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Elucidating the Role of Hyposalivation and Autoimmunity in Oral Candidiasis

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Abstract

Introduction—Oral candidiasis (OC) is a potential oral complication in Sjögren's Syndrome (SS). Some studies indicate that the low stimulated salivary flow and not low unstimulated salivary flow is associated with OC in SS, while others report that the underlying autoimmune disorders contributes to OC, based solely on correlation coefficients. Given the conflicting and limited existing evidence, we purposed to ascertain the role of both salivary gland dysfunction (hyposalivation based on unstimulated and stimulated flow rates) and autoimmunity (SS, other autoimmune disorders) in OC among those with SS, other salivary gland dysfunction and non-salivary gland dysfunction controls (NSGD).

Methods—A nested case-control study was designed within a larger NIH/NIDCR cohort. Descriptive analyses, non-parametric tests, comparative analyses, and multivariate logistic regression analyses were undertaken.

Results—Data on 1,526 subjects (701 SS, 247 ISS, 355 Sicca, and 223 NSGD) were obtained from the source cohort of 2,046 and analyzed for this current study. The median whole unstimulated salivary flow rate (WUS, ml/15min) was lower in SS (0.8, interquartile range (IQR) 1.8) compared to ISS (5.5, IQR 5.2, $p<0.001$) and NSGD (3.8, IQR 3.8, $p<0.001$) but comparable with that of Sicca (1.0, IQR 1.5, $p=0.777$) participants. The median total stimulated salivary flow rate (TSS, ml/15min) was lowest in SS (7.0, IQR 12.4, $p<0.001$) compared to other groups. Of the 45 OC cases in this cohort, 71.1% ($n=32$) were from the SS group. The prevalence of OC was highest in the SS group (4.6%, $p=0.008$). SS group had twice the risk of OC than NSGD (OR =2.2, 95%CI: 1.1–4.2, $p=0.02$) and Sicca (OR =2.2, 95%CI: 1.0–4.8, $p=0.03$), adjusting for confounders; hyposalivation [WUS (OR=5.1, 95%CI: 2.5–10.4, $p<0.001$), TSS (OR=1.9, 95%CI: 1.0–3.5, $p=0.04$)], history of other autoimmune disorders (OR=4.4, 95%CI: 1.7–11.3, $p=0.002$), medications for extraglandular manifestations (OR=2.3, 95%CI: 1.1–4.9, $p=0.03$), and diabetes mellitus (4.2, 95%CI: 1.2–15.2, $p=0.02$) were independent predictors of OC; females had a lower risk than males (OR =0.29, 95%CI: 0.13–0.67, $p=0.004$). Age, race, anti-SSA/SSB autoantibodies,

focus score, other medications, anxiety, fatigue, cigarette smoking, alcohol, and caffeine use were not associated with oral candidiasis.

Conclusion—Salivary gland dysfunction (hyposalivation with WUS being a stronger predictor than TSS) and autoimmunity (SS, other autoimmune disorders, medications i.e., DMARDS) are both independent predictors of OC. Diabetes mellitus is an independent predictor of OC among those with salivary gland dysfunction. Our findings suggest that these independent predictors should be considered in the prevention and management of OC in this population.

Introduction

The prevalence of oral Candida carriage in the normal population has been estimated to range from 23% to 68% and 68% to 100% among Sjögren's syndrome (SS) patients (Tapper-Jones et al., 1980, Radfar et al., 2003, MacFarlane & Mason, 1974, Lundstrom & Lindstrom, 1995, Soto-Rojas et al., 1998, Hauman et al., 1993). Studies have attributed the higher prevalence of oral Candida carriage in Sjögren's Syndrome to hyposalivation. The study by Radfar et al on 103 SS patients found that a low stimulated salivary flow rate and not a low unstimulated salivary flow rate was negatively correlated with oral Candida load ($\Gamma = -0.47$, $p = 0.0001$) (Radfar et al., 2003). On the other hand, the study by Navazesh et al on 100 ambulatory subjects, not limited to SS patients, reported that unstimulated whole ($\tau_c = -0.31$, $p < 0.001$) and not stimulated parotid ($\tau_c = -0.03$, $p > 0.05$) flow rate was negatively correlated with oral Candida load (Navazesh et al., 1995). Furthermore, some studies have not found a correlation between Candida colony-forming unit (CFU) counts and whole unstimulated or stimulated salivary flow rates in the carriage state (where, CFU/ml count 400 or 500) or among healthy subjects, but have found a negative correlation between these parameters only in the state of infection (where, CFU/ml count > 400 or > 500) (Torres et al., 2002, Navazesh et al., 1995). This was further confirmed by the nonlinear association between CFU count and salivary flow rate reported in studies (Tapper-Jones et al., 1980, Torres et al., 2002).

