are increasingly implicated as causative agents of candidiasis(12). The shift of species from *C. albicans* to non-*C. albicans* species can potentially cause treatment challenges, especially in resource-limited areas where treatment is usually empirical. In addition, studies have shown that *C. albicans* and non-*C. albicans* though closely related, differ in their antifungal susceptibility profiles(7,13). Therefore, identifying a specific causative agent can help inpatient management.

There is limited data on the predominant *Candida* species colonising cancer patients undergoing cancer treatment in our geographical area. Therefore, we conducted the present study to determine the current prevalence of oral carriage of *Candida* species among cancer patients receiving cancer treatment and evaluate the association between some factors and oral carriage.

MATERIALS AND METHODS

Study design and settings

A hospital-based cross-sectional study was conducted from July to August 2019 at Ocean Road Cancer Institute (ORCI) in Dar es Salaam, Tanzania. ORCI is located along the Indian Ocean in Ilala district, Dar es Salaam, Tanzania. It is a public national referral hospital for cancer treatment in Tanzania. Currently, ORCI serves more than 50,000 patients, including about 28,000 cancer patients, 10,000 cancer screening patients, and 12,000 non-cancer patients. In addition, ORCI attends to over 15,000 clients in the outreach programs in the Tanzania mainland regions.

Study population, sample size, and sampling procedure

Adult patients aged 18 years and above on anticancer therapy present at the clinic or ward on the day of data collection were eligible for inclusion in the study. Participants were randomly selected, and those consented were included in the study until the sample size was reached. Sample size estimation was done using the Kish Leslie formula for cross-sectional study (N =z2pq/ε2); considering the prevalence of 15% (12), 95% CI and ε at 0.05, the estimated sample size was 196. To avoid underestimating candida oral carriage, we excluded cancer patients who had taken antifungal agents in the past four weeks. In addition, patients with signs and symptoms of dry mouth were also excluded to avoid overestimating oral candida carriage. These included: mouth sores, thirstiness, cracks and cuts on lips, difficulty swallowing and loss of sense of taste.

Data collection

Data were collected by two research assistants who were medical doctors trained in the study protocol. A well-structured questionnaire was used to collect socio-demographic information such as age, sex, education status, employment status, and clinical data, including the type of

malignancy, type of anticancer treatment, stage of malignancy, salivation status, inpatient and outpatient services and diabetes status.

Sample collection and laboratory procedures

Oral swabs were collected from each participant as per standard procedures. Briefly, a sterile cotton wool swab was used to collect the sample from the mouth of the patient. Swabs were not taken from oral lesions to avoid overestimating oral carriage. After collection, the samples were transported to Muhimbili University of Health and Allied Sciences (MUHAS) and processed in a Microbiology laboratory.

The oral swabs were inoculated into Sabouraud dextrose agar (SDA) media supplemented with 50 mg/ml gentamicin and 50 mg/ml chloramphenicol (Oxoid, Basingstoke RG24 8PW, UK) and chromogenic candida agar (CHROMagar Candida Oxoid). All media were incubated aerobically at 37^0C for 24-48 hours for phenotypic identification of *Candida* species. *Candida* species were identified based on colour and colonial morphology on CHROMagar as per the manufacturer's instructions. *C.albicans* isolates were further confirmed by germ tube test. Growth of colonies in less than three quadrants of the plate and absence of pseudo hyphae in Gram stain indicated oral candida carriage.

Quality control

All the reagents were prepared following the manufactures instructions. In addition, we performed sterility and performance tests to check for the quality of the prepared media.

Variables

The independent variables were age, sex, education status, employment status, type of malignancy, type of anticancer treatment, stage of malignancy, treatment services (inpatient

or outpatient) and diabetes status. The dependent variable was the detection of candida species by the phenotypic method.

