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Prevalence and predictors of oral thrush among Maasai in Ngorongoro Conservation Area: A preliminary consideration

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Abstract

Background: It was reported by clinicians working in the Ngorongoro Conservation Area that about 40% of Maasai pregnant women reporting at the Antenatal Clinic were diagnosed with oral thrush. However, it was not immediately determined what factors predispose these women to oral thrush. This study aimed at finding out the prevalence of oral thrush in the Maasai women community and the identification of predisposing factors.

Methods: This was a cross sectional study done in the Ngorongoro Conservation Area among Maasai women of reproductive age (15–49 years). Convenience sampling technique was used to select participants from all participating villages. Participants were clinically examined for oral thrush lesions and a structured interview guide was used to collect information on age, parity, pregnancy status and history of breastfeeding. To explore the possible predisposing factors for oral thrush, anthropometric measurements including weight and height were taken and also blood samples were collected from participants and tested for HIV, syphilis and blood sugar. Multivariate logistic regression was used to obtain independent predictors of oral thrush.

Results: The prevalence of oral thrush among study participants was 32%. Oral thrush prevalence was significantly higher in non-pregnant than pregnant women ($p=0.000$). Amongst the non-pregnant women, oral thrush was significantly higher in breastfeeding than the non-breast feeding participants. Univariate logistic regression showed that non-pregnant women had higher odds of getting oral thrush compared to pregnant women [OR 21.8; (95% CI 9.6, 49.6)]. Amongst the non-pregnant women, breastfeeding women had increased odds

or higher chances of getting oral thrush compared to the non-breastfeeding women [OR 15.8; (95% CI: 7.2, 34.8)]. In the multivariate analysis, breastfeeding women adjusted odds ratio (AOR) 2.88; (95% CI: 0.99, 8.3) and non-pregnant women AOR 0.09; (95% CI: 0.03, 0.27). Two factors that predicted oral thrush are breastfeeding and not being pregnant.

Conclusions: From the results, breastfeeding non pregnant women were at a higher risk of developing oral thrush. Possible predisposing factors are discussed.

Key words: women, oral thrush, pregnancy, Tanzania, Maasai, Ngorongoro.

Background

Oral candidiasis (also known as “oral thrush”) is a fungal infection caused mainly by *Candida albicans* (*c.alb*) [1]. Reports of high incidences of oral thrush amongst pregnant Maasai women living in villages around the Ngorongoro Crater is of great concern and therefore calls for thorough investigation. It was reported by clinicians working in the community of Ngorongoro Conservation Area (NCA) that two out of five pregnant women (about 40%) develop oral thrush during the second trimester of their pregnancy . Both the prevalence of oral thrush within the Maasai community and the identification of predisposing factors are needed if this condition is to be controlled and/or eliminated.

Literature Review

The causative agent of oral thrush is *c.alb*, which is normally present in the oral cavity, digestive tract, genitourinary mucosal surfaces, and skin of normal humans, persists as commensal in up to 60% of healthy individuals [2, 3, 4]. D’Enfer (2009) [5] suggests that asymptomatic healthy carriage rates

ranged from 3 to 70% in cross-sectional studies but predicted significantly higher prevalence to be likely. Within healthy mucosal membranes, *c.alb* co-exists with highly diverse bacterial flora which helps to balance the growth [6]. Although *c.alb* is a normal flora in the body, a simple imbalance may result in overgrowth and potentiate infection. Such imbalances may include alterations in host immunity, bacterial flora, or local environmental factors, such as oral salivary flow. These factors may contribute to *c.alb* transitioning from a commensal to an opportunistic pathogen, capable of causing a wide range of superficial to invasive mucosal states to life-threatening systemic infections [7, 8].

A range of predisposing factors to invasive *c.alb* has been reported. Salivary contribution including reduced flow, glucose-richness, and acidity all promote *c.alb* adherence to human epithelial cells [9] and are predictive of high candida colonization, as is tobacco use [10]. Specific conditions such as diabetes mellitus [11], HIV infection, cancer, dry mouth, or pregnancy [12, 13], IgA deficiencies, disrupted/diseased oral epithelial condition [14, 15] iron and folic acid deficiencies [16] as well as Vitamin A [11],

Vitamin C [17] and zinc depletion [18] predispose individuals to *c.alb* colonization. There is also a literature suggesting that individuals with blood type O show increased susceptibility to oral thrush [19]. It is noted that there is a lack of recent research on nutrition an *c. alb* outside of opportunistic and comorbidities. Further, there are no known reports on prevalence of these predisposing factors in Maasai communities.