Positive correlations between CFU and autoantibodies anti-SSA ($\Gamma = 0.27$; $p = 0.05$) and anti-SSB ($\Gamma = 0.42$; $p = 0.002$), IgG ($\Gamma = 0.42$; $p = 0.002$), and focus score ($\Gamma = 0.33$; $p = 0.02$) have been reported (Radfar et al., 2003). Studies have not reported or found an association between fungal load and gender or age (Torres et al., 2002). Furthermore, studies have solely attributed the higher prevalence of oral candidiasis in autoimmune disorders such as SS to hyposalivation. The study by Almstah et al counters this premise with the findings that the underlying autoimmune dysfunction is associated with the changes in fungal load and not hyposalivation (Almstah et al., 2003).

Given the heterogeneity of findings reported in the literature and the ambiguity over predictors of oral candidiasis, we focused not on oral Candida carriage but rather on infection and sought to investigate the following questions: (1) Are hyposalivation and autoimmunity independent predictors of oral candidiasis among SS patients? (2) What are the other independent predictors of oral candidiasis among SS patients? (3) Is low unstimulated or stimulated salivary flow rate associated with oral candidiasis among SS patients? Findings were compared with three comparison groups: (i) non-salivary gland

dysfunction controls (NSGD); those with other salivary gland dysfunction namely (ii) Sicca and (iii) Incomplete SS (ISS).

Methods

Study Population

The NIDCR Sjögren's Syndrome and Salivary Gland Dysfunction Unit (SSGDU) has been evaluating and enrolling participants with clinical suspicion of SS or salivary gland dysfunction since 1984, making the NIDCR/NIH SS cohort one of the largest in the US. To investigate the proposed objectives we designed a nested case-control study within the NIDCR/NIH source cohort (N=2,046). All participants with SS, Sicca, ISS, and NSGD enrolled before August 27, 2015 within the source cohort were included in the nested case-control study (N=1,526). Sjögren's Syndrome was defined according to the American-European consensus group classification criteria (AECG) (Vitali et al., 2002). Sicca was defined as the presence of oral dryness determined by a whole unstimulated salivary flow rate (WUS) of ≤ 1.5 ml/15min or ocular dryness determined by a Schirmer's test of ≤ 5 mm/5 minutes without anesthesia or Van Bijsterveld score of ≤ 4 in at least one eye, with the absence of focal lymphocytic sialadenitis (focus score < 1 per 4mm²) and the absence of anti-SSA(Ro) and anti-SSB (La) autoantibodies. Incomplete Sjögren's syndrome (ISS) was defined as the presence of a focus score of ≥ 1 per 4mm² or autoantibodies SSA or SSB but not meeting the AECG criteria for SS classification. Non-salivary gland dysfunction controls included healthy volunteers over 18 years of age without any systemic disease other than controlled hypertension or hyperlipidemia and those who are non-SS, non-Sicca and non-ISS without fulfilling any of the SS classification criteria.

Study Procedures

A comprehensive oral and medical history was recorded. Head and neck and oral examination, and physical examination were performed. Salivary gland function evaluation (unstimulated followed by stimulated salivary flow rates), labial minor salivary gland biopsy, assessment of lacrimal function and standard eye examinations were performed, following standard operating procedures (SOPs) to maintain consistency in the procedures and data collection. Clinical laboratory studies were done to test the general health of the participant and to assess for autoimmune markers. Testing for hepatitis B and C, and HIV was also undertaken. Information from all procedures gathered at enrollment was used in our analyses. All study participants provided Informed Consent prior to the initiation of any study procedures and clinical protocols were approved by the NIH Institutional Review Board (84-D-0056, 1984; 94-D-0018, 1994; 99-D-0070, 1999; 11-D-0172, 2011; 15-D-0051, 2015) conforming to the standards indicated by the Declaration of Helsinki.

Main Exposure Variables—Hyposalivation was defined as whole unstimulated salivary flow rate (WUS) of ≤ 1.5 ml/15min (Vitali et al., 2002). Hyposalivation based on total stimulated salivary flow rate (TSS), which is the sum of parotid and submandibular/sublingual stimulated salivary flow was defined as TSS of ≤ 7.5 ml/15 min. (Manuel Ramos-Casals., 2012).