Statistical analysis

We used STATA version 15.1 software for statistical analysis. Continuous variables were summarised as the median and interquartile range (IQR), while proportions were used to describe categorical variables. Group differences were determined using Fisher's exact test for categorical variables. Binary logistic regression was performed to identify factors associated with oral colonisation. In addition, multivariable logistic regression was performed to examine the associations between the outcome variable and independent variables after adjustment for other variables. At a 95% confidence level, factors with a *p-value <0.05* were considered statistically significant.

Patient and public involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research study.

RESULTS

Socio-demographic and clinical characteristics

A total of 196 cancer patients with a mean age of 54 years, a standard deviation (SD) ± 14.2, were enrolled in the study. Of the 196 participants, 69.9% were female, and nearly half (87/196, 44.4%) had acquired primary education. The majority, 143/196 (73%) of the participants, were inpatients, and about three-quarters, 151/196 (77%), received both chemotherapy and radiotherapy treatment. Head and neck cancers were the most prevalent type of malignancies 100/196 (51%), whereas only a few participants had gastrointestinal

cancer 7/196 (8.7%). Many participants were either in stage 2 (78/196;39.8%) or stage 3 (73/196;37.2%). Twenty-two participants (11.2%) had diabetes (Table 1).

Table 1: Distribution of sociodemographic and clinical characteristics among cancer patients (N = 196)

Variable	Total number(N)	Percentage (%)
Age group (Mean =54; SD± 14.2)		
<54	101	51.5
>54	95	48.5
Gender		
Male	59	30.1
Female	137	69.9
Educational level		
Primary	87	44.4
Secondary and above	80	40.8
Non formal	29	14.8
Smoking		
No	165	84.2
Yes	31	15.8
Oral hygiene practices		
Frequency of tooth brushing		
1 time a day	4	2
2 times a day	192	98
Tooth cleaning material		
Mouth wash	0	0
Toothpaste	196	100
Type of toothbrush		
Plastic	196	100
Chewing stick	0	0
Denture		
No	196	100
Yes	0	0
Patient care		
Inpatient	143	73.0
Outpatient	53	27.0
Treatment type		
Chemotherapy	30	15.3
Radiotherapy	15	7.7
Chemotherapy and Radiotherapy	151	77.0
Type of Malignancy		
Head and neck*	100	51.0
Gastrointestinal	17	8.7
Breast, cervical & prostate	26	13.3
Other	53	27.0
Cancer stage		
1	29	14.8
2	78	39.8
3	73	37.2
4	16	8.2
Diabetes status		

No	174	88.8
Yes	22	11.2

*Others: leukaemia, lymphoma, liver, kaposi sarcoma, * also includes oropharyngeal cancer and oral squamous cell carcinoma*

Prevalence of oral colonisation of *Candida* species

All 196 cultured plates showed growth in less than three quadrants making the overall prevalence of oral colonisation of *Candida* species to be 37.8% (74/196). A higher carriage rate of 44.4% (67/151) was observed in patients treated with both chemotherapy and radiotherapy compared to each treatment separately;13.3% (4/30) and 20% (3/15) for chemotherapy and radiotherapy, respectively (*p=0.02*). Patients with head and neck malignancies had a higher oral carriage, 54% (54/100) of *Candida* species, than other types of malignancies (*p<0.0001*). Although not statistically significant, the detection of *Candida* species was more prevalent among diabetic patients than non-diabetic; 54.5 % (12/22) vs 35.6 % (62/174) (*p=0.08)*. There was no difference in the carriage rate of *Candida* species in other parameters such as age, gender, smoking habits, education level, and cancer stage (Table 2).