In this study we sought to the prevalence of oral thrush and to identify the possible predisposing factors associated with the Maasai culture or environmental conditions around NCA which may contribute to that prevalence rate.

Methods

Study area

This study was conducted in the NCA which is located west of Arusha City in the Crater Highlads area of Tanzania. Ngorongoro District has a population of 174,278 of which males are 82,610 and females 91,668 with 46,750 of these being women of reproductive age [12]. The majority of the population of Maasai live in the rural areas as pastoralists with primary dependence on their cattle for their livelihoods (00). They comprise approximately 45 % of the Ngorongoro District population (National Bureau of Statistics, 2012).

Ethics

Ethical approval was sought and obtained from the National Institute for Medical Research (NIMR) Tanzania, Certificate number NIMR/HQ/R.8a/Vol. IX/1474. Permission to conduct the study was also sought from Tanzania Wildlife Research Institute (TAWIRI) and also received from respective village/ward governments. All study participants provided a written informed consent.

Study Design

This cross-sectional study is part of a larger initiative entitled the "Mama Kwanza Socio-economic Health Initiative" which is undertaking an integrated, inter-sectoral approach to maternal, infant, and child health continuum in Arusha Region (which includes Ngorongoro District) in Tanzania. The present study considered the prevalence of oral thrush and its predictors among Maasai women of reproductive age (15-49 years) in the NCA. Data was collected in March 2013 involving target Maasai when living in or near one of five villages in the NCA, specifically Olbalbal, Alelayli, Misigiyo, Olorobi, and Makoromba.

Sample size

The sample size was calculated using the formula $= Z^2P(1-p) / d^2$ where p = anticipated proportion of population with oral thrush (40%); as per a study carried out in South India (Reshmi et al., 2012); d = Absolute precision (10%); (Confidence interval) I = 90% and Z = 1.645. As a result, the calculated sample size targeted 260 participants.

Sampling

A convenience sampling technique was used in which a recruitment call through 'word of mouth' was made in each participating village for all women of reproductive age with and without oral thrush. The call was made between 1900 – 2000h by a person appointed by the village leader using a loud speaker. This call was repeated for two consecutive days in each village. Participants were asked to gather at the Village Office (which is an administrative centre) on a given day. All women that came and agreed to participate in the study were enrolled as participants. These women were categorized into two mutually exclusive groups - pregnant and non-pregnant.

Interviews

A structured interview guide was used to collect information from participating women. Data on age, parity, pregnancy status, weight, height, and history of breastfeeding was collected from each participant. The age of the youngest child was estimated based on mother's memory, as this is not a typical demographic indicator in this community.

Clinical examination

Pregnancy status

For pregnant participants, a female medical doctor measured fundal height (in centimetres) to determine pregnancy age from twelve weeks gestation. For those with a pregnancy of less than twelve weeks, gestational age was calculated based on the woman's recall of her last normal menstrual period.

Oral thrush scoring

Oral thrush was determined through examination by an Assistant Medical Officer who used a clinical torch to observe the oral cavity. If an individual had oral thrush, a swab of the infected area(s) was taken. Swabs were preserved in a media until future analysis was possible. In each positive case, oral thrush infiltration was scored using a 0 to 4 scale as described by Wright et al., [2] (See **Table 1**). Those with confirmed oral thrush were prescribed

and provided with Nystatin tablets by the Assistant Medical Officer, with dosages dependent upon the severity of the condition.

