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Salivary Flow Rate, Ph Level, and Candida Colonization in Elderly Patient at Tuntungan Public Health Center, Sumatera Utara

Pocut Astari[1], Wilda Hafny Lubis[1], Nurdiana[1]

[1]*(Department of Oral Medicine, Faculty of Dentistry, Universitas Sumatera Utara, Indonesia)*

Corresponding Author:
Pocut Astari

Abstract : Salivary flow rate and pH level are pivotal parameters influencing oral microbial equilibrium and defense mechanisms against pathogens, including Candida species. With advancing age, elderly individuals encounter physiological changes that often lead to decreased salivary flow and altered pH levels. These changes can potentially foster an environment conducive to Candida colonization and subsequent infections. The aim of this study is to investigate the potential correlation between salivary flow rate, pH levels, and the count of oral Candida colonies in elderly. Furthermore, the study aims to identify the Candida species present in the oral cavities of elderly individuals. This analytical cross-sectional study involved 105 elderly patients visiting Tuntungan Public Health Center, Sumatera Utara. Unstimulated saliva was collected by spitting method for 5 minutes to measure salivary flow rate and pH level. Subsequently, the samples were cultivated on Saboraud Citrus agar medium, and over a period of 24-48 hours, the development of Candida colonies was monitored, and their quantities were tallied. Identification of each Candida colony has been conducted using Vitek® 2 Compact system. Statistical analyses were performed to compare the salivary flow rate (SFR) and pH between male and female participants using T-test. The relationship between SFR and salivary pH level was examined using the Fisher Exact test. Additionally, Chi-Square tests were utilized to examine the relationship between SFR and pH level with the presence of Candida colonies. There are no differences found in the mean SFR ($p=0.87$) and salivary pH ($p=0.18$) between male and female groups. There was a significant relationship found between salivary flow rate (SFR) and salivary pH level ($p<0.0001$). There was a significant relationship found between candida colonization and SFR ($p=0.03$) but not with salivary pH level ($p=0.53$). In conclusion, the reduction in salivary flow rate may increase the risk of acidity and Candida infections in elderly patient's oral cavity and the most Candida species inhabiting elderly oral cavity is Candida albicans. Therefore, preventive measures should be taken to maintain good oral hygiene and overall health in elderly.

Keywords –*Salivary flow rate, pH level, Candida, Elderly*

I. INTRODUCTION

People worldwide are living longer. Every country in the world is experiencing growth in both the size and the proportion of older persons in the population. Between 2015 and 2050, the proportion of the world's population over 60 years will nearly double from 12% to 22%.(1) Based on data from the Indonesian Central

Statistics Agency, the number of elderly individuals in the year 2022 has reached more than 31 million people.(2) Physiologically, old individuals experienced the aging process. This aging process, which involves the accumulation of cellular and molecular damage over a long period of time, is often associated with the alteration of elderly overall health.(1,2)

Saliva plays a crucial role in maintaining oral health especially in elderly by aiding in mechanical cleaning and providing protection through various physiological and biochemical mechanisms.(3) Insufficient saliva production, known as hyposalivationcan lead to the emergence of several health issues that significantly impact the patient's quality of life. These problems can affect eating habits, nutritional status, taste perception, speech, and the ability to tolerate dental prostheses. Additionally, these effects can elevate the risk of oral infections, such as candidiasis. (3–5)

Multiple research studies have investigated the correlation between low salivary flow rate (SFR) and pH with Candida colonization in the elderly. Nonetheless, the findings have remained inconclusive, and the identification of Candida species in the oral cavities of the elderly is still constrained. As a result, this study aims to establish a connection between SFR and salivary pH with Candida colony counts, as well as to identify Candida species in the oral cavities of the elderly.

II. MATERIAL AND METHODS

This observational analytic study was carried out on patients of Tuntungan Public Health Center, Pancur Batu, Deli Serdang, Sumatera Utara from August 2022 to December 2022. A total 105 elderly subjects (both male and females) of aged ≥ 60 years who were in a good health and willing to participate in this study were selected in this study. Patients that on medication such as corticosteroids and/or antibiotics within 3 months, who wear dentures and uncooperative were excluded from this study.