Table 2: Prevalence of oral candida carriage among cancer patients by social-demographic and clinical factors

Variable	Total number	Candida colonisation n (%)	*P-value*
Overall	**196**	**74 (37.8)**	
Age group			
<54	101	41 (40.6)	0.46
>54	95	33 (34.7)	
Gender			
Male	59	18 (30.5)	0.20
Female	137	56 (40.9)	
Educational level			
Primary	87	38 (43.7)	
Secondary and above	80	29 (36.3)	0.26
Non formal	29	7 (24.1)	
Smoking			
No	165	62 (35.6)	0.90
Yes	31	12 (38.7)	

Patient care			
Inpatient	143	52 (36.4)	0.51
Outpatient	53	22 (41.5)	
Treatment type			
Chemotherapy	30	4 (13.3)	
Radiotherapy	15	3 (20.0)	**0.02**
Chemotherapy and Radiotherapy	151	67 (44.4)	
Type of Malignancy			
Head and neck*	100	54 (54.0)	
Gastrointestinal	17	8 (47.1)	
Breast, cervical& Prostate	26	2 (7.7)	**< 0.01**
Other	53	10 (18.9)	
Cancer stage			
1	29	10 (34.5)	
2	78	29 (37.2)	0.85
3	73	30 (41.1)	
4	16	5 (31.3)	
Diabetes status			
No	174	62 (35.6)	0.08
Yes	22	12 (54.5)	

*Others: leukaemia, lymphoma, liver, kaposi sarcoma; In bold p-value of less than 0.05 that indicates statistically significant association (Fisher's exact test), * also includes oropharyngeal cancer and oral squamous cell carcinoma.*

Candida species isolated from cancer patients

A total of 74 patients had one type of *Candida* spp. in their oral cavity, making 74 candida isolates. Of the 74 *Candida* spp isolated, 61(82.4%) were non-C. *albicans. Candida krusei* was the dominant species accounting for 48.6% (36/74), followed by *Candida tropicalis* (33.8%, 25/74) and lastly, *C. albicans* (17.6%, 13/74) (Figure 1).

Predictors of *Candida* species oral colonisation

On bivariate analysis, participants receiving both chemotherapy and radiotherapy treatment were five times more likely to have oral carriage of candida than other treatment types (cOR5.18, 95%CI 1.72-15.58, *p<0.0001*). Likewise, in comparison to breast, cervical and prostate malignancies, patients with head and neck malignancies (cOR,14.09, 95%CI 3.16-62.83, p<0.0001) and those with gastrointestinal cancer (cOR, 10.67, 95%CI 1.89-60.08, *p=0.01*) had an increased probability of having oral carriage of *Candida spp* (Table 3).

After adjusting the effect of confounding factors on multivariable analysis, some types of malignancies remained associated with the oral carriage of *Candida* species among cancer patients. Participants with head and neck malignancies were 15 more likely (aOR, 15.09, 95%CI 3.05-74.59, p<0.0001) to have oral carriage of *Candida* species, while those with gastrointestinal cancer were fourteen more likely (aOR, 14.14, 95%CI 2.25-88.63, p<0.0001) to have candidiasis as compared to those with breast, cervical and prostate malignancies. In addition, the probability of being colonised by *Candida* species was three times higher among diabetic patients than non-diabetic patients (aOR=3.18, 95% CI=1.03-9.77, p=0.04) (Table 3).

Table 3: Bivariate and Multivariate logistic regression for the factors associated with candida oral carriage

Variable	Detection of *Candida* spp, n (%)	Univariate cOR	95% CI	p-value	Multivariate aOR	95% CI	*p*-value
Gender (p=0.2)							
Male	18 (30.5)	Ref			Ref		
Female	56 (40.9)	1.57	0.82-3.02	0.21	1.69	0.82-3.49	0.16
Treatment type (p=0.02)							
Chemotherapy	4 (13.3)	Ref			Ref		
Radiotherapy	3 (20.0)	1.63	0.31-8.43	0.56	1.97	0.31-12.55	0.47
Chemotherapy and Radiotherapy	67 (44.4)	5.18	1.72-15.58	**<0.01**	2.22	0.52-9.56	0.28
Type of Malignancy (p=0.00)							
Head and neck*	54 (54.0)	14.09	3.16-62.83	**<0.01**	15.09	3.05-74.59	**<0.01**
Gastrointestinal	8 (47.1)	10.67	1.89-60.08	**0.01**	14.14	2.25-88.63	**<0.01**
Breast, cervical & Prostate	2 (7.7)	Ref			Ref		
Other	10 (18.9)	2.79	0.56-13.80	0.21	4.45	0.80-24.98	0.09
Diabetes status (p=0.08)							
No	62 (35.6)	Ref			Ref		
Yes	12 (54.8)	2.17	0.89-5.30	0.08	3.18	1.03-9.77	**0.04**

cOR *stands for crude odd ratio(Binary logistic regression),* ***aOR*** *stands for adjusted odd ratio (Log-likelihood), and* ***Ref*** *stands for reference association, * also includes oropharyngeal cancer and oral squamous cell carcinoma*