Main Outcome Variables—Oral candidiasis was defined as clinically diagnosed oral candidiasis. Culture growths by the semiquantitative 4-quadrant method were quantified as: 1 colony, scant (2 colonies), light (3–10 colonies), moderate (>10 colonies primarily in the first two quadrants), and heavy (>10 colonies into the third or fourth quadrants) growth. In addition, direct smear by Gram stain or wet-mount preparation for the presence of fungi was undertaken.

Covariates—Demographic data on age, gender, race, and ethnicity of participants were collected. History of autoimmune disorders apart from SS, diabetes mellitus type I and II was ascertained. Current use of medications was categorized into five mutually exclusive categories: i) medications that reduce salivary flow, ii) sialagogues, iii) medications for extraglandular manifestations, iv) combination of medications of the three categories, and v) other medications. In addition, current use of antifungal medications was ascertained. Cigarette smoking, alcohol use, and caffeine use status was defined as any use of cigarettes, any alcoholic drinks per week, and one or more caffeinated beverages per day respectively.

Statistical Analysis—Descriptive data analysis was performed using standard summary statistics. Exploratory data analyses were performed on all variables of interest, including checks for normality (Shapiro Wilk test). Non parametric tests (Wilcoxon rank-sum test) were employed when indicated. Comparisons of proportions were tested with Fisher exact and Pearson's Chi-squared tests. Multivariate logistic regression models were constructed after checking for collinearity and forward stepwise selection determined the final model. Statistical significance was assessed on a set two-tailed p-value of 0.05. All statistical analyses were performed using STATA 14.0 (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP).

Results

Information from 701 SS, 247 ISS, 355 Sicca, and 223 NSGD participants were analyzed. Table 1 presents the demographic characteristics of these groups. The age of participants ranged from 7–92 years with the median age falling into the 4th and 5th decade of life. Females, whites, and non-Latino/Hispanic ethnicity were significantly more prevalent than males, other races and ethnicities.

Figure 1 illustrates the unstimulated and stimulated salivary flow rates among groups. The median WUS of the SS group (0.79, interquartile range (IQR) 1.76) was significantly lower than that of the ISS (5.49, IQR 5.17, $p<0.001$) and NSGD (3.83, IQR 3.84, $p<0.001$) groups but comparable with that of the Sicca group (1.00, IQR 1.53, $p=0.777$). The median WUS of the Sicca group was also significantly lower than that of the ISS and NSGD groups ($p<0.001$). While the median WUS of the ISS and NSGD groups were comparable ($p=0.102$). When TSS was compared across groups, it was found that the median TSS was significantly lowest in the SS group (7.03, IQR 12.36) compared to Sicca (10.78, IQR 11.47, $p<0.001$), ISS (16.46, IQR 13.94, $p<0.001$) and NSGD (14.81, IQR 12.86, $p<0.001$) groups. Sicca group had a significantly lower median TSS than that of ISS and NSGD groups ($p<0.001$). The median TSS was comparable between ISS and NSGD groups ($p=0.823$).

There were 45 cases of clinically diagnosed oral candidiasis among the study participants (Table 2). A significant proportion of these oral candidiasis cases had SS (71.1%, 95% CI: 55.69%–83.63%), while a smaller proportion of the cases came from the Sicca (15.6%, 95% CI: 6.49%–29.46%), ISS (8.9%, 95% CI: 2.48%–21.22%), and NSGD (4.4%, 95% CI: 0.54%–15.15%) groups ($p=0.008$). The prevalence of oral candidiasis in the SS group was also the highest compared to the other groups (SS: 4.6%, 95% CI: 3.14%–6.38%; Sicca: 2.0%, 95% CI: 0.80%–4.02%; ISS: 1.6%, 95% CI: 0.44%–4.09%; NSGD: 0.9%, 95% CI: 0.11%–3.20%; $p=0.008$). When stratified by the type of oral candidiasis, in the SS group there were 13 cases of pseudomembranous candidiasis, 9 cases of erythematous candidiasis, and 16 cases of other types of candidiasis. Although the SS group had a higher proportion of each type of oral candidiasis compared to the other groups, it was not of statistical significance given the low number of cases in each cell.