The research proposal received approval from the Institutional Ethical Committee No. 1002/KEPK/USU/2022 and all participants were adequately informed about the study through both verbal and written explanations. After fully understanding the purpose and details of the research, the subjects provided written consent to participate.

Participants were given specific instructions not to eat, drink, smoke, or perform oral hygiene activities for 1 hour prior to saliva collection. Unstimulated saliva was gathered by allowing it to accumulate in the mouth for 5 minutes, followed by spitting it into a sterile container. The salivary flow rate (SFR) was computed in milliliters per minute. Hyposalivation was characterized as an SFR of less than 1 ml/min. After collection, the samples were promptly transported to the laboratory on ice packs within 2 hours for further examination process.

Salivary pH level was determined using a Digital pH meter (Mettler Toledo Seven Multi pH-meter with Lab®413 electrode). The saliva pH results were categorized in three groups based on the pH value obtained. A pH value of 6.9 or less was considered 'acidic', a pH value of 7 was labeled as 'neutral', and a pH value greater than 7 was classified as 'alkaline'.

Saliva samples were subjected to centrifugation for 2 minutes. From the separated saliva, 0.1 ml was placed onto Sabouraud Citrus chloramphenicol agar culture plates and then incubated at 37°C for 24 to 48 hours. The presence of creamy white-colored, smooth colonies with a yeasty odor indicated the potential growth of Candida. After 48 hours, candida colonies were counted manually (4 quadrants) and the data were recorded.

Candida species were identified using the Vitek® 2 Compact system. Saline was added to the ID tube and a Candida colony was transferred and homogenized. The turbidity level was checked using DensiCHEK™, with expected levels of 1.00-2.20 McF. 280 µl of the solution was transferred to the AST tube and the ID & AST card were inserted into the cassette and placed in the Vitek 2 Compact® system. Candida species were identified in 4-6 hours and the results were printed after data input.

The collected data was organized, and statistical analyses were performed to compare the salivary flow rate (SFR) and pH between male and female participants using t-tests. Additionally, Chi-Square tests were utilized to examine the relationship between SFR and pH with the presence of Candida colonies. A p-value of less than 0.05 was considered to be statistically significant, indicating meaningful differences or associations in the results. The level $P < 0.05$ was considered as the cutoff value or significance.

III. RESULTS

This study conducted on 105 elderly patients visited Tuntungan Health Center, Pancur Batu, Deli Serdang, Sumatera Utara, Indonesia. There were 19 (18%) male patients and 86 (82%) were females contributed to this research. The overall mean age of the subjects was 66.69 years where mean age of the male subjects is 66.52 years and female are 66.72 years.

Table 1 shows the average of salivary flow rate (SFR) and salivary pH based on gender. Salivary flow rate (SFR) and salivary pH of each subject were measured, and the data collected were analyzed to determine a difference between gender groups. Average SFR in male was 0.19±0.08 (mean±SD) while average SFR in females subject was 0.19±0.06. The average salivary pH in male was 5.65±0.70, while in female there was 5.64±0.53. (Table 2). There are no significant differences found in the average of SFR (p=0.87) and salivary pH (p=0.18) between both groups.

Table 1. Differences in Salivary Flow Rate (SFR) and Salivary pH Based onGender.

Salivary Profile	**Male (Mean±SD)**	**Female (Mean±SD)**	***p-value***
Salivary flow rate (SFR)	0.19±0.08	0.19± 0.06	0.87
Salivary pH	5.65±0.70	5.64±0.53	0.18

The study analyzed saliva flow rate (SFR) and salivary pH level to understand their relationship. SFR below 1 ml/min was considered hyposalivation, while SFR greater than or equal to 1 ml/min was considered normal. Salivary pH was categorized as acidic, neutral, or alkaline. Table 2 shows the relationship and distribution of the subject's salivary pH level based on SFR. There are 88 (84%) subjects that experience hyposalivation have an acidic saliva and 4 (4%) subjects have neutral saliva. For subjects that have normal SFR, there are 12 (11%) subjects have acidic saliva and 1 (1%) subject has neutral saliva. There are no subject have alkaline saliva found in both groups. The findings revealed a significant relationship between SFR and salivary pH ($p<0.0001$).