Blood collection

Five millilitres of blood was collected from all participants who consented. The collected blood was used to test for some of the known predisposing factors to oral thrush. These included HIV sero status, blood glucose (diabetes mellitus), syphilis, and anaemia.

a) Serology for HIV status

HIV sero-status was determined using a rapid test called Alere Determine HIV-1/2 which is an immuno-chromatographic test for the qualitative detection of antibodies to HIV1/2[23]. A blood drop was applied to the sample pad and after a minute a drop of chase buffer was added to the sample pad. Results were read after 15 minutes.

b) Serology for Syphilis

Syphilis was tested by the "One Step Strip Style ANTI-TP Rapid Screen Test". This is a chromatographic immunoassay for the qualitative detection of antibodies to *Treponema Pallidum* human serum [24]. A blood drop was applied to the sample pad and after a minute a drop of buffer was added to the sample pad. Results were read after 15 minutes.

Table 1. Oral Thrush Scale.

Grade	Definition
0	No thrush
1	Painless white, curdy-like plaques in the throat area
2	White, curdy-like plaques in throat and spread over tongue
3	Severe thrush in throat, tongue with difficulty to swallow
4	Severe thrush in throat, over tongue, with ulcerations and bleeding and/or angular cheilitis, difficulty to swallow

c) Quantitation of Blood Glucose

A drop of blood was placed at either left or right side of the glucostrip which was pre-inserted in the Glucoplus™ machine (Glucoplus Inc., Canada). A reading appeared after 15 seconds showing the amount of blood glucose in mmol/L.

d) Determination of Haemoglobin Level

Haemoglobin was tested using a Hb 201+™ machine (HemoCue AB, Sweden). The individual participant's finger was pricked using a lancet device and a hemocue cuvette was filled with blood and inserted in the Hb 201+™ machine. Readings appear on the screen and report the haemoglobin in g/dL.

Anthropometric measurements

Anthropometric measurements (i.e., weight in kg and height in cm) were taken by one trained person using a SECA™ scale (Seca GmbH & Co. KG, Germany).

Data Analysis

Data was analysed using Predictive Analytical Soft Ware (PASW) Version 20. Descriptive statistics (i.e., frequency, cross tabulation) were used to summa-

rize the data. Chi square test was used to reveal association between variables. Logistic regression was performed to control for confounding variables and all factors that had a p value < 0.05 in the binary/univariate logistic regression were included in the multivariate analysis in order to identify independent predictors of oral thrush.

Results

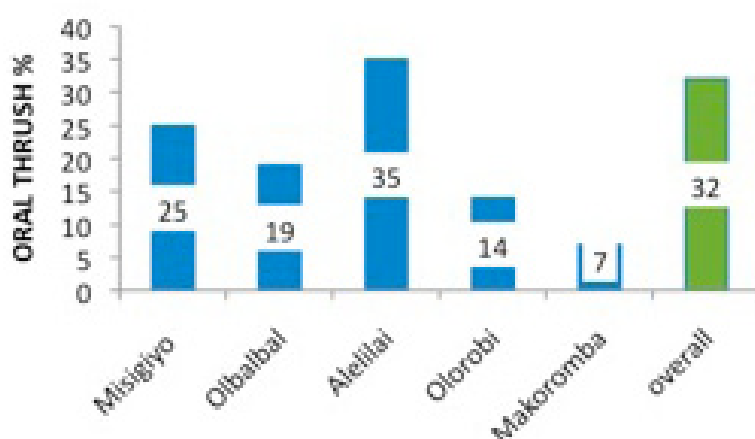
Demographics

In total, 181 women of reproductive age (15-49 years) participated in the study and were included in this analysis. The majority of participants were between 15 and 34 years of age.

Prevalence

The number of participants with oral thrush was 57 (32%). Oral thrush prevalence ranged between 7% to 35% in Makoromba and Alelilai villages, respectively. Pearson's chi-square test results revealed a statistically significant difference in oral thrush occurrence among villages (p value =0.022).

Figure 1. Prevalence of oral thrush in villages.