C. albicans (24 cases, 18/24 from SS group), *C. tropicalis* (2 cases, 2/2 from SS group), *C. glabrata* (5 cases, 4/5 from the SS group), and *C. lambica* (1 cases, 1/1 from SS group) were isolated from oral rinse specimens of oral candidiasis cases. Not every case of clinically diagnosed oral candidiasis had specimens sent for isolation of fungi. Therefore, only 32 of the 45 cases of oral candidiasis had candidiasis species identified. The 4-quadrant method indicated that in the SS group, 52% ($n=13$) had moderate growth, 36% ($n=9$) had severe growth, 8% ($n=2$) had one colony and 4% ($n=1$) had scant growth (Supplementary Content, Figure 1). There was only one case of heavy growth in the Sicca group, two cases of moderate growth in the ISS group, and one case of moderate growth in the NSGD group. A statistical comparison of fungal load among groups was not possible due to a low number of cases.

Table 3 presents the independent predictors of oral candidiasis selected by the forward selection stepwise logistic regression models. In Model 1, after adjusting for significant confounders, it was found that females were less likely to have oral candidiasis compared to males (OR: 0.29, 95% CI: 0.13–0.67, $p=0.004$). Race was not an independent predictor of oral candidiasis. Although the Black or African American race had a lower risk of oral candidiasis compared to the White race it was not of statistical significance (OR: 0.32, 95% CI: 0.09–1.12, $p=0.074$). Those with SS were twice as likely as NSGD to have oral candidiasis (OR: 2.15, 95% CI: 1.11–4.18, $p=0.023$). Those with hyposalivation based on WUS were five times more likely to have oral candidiasis compared to those without WUS hyposalivation (OR: 5.11, 95% CI: 2.50–10.45, $p<0.001$). Further, when WUS was modeled as a continuous variable, it was found that the risk of oral candidiasis decreased by 39% for every unit (1ml/15min) increase in WUS (OR: 0.61, 95% CI: 0.46–0.82, $p=0.001$). Also, those with hyposalivation based on TSS were twice as likely to have oral candidiasis compared to those without TSS hyposalivation (OR: 1.89, 95% CI: 1.01–3.53, $p=0.047$). The risk of oral candidiasis decreased by 6% for every unit (1ml/15min) increase in TSS, when TSS was modeled as a continuous variable (OR: 0.94, 95% CI: 0.90–0.98, $p=0.006$). Those with a history of other autoimmune disorders were four times as likely as those without such a history to have oral candidiasis (OR: 4.45, 95% CI: 1.75–11.30, $p=0.002$). Finally, those on medications for extraglandular manifestations were twice as likely to have oral candidiasis as those not on any medications (OR: 2.31, 95% CI: 1.08–4.94, $p=0.031$).

In Model 2, those with SS had twice the risk of oral candidiasis than those with Sicca (OR: 2.23, 95% CI: 1.05–4.76, $p=0.037$). The other independent predictors of oral candidiasis were: gender with females having a lower risk than males (OR: 0.33, 95% CI: 0.13–0.87, $p=0.024$); hyposalivation based on WUS (OR: 3.52, 95% CI: 1.66–7.49, $p=0.001$) and TSS (OR: 2.06, 95% CI: 1.08–3.95, $p=0.029$); when modeled as continuous variables, there was a 40% reduction in the risk of oral candidiasis for every unit (1ml/15min) increase in WUS (OR: 0.60, 95% CI: 0.41–0.87, $p=0.007$) and a 7% reduction in the risk of oral candidiasis for every unit (1ml/15min) increase in TSS (OR: 0.93, 95% CI: 0.89–0.98, $p=0.009$); history of other autoimmune disorders (OR: 3.63, 95% CI: 1.29–10.22, $p=0.014$); diabetes mellitus (OR: 4.24, 95% CI: 1.18–15.22, $p=0.027$); medications for extraglandular manifestations (OR: 2.15, 95% CI: 0.99–4.68, $p=0.054$).

In Model 3, SS group was compared to ISS group. The risk of oral candidiasis did not differ between the two groups when adjusted for other confounders. However, the risk of oral candidiasis was dependent on hyposalivation and diabetes mellitus. Those with hyposalivation based on WUS were twice as likely to have oral candidiasis as those without WUS hyposalivation (OR: 2.41, 95% CI: 1.09–5.31, $p=0.029$) with a 38% reduction in the risk of oral candidiasis for every unit (1ml/15min) increase in WUS (OR: 0.62, 95% CI: 0.42–0.90, $p=0.013$). Also, those with hyposalivation based on TSS were twice as likely to have oral candidiasis as those without TSS hyposalivation (OR: 2.38, 95% CI: 1.16–4.87, $p=0.018$) with a 7% reduction in the risk of oral candidiasis for every unit (1ml/15min) increase in TSS (OR: 0.93, 95% CI: 0.88–0.98, $p=0.012$). Furthermore, among those with SS or ISS, those with diabetes had a 7-time higher risk of oral candidiasis (OR: 6.73, 95% CI: 1.52–29.79, $p=0.012$) controlling for hyposalivation and other confounders.