Table 2. Relationship between Salivary Flow Rate (SFR) and Salivary pH Level

Salivary Flow Rate	**Salivary pH Level**			***p-value***
	Acidic, n(%)	**Neutral, n(%)**	**Alkaline, n(%)**	
SFR ≤1ml/min	88 (84)	4 (4)	0 (0)	<.0001*
SFR >1ml/min	12 (11)	1 (1)	0 (0)	
Total	**100 (95)**	**5 (5)**	**0 (0)**	

* Statistically significant (Fisher Exact test)

Candida colony count categories are divided into 2 categories, overgrowth for ≥100 Candida colony forming unit (CFU) and normal for <100 CFU. Table 3 shows the relationship between SFR and salivary pH to

Candida colony counts. A significant correlation was observed between SFR and Candida overgrowth (p=0.03), but there was no apparent association between pH and Candida overgrowth (p=0.59).

Table 3. Relationship between SFR and salivary pH with Candida Colony Counts

Salivary Profile		**Candida Colony Counts**		***p-value***
		Normal n(%)	**Overgrowth n(%)**	
Salivary flow rate (SFR)	SFR ≤1ml/min	79 (75)	13 (12)	.03*
	SFR ≤1ml/min	8 (8)	5 (5)	
Salivary pH	Acidic	82 (78)	18 (17)	.59
	Neutral	5 (5)	0 (0)	
	Alkaline	0 (0)	0 (0)	

* Statistically significant (Chi-square test)

Among the 105 samples analyzed, Candida colonies were observed in only 55 of them, representing 52% of the total samples. However, the remaining 50 samples, accounting for 48%, did not display any growth of Candida colonies. Identification of Candida species were conducted, and the results were seen in Table4. Out of the 55 samples, the majority (44%) were identified as *Candida albicans*, followed by *Candida dubliniensis* (16%), *Candida parapsilosis* (14%), and *Candida glabrata* (9%). Three samples (5%) were identified as an unidentified Candida species, *Candida giuliermondii* (4%), while *Candida cryptococcuslaurentii*, *Candida lusitaniae*, *Candida spherica*, and *Candida tropicalis* each accounted for 2% (1 sample) of the total.

Table 4. Identification of Candida Species

Candida Species	**Amount of samples n (%)**
Candida albicans	24 (44)
Candida dubliniensis	9 (16)
Candida parapsilosis	8 (14)
Candida glabrata	5 (9)
Candida species (unidentified)	3 (5)
Candida giuliermondii	2 (4)
Candida cryptococcuslaurentii	1(2)
Candida lusitaniae	1(2)
Candida spherica	1(2)
Candida tropicalis	1(2)
Total	**55 (100)**

IV. DISCUSSION

Saliva performs a vital function in maintaining good oral health.(4,6)The process of aging leads to numerous biological, chemical, and physiological shifts that could potentially influence changes in salivary traits.(7,8)Hyposalivation may contribute to several oral complaints, such as xerostomia, generalized oral discomfort, burning mouth and tongue, traumatic oral lesions, halitosis, intolerance to acidic and spicy foods, poor retention of dentures, disturbances in taste and mastication, polydipsia, dysgeusia, dysphasia and dysphonia.(9,10)Additionally, these effects can elevate the risk of oral infections, such as bacterial infection and candidiasis.(3,6,9)