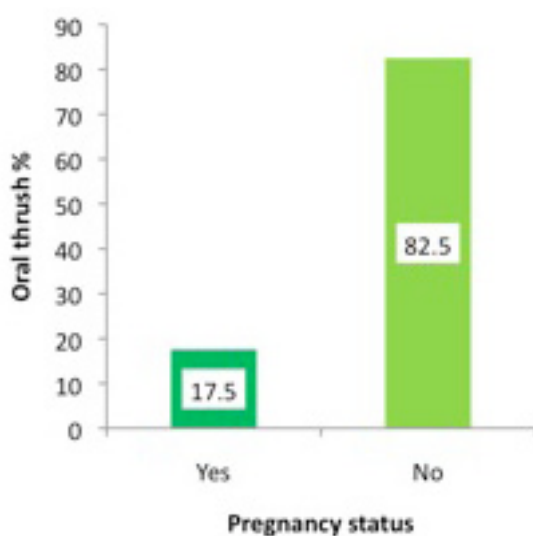


Figure 2. Oral thrush prevalence by pregnancy status.

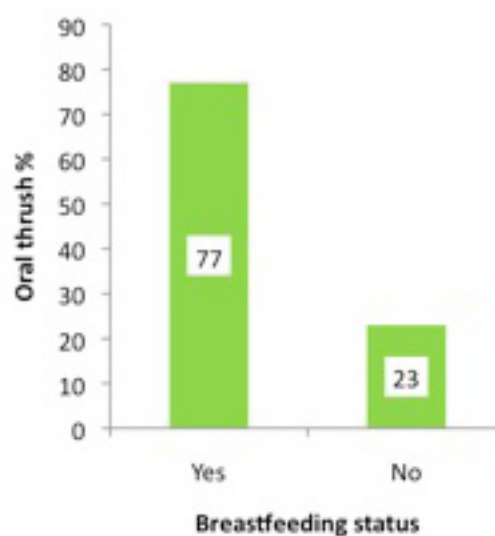


Figure 3. Oral thrush prevalence by breastfeeding status.

Predisposing factors

a) Pregnancy status

The prevalence of oral thrush was higher in non-pregnant as compared to pregnant women ($p=0.000$). Univariate logistic regression showed that non-pregnant women had higher odds of getting oral thrush compared to their pregnant counterparts [OR 21.8; (95% CI 9.6, 49.6)].

However, within the non-pregnant group, the prevalence of oral thrush was significantly higher in those women who were breastfeeding as compared to those not breastfeeding ($p=0.000$). Further analysis showed that breastfeeding women had increased odds of getting oral thrush compared to the non-breastfeeding women [OR 15.8; (95% CI: 7.2, 34.8)].

Pregnant women were also differentiated based on their pregnancy stage. Oral thrush was seen to increase with advancement in pregnancy, peaking during lactation and dropping in non-breastfeeding women (**Figure 4**). Chi square tests showed a sig-

nificant difference in oral thrush occurrence in the pregnancy intervals (i.e., first trimester to after lactation) at $p=0.000$.

Among breastfeeding women, parity was assessed (see **Figure 5**), but no significant difference ($p=0.919$) was found between parity and oral thrush occurrence.

b) HIV sero-status and Syphilis

HIV sero-status and syphilis are other predisposing factors for oral thrush considered in this study. Test results for these were all negative showing no association with oral thrush occurrence in this sample.

c) Quantification of blood glucose

Results showed that the prevalence of oral thrush did not differ by blood glucose levels ($p=0.606$).

d) Anaemia

Results showed that anaemia status had no significant association ($p = 0.769$) with oral thrush prevalence in this sample.