In all three multivariable logistic regression models covariates such as, age, race, anti-SSA/SSB autoantibodies, focus score, medications that reduce salivary flow, sialagogues, combination of medications, other medications (which included antibiotics), antifungal medications, anxiety, fatigue, cigarette smoking, alcohol, and caffeine use were not significant predictors of oral candidiasis.

Discussion

Oral candidiasis in our study population was most prevalent among those with SS. We defined oral candidiasis as clinically diagnosed oral candidiasis rather than a certain cutoff of *Candida* colony count. This was because studies had shown an association between low salivary flow rate and CFU only above a cutoff threshold of > 400 or 500 colonies per ml and not below this cutoff. Further, it is implausible to have an infection of oral candidiasis with a CFU of < 400 or 500/ml. This is reiterated by the findings from this study, where *Candida* growth, quantified by the 4-quadrant method, was of moderate to heavy growth in the SS group.

In this study we modeled hyposalivation based on both unstimulated (WUS) and stimulated (TSS) salivary flow rates as there was heterogeneity in correlation between these parameters and *Candida* load. We found that hyposalivation based on both WUS and TSS was significantly associated with oral candidiasis, after adjusting for potential confounders.

These findings are consistent with the underlying rationale that the protective properties of saliva are diminished in a state of reduced salivary flow or hyposalivation. The fungicidal property of histatins, particularly histatin 5 present in saliva has been well documented (Xu et al., 1991). Histatins have been found to be potent fungicidals even against azole-resistant *Candida* species due to their unique mechanism of action on the mitochondria (Kavanagh & Dowd, 2004). Furthermore, for invasion of mucosal lining to occur, adhesion and colonization of *Candida* spp. to the mucosal epithelial surface needs to first occur. The fungi must overcome the protective properties of saliva which include mechanical washing away of desquamated surface epithelial cells with yeast colonies (Glick, 2015). The buffering capacity of saliva is another protective feature as low pH facilitates the growth of *Candida* species (Young et al., 1951) and salivary flow rate is positively associated with salivary pH (Jenkins, 1960).

Further, hyposalivation based on WUS was a stronger predictor than hyposalivation based on TSS. Whole unstimulated saliva which coats the oral mucosa and responsible for the maintenance of oral tissues, reflects resting flow; while stimulated saliva, secreted in response to a stimulus, reflects functional flow. The inherent difference in salivary flows could explain their differences in risk of association with oral candidiasis. For example, it is known that the majority of WUS contributed by the submandibular/sublingual glands is rich in mucins compared to the serous quality of TSS secreted by the parotids (Jenkins, 1978). Mucins are responsible for the lubrication and protection of oral tissues. The oral defense mechanism of mucins has been attributed to their selective control on fungal adhesion and direct killing capacity via pore formation and intracellular targeting (Bobek & Situ, 2003, Satyanarayana et al., 2000, Situ & Bobek, 2000). Therefore, the role of mucins could explain the stronger predisposition of reduced WUS, than reduced TSS, to oral candidiasis.

We also found that the history of other autoimmune disorders was significantly associated with oral candidiasis, after adjusting for hyposalivation, SS, and other confounders. In turn, SS was found to be an independent predictor of oral candidiasis, after adjusting for hyposalivation, history of other autoimmune disorders, and other confounders. Those with SS had a higher risk of oral candidiasis compared to NSGD controls. In addition, the SS group had a higher risk of oral candidiasis compared to the Sicca group, although the median WUS were comparable between these two groups. These findings indicate that low salivary flow is not the only predisposing factor to oral candidiasis and that this autoimmune syndrome by itself is associated with oral candidiasis. The biologic plausibility may be explained by the dysfunctional immune system prevalent in an autoimmune condition that ineffectively prevents *Candida* infections. Studies have shown that immunocompromised individuals succumb to these opportunistic fungal pathogens resulting in local or systemic infections (Lagunes & Rello, 2016, Ayatollahi Mousavi et al., 2016, Jivan & Meer, 2016). *Candida* spp. forming the normal oral mycobiome, causes infection in the host when the intricate balance of host-commensal is disrupted (Lagunes & Rello, 2016). Defective cytokine response, phagocytic function of neutrophilic granulocytes and macrophages, T-cell-mediated immunity, B-lymphocyte antibody response, SIgA, and complement function lead to infection by *Candida* spp. (Lagunes & Rello, 2016, Conti & Gaffen, 2015, Huppler et al., 2012, Glocker & Grimbacher, 2010, Glick, 2015, Chilgren et al., 1967, Steensma et al.,