This study has found that the mean SFR in elderly was ±0.19 ml/min which indicated a low salivary flow rate or hyposalivation. The same result also seen in other studies, that exhibited low salivary flow rate in elderly, especially menopausal women.(11,12)As people age, the oral cavity undergoes anatomical changes, and elderly individuals often experience a decline in saliva flow rate. These changes are attributed to the atrophy of acinar cells within the salivary glands, leading to a deterioration in salivary gland function. Consequently, there is a loss of parenchymal tissue in the glands, which is replaced by adipose and connective tissue, resulting in reduced saliva production.(13) Studies have also shown that systemic diseases can further contribute to the decline in salivary flow rate among the elderly. (3,4)As a consequence of reduced saliva production, elderly individuals become more vulnerable to oral infections.(13)

The average salivary pH level in this study were 5.65±0.70 for male and 5.64±0.53 for female subjects, which indicated acidic oral condition in elderly patients. Studies have shown a decrease in elderly salivary pH.(11,12) The notable reduction in salivary pH level can be attributed to xerostomia, which arises from reduced fluid volume and electrolyte loss due to increased urination and dehydration.(12) However, there are no differences detected in SFR and salivary pH between male and female subjects. This result is in accordance with a study in Mexico, which demonstrated that there were no significant differences found in salivary flow rate (SFR) and salivary pH level between both genders.(14)

This study showed that there was a statistically significant relationship found between elderly SFR and salivary pH level. The same result also shown in a study in India.(15) The reduction in SFR impacts salivary pH level by diminishing the secretion of bicarbonate, leading to a subsequent decrease in saliva bicarbonate levels, which in turn lowers the salivary pH level.(15)

This study exhibited a significant relationship between saliva flow rate and the number of Candida colony counts (p=0.03). The development of Candida colonies may be due to decreased salivary gland function inhibits saliva secretion. Low salivary secretion means the low of anti-fungal components in saliva such as lactoferrin and lysozyme. Altered salivary gland function leads to a low saliva flow rate, resulting in an increased number of Candida colonies.(4,16,17)Not only the increased number of Candida colony growth, the impaired salivary gland function in the elderly also leads to increased infections in the oral cavity such as Candidiasis.(4,5,16)This study shows no significant relationship between salivary pH and the number of Candida colonies (p=0.59). While acidic pH can accelerate bacterial growth in the oral cavity, it does not have a significant impact on the growth of Candida colonies in the oral cavity of elderly patients.(18)

Candida are normal commensal organisms of the mouth and generally did not cause any problems in healthy people.(19) However, several factors, local and systemic alterations such as low salivary flow rate, medications and decreased immune system may cause the overgrowth of Candida. Over 40 species of Candida, most commonly *Candida albicans*, can cause infections in humans. In addition, other major pathogenic Candida species are *C. tropicalis*, *C. parasilposis*, *C. krusei*, and *C. glabrata*. (19,20)

In this study, only 55 of 105 samples showed colony candida growth, identification of each colony has been conducted using Vitek® 2 Compact system. The most Candida species detected in elderly saliva was *Candida albicans* (44%). This result is in accordance with many studies that exhibited *Candida albicans* as the most commonly species found in elderly oral cavity.(6,19,21,22) Furthermore, the three most commonly non-albicans Candida identified in this study were *Candida dubliniensis* (16%), *Candida parapsilosis* (14%), and

Candida glabrata (9%). This result is also in accordance with research in Japan that showed *C.dubliniensis*, *C. Parapsilosis* and *C. Glabrata* were the most dominant non-albicans Candida species found in elderly oral cavity.(23) Individuals who have preexisting medical conditions or factors that make them more susceptible are at an elevated risk of the spread of these microorganisms from the reservoir in the mouth into the bloodstream, potentially resulting in a condition known as candidaemia.(24) The rare species identified were *Candida giuliermondii* (4%), *Candida cryptococcuslaurentii*, *Candida lusitaniae*, *Candida spherica*, and *Candida tropicalis* each accounted for 2% (1 sample) of the total. However, there are three samples that we still could not identified in this study.

V. CONCLUSION

Low salivary flow rate (SFR) may affect the acidity of saliva in elderly. There is a significant relationship between low SFR and Candida overgrowth, but no relationship found between pH levels and Candida overgrowth and the most Candida species detected in elderly oral cavity was Candida albicans.

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