DISCUSSION

Oral candidiasis, usually preceded by colonisation, is a problem among immunocompromised patients with cancer, especially in cytotoxic therapy. In the present study, we report a prevalence of oral *Candida* species colonisation among cancer patients at ORCI undergoing chemotherapy and/or radiotherapy to be 37.8%. Our finding is slightly higher compared to 25%, reported in Nagasaki, Japan, by Kawashita, Y *et al.,* 2011(14), and 30.1% reported in France by Grigorov J *et al.,*2010(15). The high prevalence reported here might be attributed to the high number of patients with advanced cancer stages (2 and 3). In the present study, most participants were either in stage 2 (37.2%) or stage 3 (41.1%) of cancer, making them more prone to oral candida carriage. On the other hand, Al-Abeid *et al*., 2004 reported a much higher prevalence of candida colonisation, i.e., 72.6% in Jordanian cancer patients(16). The observed differences may be attributed to geographical location, population characteristics, and sampling protocol.

The limited literature on oral candida carriage exists in the study settings; locally and in nearby geographical areas. However, the prevalence of oral candida carriage has been reported to be 10.3% among people living with HIV in Mwanza, while, that of the control group in the same study was reported to be 4.5%(12). Different methodological approaches might be a contributing factor for the observed difference, whereby a study conducted in Mwanza used a more sensitive test (Matrix-assisted laser desorption ionization-time of flight mass spectrometry) for confirmation of candida isolates versus the use of CHROMagar in the present study which might have overestimated the reported prevalence. Furthermore, our study participants were on either chemotherapy and/or radiotherapy, a risk factor for oral candida colonisation, compared to the population used in Mwanza who were not in such therapy.

The role of cell-mediated host immunity (CMI) in controlling fungal infections is well known. Scientific evidence shows that cytotoxic chemotherapy and radiation used in treating malignancies compromise CMI, thus predisposing a person to fungal infections. In the current study, nearly all patients who had head and neck malignancy received radiotherapy and chemotherapy. As a result, oral colonisation was highest in this group (54.0%) among all patients. This result is comparable to the studies done by Lone M S*et al.,* who found oral candidiasis was highest in head & neck cancer patients compared to other types of malignancies(11).

In the present study, a higher colonisation rate (44.4%) was seen in patients receiving chemotherapy and radiotherapy together than in patients receiving monotherapy (either chemotherapy or radiation therapy). Similar results were obtained in a study conducted by Manish Jain *et al.,*2016 which observed a significant increase in oral carriage of *Candida* species in patients taking both radiation and chemotherapy(1). This observation may be explained by the fact that cytotoxic drugs given during chemotherapy cause dryness of oral mucosa facilitating infections by various pathogens, including fungi, and at the same time, radiation causes mucositis and changes in salivary glands, which leads to quantitative and qualitative changes in saliva, whereby thick saliva makes the oral environment conducive for fungal colonization(17). Hence, taken together, these factors increase the chances of fungal colonisation.

Other researchers have identified *Candida albicans* as the most common species causing oral colonization(10,18). However, this was not the case in this study; we report *Candida krusei* as the predominant species detected in our study setting. In addition, the predominance of *Candida krusei* colonising patients on cancer therapy in the area where fluconazole is the main therapy is alarming considering the intrinsic resistance of *Candida krusei* to

fluconazole(12). We also report the detection of *Candida tropicalis,* which has been associated with a high predictive value for invasive fungal infection(19).