2000, van der Wielen et al., 2016). Hence, a dysfunctional immune response inherent in an autoimmune syndrome renders it a risk factor for oral candidiasis.

The SS and ISS groups, which both have an autoimmune component, when compared did not differ in their risk for oral candidiasis. However, it was found that hyposalivation (based on WUS and TSS) and diabetes mellitus were significantly associated with oral candidiasis, irrespective of SS or ISS status, adjusting for confounders. Diabetes mellitus was an independent predictor of oral candidiasis among those with salivary gland dysfunction, namely SS, Sicca and ISS.

Medications for extraglandular manifestations of SS include the classification of disease-modifying antirheumatic drugs (DMARDs) including glucocorticoids, immunosuppressives, as well as biological agents, NSAIDs, and hormone replacement therapy (HRT). Although DMARDs may not be the mainstay treatment in SS, many of the DMARDs have a direct influence on the immune system. Therefore, it was not surprising to find that this category of medications was an independent predictor of oral candidiasis in the model with NSGD controls. However, these medications were not significant in the models limited to salivary gland dysfunction groups, indicating that the other predictors in models 2 & 3 had a stronger influence on the outcome, oral candidiasis.

In the multivariable regression modeling which adjusts for confounding, we did not find a significant association between age, race, anti-SSA/SSB autoantibodies and focus score with oral candidiasis. Since auto-antibodies SSA/SSA and focus score were not significantly associated with oral candidiasis albeit SS was an independent predictor, it could be possible that the serology and focus score variables were captured by the SS status in the multivariate modeling.

We chose to study clinically diagnosed oral candidiasis and its risk factors and therefore excluded the non-disease causing colonized state. Hence, CFU counts were not imperative for our study objectives. However, the lack of CFU counts limits the comparability to studies that report CFU counts and correlations with salivary flow rates. The semiquantitative 4-quadrant method that quantifies *Candida* growth reported in this study provides insight into the *Candida* load among SS participants, although not useful for comparison with other published studies. On the other hand, this study is the first to report associations between oral candidiasis and salivary flow rates, autoimmunity, and other risk factors, adjusting for the confounding effect of each individual predictor. Such effect measures are more statistically powerful than simple correlation coefficients reported by prior studies. Therefore, this study is not only a comprehensive synthesis of individual predictors of oral candidiasis reported in studies but also resolves some of the heterogeneity of findings found in the literature. Therefore, this study provides information that exceeds the purpose of comparability with other studies.

In this study, the prevalence of oral candidiasis in the SS group was lower than that reported in other studies (Radfar et al., 2003, Likar-Manookin et al., 2013). It must be noted that in our study we defined oral candidiasis as clinical infection of oral candidiasis and not just colonization as reported by other studies (Radfar et al., 2003, Tapper-Jones et al., 1980). The

difference in prevalence estimates could also be a result of selection bias or surveillance bias. In other studies, SS participants were evaluated for colonization of oral *Candida* spp. at the time of enrollment; whereas in our study, subjects were enrolled only on the basis of salivary dysfunction assessment and the detection of infection caused by the fungus was not the primary aim while setting up the cohorts. Furthermore, in studies ascertaining the prevalence of oral candidiasis in SS, patients were selected from clinics or hospitals where reporting of symptomatic oral lesions is more likely than at a research institute following-up a longitudinal cohort at set intervals of time. The clearance of colonization and infection by *Candida* Spp. between visits need to be considered while comparing prevalence estimates of this study with other clinic-based studies.

Given that this study was nested within a large cohort, data on certain variables of interest such as oral prostheses use were not collected. Consequently, we could not control for denture stomatitis in our modeling. Furthermore, not every case of oral candidiasis was evaluated for type of infection therefore yielding variable denominators for each type, which was another limitation of this study.

