Original Research

Relationship between the quantity of oral *Candida* and immunological vigor

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ABSTRACT

Objective: Oral *Candida* asymptomatically colonizes approximately 35–80% of individuals in a population at any given time. It is speculated that increases in oral *Candida* may be closely related to host immunity. Therefore, the aim of the present study was to determine whether a relationship exists between the number of oral *Candida* colonies and immunological status of the host.
Materials and methods: We analyzed 32 subjects and their immunity was assessed and scored using the "Scoring of Immunological Vigor (SIV)". Amount of oral candida was detected by culture of concentrated oral rinse solutions. The relationship between SIV and the count of oral *Candida* was investigated.
Results: Oral *Candida* was detected in cultures of samples from all subjects. The median number of *Candida* colonies was 6.5 CFU (IQR: 28.5 CFU, range: 1–3480 CFU). There was a significant correlation between the count of oral *Candida* and the grade of SIV. The amount of oral candida was higher in the lower immunological status of the host. Significant correlations were also found between the amount of *Candida* and the number of T cells, naïve T cells, and Natural Killer (NK) cells.
Conclusions: The results of this study suggested that the detection of oral *Candida* may be a possible marker for determining immunological vigor of the host.

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1. Introduction

Candida habitually resides in the oral cavity and the reported rate of oral *Candida* among healthy individuals varies between 35% and 80% depending on the population studied and detection methods used [1,2]. *Candida* species asymptomatically colonize individuals in a population at any given time, and they may cause mucosal and systemic infections in the condition where oral candida increased and/or host defenses are weakened.

It is reported that the amount of oral *Candida* increased in the local condition including denture wearing, hypoptyalism, low saliva pH, and the presence of dental carious lesions [3–8]. It is also suggested that increase of oral *Candida* may be closely related to host immunity. Previous studies reported that oral *Candida* increased in immunocompromised individuals, including HIV-positive and AIDS patients, organ transplant recipients, and patients with some factors that primarily act by inducing immunosuppression (e.g., corticosteroids, chemotherapy, malnutrition, malignancy, and neutropenia) [9–15]. These results strongly speculated that increase of oral *Candida* may be closely related to host immunity; however, this relationship has not yet been examined. Therefore, the aim of the present study was to determine whether a relationship exists between the number of oral *Candida* colonies and immunological status of the host by examining the number of *Candida* colonies in the oral cavity and immunological vigor of the host, as assessed by immunological indices.

2. Materials and methods

The Committee for Ethics at Shinshu University Hospital (#2795) and Aizawa Hospital (#2012-091) approved this study protocol.

Table 1
Relationship between an amount of oral Candida and subjects' sex, age, oral symptoms/sings, and systemic diseases.

Characteristics	(n)	Amount of Candida (CFU) Median (IQR)	
Sex	Women (19)	6 (1.5–160)	$p = 0.81$[a]
	Men (13)	10 (4–15)	
Age	20–59 (19)	4 (1–6)	$p < 0.01$[a] $p < 0.001$[b]
	60< (13)	285 (20–2160)	(r = 0.64)
Oral symptoms/signs	Absence (19)	4 (1.25–6)	
	BMS (6)	10 (1–162)	$p = 0.19$[a] (vs absence)
	Oral candidiasis (7)	2320 (160–2900)	$p < 0.001$[a] (vs absence)
Denture wearing	(−) (19)	4 (1–6.5)	$p < 0.001$[a]
	(+) (13)	285 (15–2610)	
Systemic disease	Absence (14)	4 (1.3–5.8)	$p < 0.01$[a]
	Presence (18)	24.5 (10.3–1813.8)	

BMS: burning mouth syndrome.
[a] Mann–Whitney's U test.
[b] Spearman's rank correction test.

2.1. Subjects

Samples were obtained from 38 subjects who agreed to participate in the present study. Of these, 24 were recruited from consecutive patients who visited the department of dentistry and oral surgery at Aizawa Hospital or Shinshu University Hospital complaining of dental and oral diseases (between February 2013 and November 2014). The other 14 subjects were healthy volunteers. Informed consent was obtained from all subjects.