These results are worrisome as colonisation is a risk factor for infection, putting colonised patients at risk of subsequent infection. Therefore, the detection of non-*C. albicans* species, especially *Candida krusei,* empathise the need for species identification and drug susceptibility testing of the infecting *Candida* species in cancer patients before starting empirical therapy.

This study had limitations; we did not collect information about variables that could affect oral candida carriage. These variables include; duration of cancer treatment, prolonged use of antibiotics, history of dental caries and periodontal diseases. Furthermore, we did not perform biochemical and molecular tests to confirm further/differentiate *Candida* species. Also, antifungal susceptibility testing was not performed in the present study to show the antifungal profile among *Candida* species. Nonetheless, the study has demonstrated the contribution of non-*C. albicans* in the oral cavity of cancer patients, potentially leading to subsequent infections that might be difficult to treat due to their intrinsic resistance to conventional antifungal agents.

CONCLUSION

Oral non-*Candida* species colonisation is high among cancer patients at ORCI. Patients with head and neck malignancies are at increased risk of colonisation, a risk factor for subsequent infections. There is, therefore, a need for prompt identification of causative agents of candidiasis among cancer patients and fungal susceptibility testing for better management of patients as resistance pattern differs between *C. albicans* and non-*C. albican*s species.

LIST OF ABBREVIATIONS

AIDS- Acquired Immunodeficiency Syndrome, **HIV-** Human Immunodeficiency Virus**, OC-** Oral Candidiasis, **ORCI-** Ocean Road Cancer Institute

DECLARATIONS

Ethics approval and consent to participate

Ethical clearance was obtained from the Senate of Research and Publications Committee of the Muhimbili University of Health and Allied Sciences (MUHAS), Ref.No. DA.25/111/01C. Permission to conduct the study was obtained from the ORCI administration. Before enrolling in the study, written informed consent was obtained from each participant. The confidentiality of the study participants was ensured using codes instead of participants' names.

Consent for Publication

Not applicable

Availability of data and materials

All data relevant to the study are included in the article.

Competing Interests

The authors declare that they have no competing interests.

Funding

No funding was received for this study.

Authors' contributions

UK and DK were involved in the study's conceptualisation and performed data collection and laboratory work. UK, DK, and AMM performed all the statistical analyses. UK, DK, AMM,

MFM, and MM were involved in drafting the manuscript. JM and MM were involved in a critical review of the manuscript.

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REFERENCES

1. Jain M, Shah R, Chandolia B, Mathur A, Chauhan Y, Chawda J, et al. The Oral Carriage of Candida in Oral Cancer Patients of Indian Origin Undergoing Radiotherapy and/or Chemotherapy. J Clin Diagn Res. 2016 Feb;10(2):ZC17-20. doi: 10.7860/JCDR/2016/15702.7180
2. Jemal A, Siegel R, Xu J, Ward E. Cancer Statistics, 2010. CA Cancer J Clin. 2010;60:277–300. doi: 10.1002/caac.20073
3. Johnson N. Tobacco Use and Oral Cancer: A Global Perspective. J Dent Educ. 2001.65(4): 328-39 doi: 10.1002/J.0022-0337.2001.65.4.TB03403.X
4. Abid A, Maatouk F, Berrezouga L, Azodo C, Uti O, El-Shamy H, et al. Prevalence and Severity of Oral Diseases in the Africa and Middle East Region. Adv Dent Res. 2015;27(1):10–7.
5. Faini D, Maokola W, Furrer H, Hatz C, Battegay M, Tanner M, et al. Burden of serious fungal infections in Tanzania. Mycoses. 2015;58:70–9.
6. Bongomin F, Gago S, Oladele RO, Denning DW. Global and multi-national prevalence of fungal diseases—estimate precision. J Fungi. 2017;3(4).
7. Jayachandran AL, Katragadda R, Thyagarajan R, Vajravelu L, Manikesi S, Kaliappan S, et al. Oral Candidiasis among Cancer Patients Attending a Tertiary Care Hospital in