In conclusion, this study not only resolves the inconsistencies reported in the literature regarding the type of salivary flow associated with oral candidiasis, the role of autoimmunity in oral candidiasis, but also reports additional independent predictors of oral candidiasis such as diabetes mellitus and medications for extraglandular manifestations. Given these multiple independent predictors of oral candidiasis, addressing and promoting higher saliva output alone will not eliminate oral candidiasis in this population. Consequently, it may be prudent to take into consideration the various independent predictors of oral candidiasis reported in this study, i.e., hyposalivation, SS, other autoimmune disorders, diabetes mellitus, and medications for extraglandular manifestations of SS while developing prevention and management strategies for oral candidiasis in this population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Median Salivary Flow Rates by Groups

Median WUS (ml/15min) of SS group is significantly lower than that of NSGD and ISS groups | Median TSS (ml/15min) of SS group is significantly lower than that of NSGD, Sicca and ISS groups.

Table 1

Demographic and Clinical Characteristics by Study Groups

A. Demographic Characteristic	SS N=701	NSGD N=223	Sicca N=355	ISS N=247	Total N=1526	p-value
Age (median, range)	53 (7–83)	47 (9–92)	52 (17–88)	48 (9–86)	-	<0.001 ^a
Gender (n, %)						
Male	57 (8.13)	33 (14.80)	63 (17.75)	37 (14.98)	190 (12.45)	<0.001 ^b
Female	644 (91.87)	190 (85.20)	292 (82.25)	210 (85.02)	1336 (87.55)	
Race (n, %)						
White	505 (72.04)	172 (77.13)	302 (85.07)	175 (70.85)	1154 (75.62)	<0.001 ^a
Black or African American	93 (13.27)	34 (15.25)	24 (6.76)	32 (12.96)	183 (11.99)	
Asian	55 (7.85)	9 (4.04)	13 (3.66)	17 (6.88)	94 (6.16)	
Other ^c	48 (6.85)	8 (3.59)	16 (4.51)	23 (9.31)	95 (6.23)	
Ethnicity (n, %)						
Not Latino or Hispanic	643 (91.73)	211 (94.62)	344 (96.90)	229 (92.71)	1427 (93.51)	0.004 ^b
Latino or Hispanic	40 (5.71)	9 (4.04)	6 (1.69)	17 (6.88)	72 (4.72)	
Unknown	18 (2.57)	3 (1.35)	5 (1.41)	1 (0.40)	27 (1.77)	
B. Clinical Characteristic	SS	NSGD	Sicca	ISS	Total	p-value
Hyposalivation (WUS 1.5ml/15min) ^d Presence (n, %)	113 (63.84)	0 (0.00)	70 (67.31)	5 (10.00)	188 (45.97)	<0.001 ^b
Hyposalivation (TSS 7.5 ml/15min) Presence (n, %)	295 (51.30)	21 (14.89)	77 (33.48)	29 (17.16)	422 (37.85)	<0.001 ^b
Other autoimmune disorders Presence (n, %)	22 (3.14)	4 (1.79)	3 (0.85)	9 (3.64)	38 (2.49)	0.054 ^b
Diabetes mellitus Presence (n, %)	14 (2.00)	2 (0.90)	13 (3.66)	5 (2.02)	34 (2.23)	0.173
Medication Use (n, %)						
No medication	490 (69.90)	215 (96.41)	327.0 (92.11)	232.0 (93.93)	1264 (82.83)	<0.001 ^b
Meds. that reduce salivary flow	23 (3.28)	0 (0.00)	2 (0.56)	0 (0.00)	25 (1.64)	<0.001 ^b
Sialagogues	16 (2.28)	0 (0.00)	3 (0.85)	1 (0.40)	20 (1.31)	0.018 ^b
Meds. for extraglandular manifestations	100 (14.27)	5 (2.24)	16 (4.51)	7 (2.83)	128 (8.39)	<0.001 ^b
Other meds	40 (5.71)	2 (0.90)	5 (1.41)	5 (2.02)	52 (3.41)	<0.001 ^b
Combination of meds	32 (4.56)	1 (0.45)	2 (0.56)	2 (0.81)	37 (2.42)	<0.001 ^b

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A. Demographic Characteristic	SS N=701	NSGD N=223	Sicca N=355	ISS N=247	Total N=1526	p-value
Antifungals	4 (0.57)	0 (0.00)	0 (0.00)	0 (0.00)	4 (0.26)	N/A ^e

^a Pearson's chi-square |

^b Fisher's exact test |

^c American Indian or Alaskan Native, Native Hawaiian and other Pacific Islander, multiple races, and unknown|

^d Based on available data

^e p-value not commutable due to 0 cells.