2.2. Detection and count of oral Candida

An oral rinse solution was collected by rinsing the mouth with 5 mL sterile saline, which was held in the mouth for 30 s before collection in a sterile container. Concentrated oral rinse solution was prepared by centrifuging the rinse solution at $3200 \times g$ for 20 min. After the supernatant was removed, the precipitate was resuspended with 250 μL saline and 50 μL of the sample was inoculated onto the Chromagar Candida agar (Kyokuto Pharmaceutical Industrial Co., Ltd., Tokyo, Japan). Candida colonies on culture agars were counted after an incubation at 37 °C for 48 h.

2.3. Assessment of host immunity

Immunity was assessed and scored using the "Scoring of Immunological Vigor (SIV)" reported by Hirokawa et al. [16–19]. Two milliliters of venous blood was collected in a tube containing ethylenediaminetetraacetic acid (EDTA-2K) at the same time as oral Candida sampling. Peripheral blood samples were sent to the Institute for Health and Life Sciences (HLS Tokyo, Japan) and SIV was measured using the patents of Tokyo Medical and Dental University (No. 4608704, No. 5030109). SIV evaluates comprehensive immunity strength by scoring various immune indexes including the total number of T cells, number of CD8+ CD28+ T cells, the ratio of CD4+ T cells to CD8+ T cells, the number of naive T cells and ratio of naive to memory T cells, number of B cells, and number of Natural Killer (NK) cells. Each index was measured by the method described below. Consequently, the measurement results of each index were reported in three ranks (scores 1–3). Score 1 means "needs improvement", score 2 means "needs observation", and score 3 means "safe". The criteria for the ranking were not shown because they were protected by the patent. Each score of seven indexes was summed to obtain the total score, with the resulting score ranging between 7 and 21. Immunological vigor was classified into 5 grades: grade 5 (total score = 21, highest immunity), grade 4 (total score = 20–18), grade 3 (total score = 17–14), grade 2

(total score = 13–10), and grade 1 (total score = 9–7, lowest immunity). Serological C-reactive protein (CRP) levels were measured in order to assess the presence of local and systemic inflammation, which may influence the assessment of immunity.

The methods used to measure each index were as follows:

WBC count: A hematological analysis performed with a PEN-TRA80 analyzer (Horiba, Kyoto, Japan).

Mononuclear cell count: Mononuclear cells were stained with a combination of 5 monoclonal antibodies (mAbs) conjugated with 5 chromophores. A flow cytometer (Navios: Beckman Coulter, Miami, FL, U.S.A) was used to count the number of mononuclear cells.

Counts of the subtypes of lymphocytes using monoclonal antibodies (mAbs): The following mAbs (Beckman Coulter, Miami, FL, U.S.A) were used: fluorescein isothiocyanate (FITC)-conjugated anti-CD8 and anti-CD20; phycoerythrin (RD1)-conjugated anti-CD3; phycoerythrin-Texas Red (ECD)-conjugated anti-CD45RA; phycoerythrin-cyanin 5.1 (PC5)-conjugated anti-CD28 and CD16; phycoerythrin-cyanin 7 (PC7)-conjugated anti-CD45; allophycocyanin (APC)-conjugated anti-CD4CD, and anti-CD56. The following combinations of mAbs were used: CD3-RD1/CD20-FITC/CD16-PC5/CD45-PC7/CD56-APC and CD4-APC/CD8-FITC/CD45RA-ECD/CD28-PC5/CD45-PC7. These mAbs enabled us to identify B cells (CD20+ cells), NK cells (CD56+ CD16+ cells), T cells (CD3+), and the following subpopulations of T cells: CD4+ T cells and CD8+ T cells.

2.4. Statistical assessment

Statistical assessments were carried out using PC running software (GraphPad Software Prism 6 for Windows). Spearman's rank correction test or Mann–Whitney's U-test was utilized to test the relationship between the variables. P-values less than 0.05 were used to indicate significance.

3. Results

3.1. Subjects

Of the 38 subjects who participated in the study, six were compromised by inflammation (CRP level higher than 0.30 mg/dL) and were excluded from the study. Therefore, 18 patients (7 males and 11 females with a mean age of 70.6 years old ranging between 48 and 88 years old) and 14 volunteers (6 males and 8 females with a mean age of 31.3 years old, ranging between 26 and 42 years old) were available for the assessments. Seven patients had oral candidiasis, 6 had burning mouth syndrome (BMS), and other