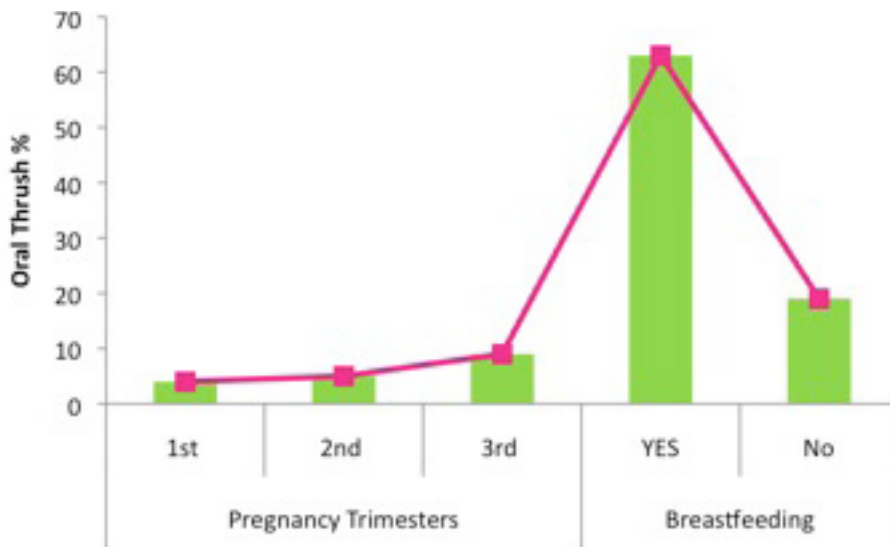


Figure 4. Oral thrush trend.

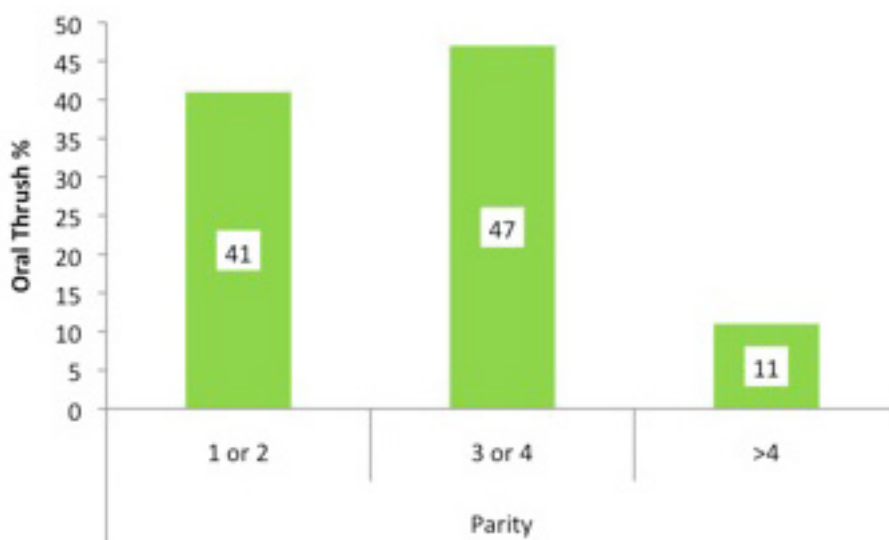


Figure 5. Oral thrush and parity among breastfeeding women.

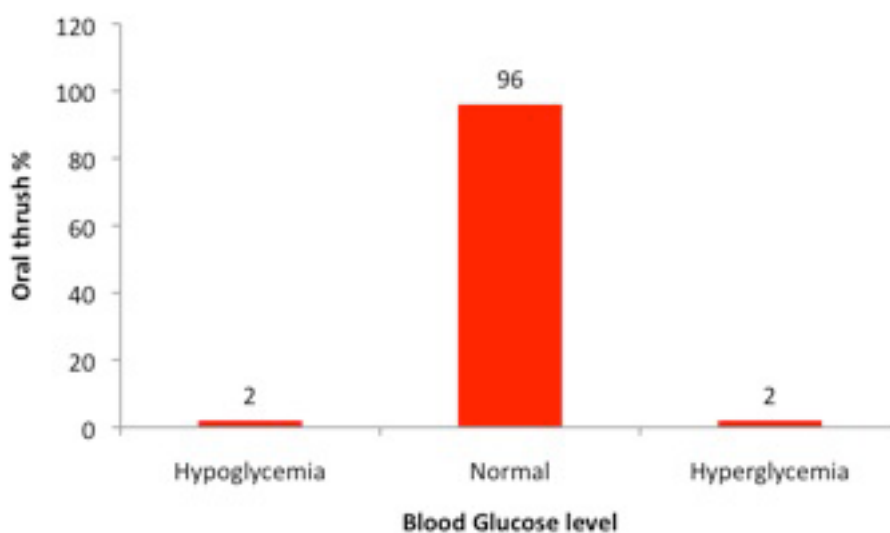
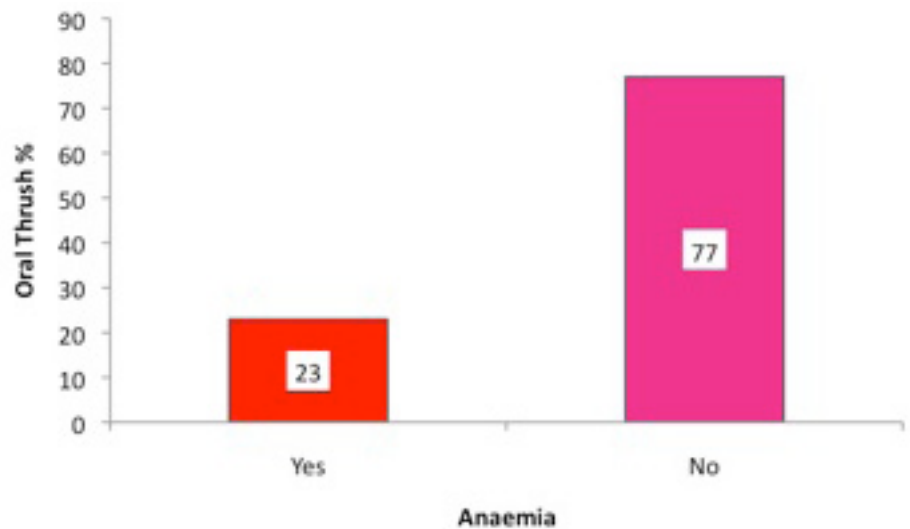