Table 2

Distribution of Oral Candidiasis by Groups

Oral Candidiasis	Group							Total	p-value*
	SS	95% CI	NSGD	95% CI	Sicca	95% CI	ISS		
Overall	n/N	32/701	2/223	7/355	4/247	45/1,526	0.008		
	Proportion of cases (%)	71.11	55.69–83.63	4.44	0.54–15.15	15.56	6.49–29.46	8.89	2.48–21.22
	Prev. in group (%)	4.56	3.14–6.38	0.90	0.11–3.20	1.97	0.80–4.02	1.62	0.44–4.09
Pseudomembranous C.	n/N	13/204	1/67	1/81	3/32	18/384	0.073		
	Proportion of cases (%)	72.22	46.52–90.31	5.56	0.14–27.29	5.56	0.14–27.29	16.67	3.58–41.42
	Prev. in group (%)	6.37	3.44–10.65	1.49	0.04–8.04	1.23	0.03–6.69	9.38	1.98–25.02
Erythematous C.	n/N	9/203	1/67	1/81	1/32	12/383	0.503		
	Proportion of cases (%)	75	42.81–94.51	8.33	0.21–38.48	8.33	0.21–38.48	8.33	0.21–38.48
	Prev. in group (%)	4.43	2.05–8.25	1.49	0.04–8.04	1.23	0.03–6.69	3.13	0.08–16.22
Other C.	n/N	16/83	0/8	5/13	1/10	22/114	0.170		
	Proportion of cases (%)	72.73	49.78–89.27	0	-	22.73	7.82–45.37	4.55	0.12–22.84
	Prev. in group (%)	19.28	11.44–29.41	0	-	38.46	13.86–68.42	10	0.25–44.50

* Fisher's exact test proportions rounded to two decimal places.

A subject may have more than one type of oral candidiasis on different locations of the oral cavity.

Table 3
Predictors of Oral Candidiasis in Sjögren's Syndrome - Multivariable Logistic Regression Modeling

Independent variable	Model 1			Model 2			Model 3		
	NSGD-comparison group			Sicca-comparison group			ISS-comparison group		
	OR	95% CI	p-value	OR	95% CI	p-value	OR	95% CI	p-value
Female ^a	0.29	0.13-0.67	0.004 *	0.33	0.13-0.87	0.024 *	--	--	--
Black or African American Race ^a	0.32	0.09-1.12	0.074	--	--	--	0.23	0.05-1.06	0.059
Sjögren's Syndrome	2.15	1.11-4.18	0.023 *	2.23	1.05-4.76	0.037 *	--	--	--
Hyposalivation WUS 1.5ml/15min ^b	5.11	2.50-10.45	<0.001 *	3.52	1.66-7.49	0.001 *	2.41	1.09-5.31	0.029 *
Hyposalivation WUS (continuous)	0.61	0.46-0.82	0.001 *	0.60	0.41-0.87	0.007 *	0.62	0.42-0.90	0.013 *
Hyposalivation TSS 7.5 ml/15min ^c	1.89	1.01-3.53	0.047 *	2.06	1.08-3.95	0.029 *	2.38	1.16-4.87	0.018 *
Hyposalivation TSS (continuous)	0.94	0.90-0.98	0.006 *	0.93	0.89-0.98	0.009 *	0.93	0.88-0.98	0.012 *
Other autoimmune disorders ^b	4.45	1.75-11.30	0.002 *	3.63	1.29-10.22	0.014 *	--	--	--
Diabetes mellitus ^b	--	--	--	4.24	1.18-15.22	0.027 *	6.73	1.52-29.79	0.012 *
Medications - extraglandular manifestations ^a	2.31	1.08-4.94	0.031 *	2.15	0.99-4.68	0.054	--	--	--

Model 1: NSGD was the control group to which SS, Sicca and ISS groups were compared

Model 2: Sicca was the control group to which SS, ISS groups were compared

Model 3: ISS was the control group to which SS group was compared

^a Male, White, no medication use as respective references

^b Binary variables with absence of the variable as reference

^c TSS - from multiple logistic regression model replacing WUS

OR - Odds Ratio

-- variable not selected by model

* Statistically significant variables.