Chennai, South India: An Evaluation of Clinicomycological Association and Antifungal Susceptibility Pattern. Can J Infect Dis Med Microbiol. 2016;2016:8758461. doi: 10.1155/2016/8758461

8. Bashir Sher-i-Kashmir G, Ahmad GMC Baramulla J. Oral Candida colonization and infection in cancer patients and their antifungal susceptibility in a tertiary care hospital. Int J Adv Res.2014;2 (5) 541-550

9. Epstein JB, Thariat J, Bensadoun R-J, Barasch A, Murphy BA, Kolnick L, et al. Oral complications of cancer and cancer therapy. CA Cancer J Clin. 2012;62(6):400–22.

10. Schelenz S, Abdallah S, Gray G, Stubbings H, Gow I, Baker P, et al. Epidemiology of oral yeast colonization and infection in patients with hematological malignancies, head neck and solid tumors. J Oral Pathol Med. 2011;40(1):83–9.

11. Suhail Lone D, Bashir D, Bali D, Sajad S, Bashir H, Ahmad D, et al. Oral Candida colonization and infection in cancer patients and their antifungal susceptibility in a tertiary care hospital. Int J Adv Res. 2014;2(5):541–50.

12. Mushi MF, Mtemisika CI, Bader O, Bii C, Mirambo MM, Groß U, et al. High Oral Carriage of Non-albicans Candida spp. among HIV-infected individuals. Int J Infect Dis. 2016 Aug;49:185-8. doi: 10.1016/j.ijid.2016.07.001

13. Mushi MF, Bader O, Bii C, Groß U, Mshana SE. Virulence and susceptibility patterns of clinical Candida spp. isolates from a tertiary hospital, Tanzania. Med Mycol. 2018 Oct 31. doi: 10.1093/mmy/myy107.

14. Kawashita Y, Funahara M, Yoshimatsu M, Nakao N, Soutome S, Saito T, et al. A retrospective study of factors associated with the development of oral candidiasis in patients receiving radiotherapy for head and neck cancer. 2018 Nov;97(44):e13073. doi: 10.1097/MD.0000000000013073

15. Gligorov J, Bastit L, Gervais H, Henni M, Kahila W, Lepille D, et al. Prevalence and

treatment management of oropharyngeal candidiasis in cancer patients: Results of the French candidoscope study. Int J Radiat Oncol Biol Phys. 2011;80(2):532–9.