Figure 6. Blood glucose in participants with oral thrush.

Figure 7. Oral thrush prevalence by anaemia status.



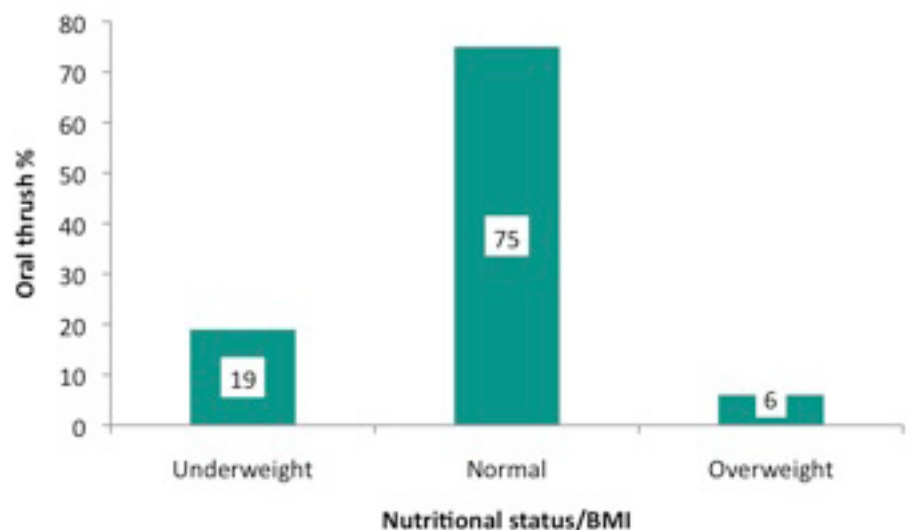
e) Being Frail/Thin

Results on assessment of nutritional status based on Body Mass Index (BMI) showed no significant association ($p=0.797$) between oral thrush and nutritional status (**Figure 8**). It is noted that this was only considered in non-pregnant women as the BMI is not corrected for the pregnant state.

Multivariate Analysis

In the multivariate analysis, breastfeeding status and non-pregnancy state were the independent predictors of oral thrush with adjusted odds ratio (AOR) of 2.88; (95% CI: 0.99, 8.3) and women AOR 0.09; (95% CI: 0.03,0.27) respectively.

Figure 8. Oral thrush prevalence by Body Mass Index in non pregnant women.



Discussion

This study revealed the prevalence of oral thrush and identified predisposing factors for the condition in Maasai community living in NCA.

Oral thrush occurred in 32% of our sample as compared to reported anecdotal observations of 40%. This difference was interpreted as incidental as previous reports were estimates without actual recording or retrospective reviews of oral thrush cases. This is the first report on oral thrush for participants who are not HIV positive in Tanzania. The prevalence of oral thrush found in this study (32%) is higher than what was reported by other studies related to immuno-compromised individuals, such as 25% [25] and 23.5% [26] among HIV infected persons in Zambia and Tanzania respectively. Considering that no participant was found to be HIV positive in our study, this clearly leads to a re-consideration of an underlying presumption of a serious predisposing factor.

Prevalence was higher in two of the five villages, specifically, Alelayli and Misigiyo. There is some question as to whether villages that have access to health services may have lower prevalence of oral thrush. For example, Olbalbal has a dispensary and had low prevalence. Other factors to be considered include differences in environmental condition and/or dietary practices in each community, which will be considered in the ongoing research.

Results from this study showed that the 'usual suspects' (i.e., HIV, anaemia, diabetes, syphilis and being frail or undernourished) were not associated with oral thrush in these communities. This finding suggests that oral thrush among Maasai women in NCA may have a unique profile or pre-dispositional factors yet to be revealed. It is possible these may be the unique Maasai cultural practices or the ecology of Ngorongoro.

Our results show that two factors - non-pregnancy and breastfeeding - were the predictors of oral thrush amongst Maasai women in Ngorongoro. Oral thrush was significantly higher in Alelayli than other villages positing that location was a potential predictor. However, this was negated by the fact that the prevalence of breastfeeding oral thrush women in this village was not significantly higher than in other villages.

Oral thrush was more prevalent in non-pregnant women who were breastfeeding in this study. Prevalence increased progressively with advancing pregnancy and peaked after birth during breastfeeding period. A possible reason for the high prevalence of oral thrush during breastfeeding period may relate to poor nutritional status of pregnant women due to fear of birth complications associated with overweight babies. It is a cultural practice within Maasai communities for pregnant women to only be allowed to eat once per day a diet which excludes foods rich in protein like milk, meat, and beans. Furthermore, pregnant women are served half a ration in the second trimester stage (between 4-6 months) and are made to vomit, using oil extracted from sheep fat [27]. These practices may cause cumulative negative energy balance and/or deficiency of essential nutrients potentially resulting in increased susceptibility to oral thrush. This deficit may be further exacerbated by higher nutritive demands of lactation [28] which precipitates increased oral thrush during breastfeeding.

In this study, most breastfeeding oral thrush patients had normal BMIs suggesting that they are not undernourished. However, during breastfeeding there is an increase in specific nutrient requirements for vitamins A, B₆, and C, and for iodine and zinc by more than 50% [29]. As stated earlier, oral thrush can be precipitated by deficiency specific micronutrients in the body such as iron and folic acid and select vitamins. Hence, we at this point

postulate that lactation may be depleting important micronutrients, especially if there is lower or no replenishment from food or environment, therefore predisposing this group of mothers to oral thrush.

The ecology of the NCA may also render breastfeeding mothers susceptible to oral thrush. Traditionally, Maasai in Ngorongoro and elsewhere were dependent on livestock for food and income [30]. However, because of drought and diseases, the numbers of livestock declined to a level that cannot sustain their livelihoods. As a result, many Maasai have turned to cultivation of crops such as maize in order to supplement their food [31]. However, the Maasai living in NCA are not allowed to cultivate crops or vegetables as they live in conservation area [32]. They must therefore purchase food shipped from elsewhere, which is not affordable for many in light of their income status [33]. So, the issue is of multiple exposures and factors, such as food insecurity, lack of nutritious foods, lower food consumption, and increased nutrient demand during lactation, may create susceptibilities to opportunistic infections including oral thrush.

Conclusion

Results from this study indicate that breastfeeding Maasai mothers living in NCA are at higher risk of suffering from oral thrush. It is hypothesized that food insecurity in the NCA and the cultural Maasai practices of reducing food serving to pregnant mothers may reduce the ability of breastfeeding mothers to replenish the essential nutrient reserves due to lactation demands hence become susceptible to oral thrush. Further studies are needed to assess levels of specific micronutrients in lactating mothers.

Acknowledgements

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