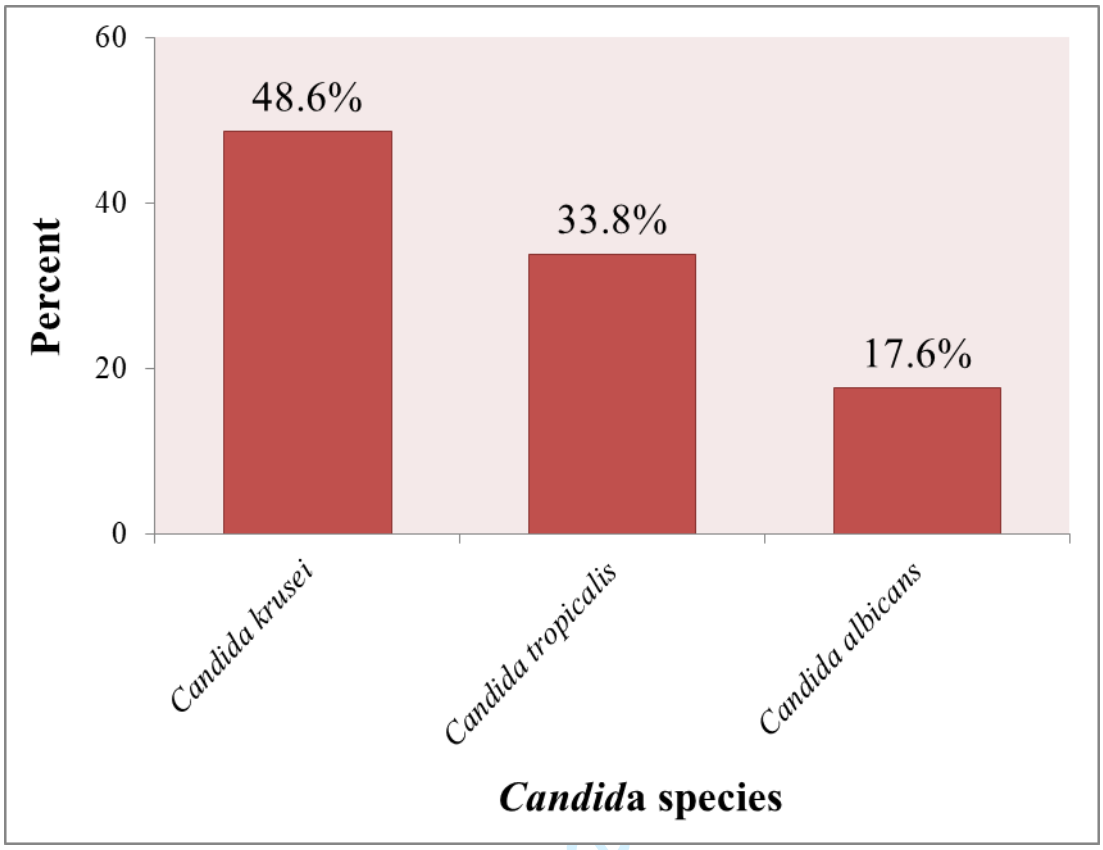
16. Al-Abeid HM, Abu-Elteen KH, Elkarmi AZ, Hamad MA. Isolation and characterization of Candida spp. in Jordanian cancer patients: Prevalence, pathogenic determinants, and antifungal sensitivity. Jpn J Infect Dis. 2004;57(6):279–84.

17. Main BE, Calman KC, Ferguson MM, Kaye SB, MacFarlane TW, Mairs RJ, et al. The effect of cytotoxic therapy on saliva and oral flora. Oral Surg Oral Med Oral Pathol. 1984 Nov;58(5):545-8. doi: 10.1016/0030-4220(84)90077-x.

18. Bensadoun RJ, Patton LL, Lalla R V., Epstein JB. Oropharyngeal candidiasis in head and neck cancer patients treated with radiation: update 2011. Support Care Cancer. 2011 Jun;19(6):737-44. doi: 10.1007/s00520-011-1154-4

19. Lalla R V., Latortue MC, Hong CH, Ariyawardana A, D'Amato-Palumbo S, Fischer DJ, et al. A systematic review of oral fungal infections in patients receiving cancer therapy. Support Care Cancer. 2010 Aug;18(8):985-92. doi: 10.1007/s00520-010-0892-z.

Figure 1: Distribution of *Candida* species isolates from cancer patients



The figure illustrates the distribution of specific *Candida* species isolates that were obtained from 196 cancer patients at Ocean road Cancer Institute (ORCI).

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Page 1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Pages 2 and 3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Page 4
Objectives	3	State specific objectives, including any prespecified hypotheses	Page 4
Methods			
Study design	4	Present key elements of study design early in the paper	Page 5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page 5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	Page 5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Page 6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Page 6
Bias	9	Describe any efforts to address potential sources of bias	Page 5
Study size	10	Explain how the study size was arrived at	Page 5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Pages 6 and 7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Pages 6 and 7
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	NA
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	NA
		(*e*) Describe any sensitivity analyses	NA
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	NA
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Pages 7 and 8
		(b) Indicate number of participants with missing data for each variable of interest	NA
Outcome data	15*	Report numbers of outcome events or summary measures	Pages 8 and 9
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Page 10
		(*b*) Report category boundaries when continuous variables were categorized	NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	Page 11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Page 12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	NA
Generalisability	21	Discuss the generalisability (external validity) of the study results	Pages 11 and 12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	NA

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.