

Salivary Secretion and Number of Facultative Anaerobic Bacterial Colony in Female Smokers

Yuyun Qurrota A'Yunina Rahmi¹, Sri Tjahajawati^{2*}, Hening Tjaturina Pramesti²

1. Faculty of Dentistry Padjadjaran University Indonesia.

2. Oral Biology Departement, Faculty of Dentistry Padjadjaran University Indonesia.

Abstract

Cigarette's smoke can affect the function of the receptor that relates to a change of salivary flow rate. The change of salivary function has the effect of increasing the number of the facultative anaerobic bacterial colony in the oral cavity of smoker that could lead to various dental and oral diseases. The purpose of this study is to measure salivary secretion and count the number of the facultative anaerobic bacterial colony of female smokers.

This research is observation descriptive. The subjects were female smokers with an age range of 19-25 years. Saliva samples were taken by spitting method. The salivary flow rate was measured based on salivary volume. Salivary samples were diluted to 10⁴ and then inoculated on the blood agar plate and then incubated at 37°C for 18-24 hours in the anaerobic facultative environment. The last step was counting the number of facultative anaerobic bacterial colony used colony counter.

The results showed that the average of salivary secretion in female smokers as much as 0.289 mL/min. The number of the facultative anaerobic bacterial colony in female smokers as much as $(167.63 \pm 43.16) \times 10^5$ CFU / mL.

The conclusion of this research is, in the mouth of female smokers was found the salivary flow rate indicated oligosialia and the number of the facultative anaerobic bacterial colony as much as $(167.63 \pm 43.16) \times 10^5$ CFU/mL.

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Introduction

The oral cavity is lined with mucous membranes and protected by saliva that secreted through the major and minor salivary gland. There are three major glands consist of the parotid gland, submandibular gland, and sublingual gland. Minor salivary glands are spread around 700-1000 in the oral cavity and pharynx. Salivary glands can produce saliva up to 1.5 liters/days. The parotid gland produces 20-25% of saliva, the submandibular gland produces 70-75% saliva, and the sublingual gland produces the lowest saliva as much as 5%.¹

Salivary secretions may be affected by a person's behavior, such as smoking. Smoking is a behavior that many people do in Indonesia.

Based on data from Riskesdas the prevalence of female smokers in Indonesia currently rises from 4.2% in 1995 to 6.7% in 2013.² Smoking is thought to be one of the risk factors for decreasing salivary secretion and leading to xerostomia.³ The Heat that produces by smoking can irritate the oral mucosa and directly causing changes in vascularization and salivary secretions. Smoking also can reduce antibodies (IgA, IgG, and IgM), enzymes (lysozyme, lactoperoxidase, and lactoferrin), mucin and histatin in saliva that serves to neutralize bacteria in the oral cavity, resulting in impaired function of body defense cells. The decrease in the quality and quantity of the salivary component causes an increase in the number of microbes in the oral cavity so that the oral cavity is susceptible to infection.⁴ Other studies have shown that in people with reduced salivary flow rates there is an increase in *Lactobacillus* and *Streptococcus mutans* bacteria which is one of the facultative anaerobic bacteria.

*Corresponding author:

Dr. Sri Tjahajawati, drg., M.Kes
Faculty of Dentistry, Padjadjaran University
Jl. Sekeloa Selatan I, Bandung. Indonesia
E-mail: sri.tjahajawati@fkg.unpad.ac.id

Facultative anaerobic bacteria are bacteria that use oxygen to produce energy, but can also grow in a lack of oxygen.⁵ These bacteria are normal flora in the oral cavity. The growth of normal flora in the body is influenced by various factors such as temperature, PH, water and nutrient availability, oxidation-reduction potential, the anatomy of the oral cavity, salivary secretions and antimicrobial substances. The existence of normal flora in the body has an important role in the body because it can inhibit the growth of other microorganisms. These bacteria can turn into pathogens in certain circumstances due to the presence of predisposing factors such as oral hygiene and bad habits.⁶

Information about the description of salivary secretions and the number of facultative anaerobic bacterial colonies in female smokers has not been widely reported, so researchers are interested in examining the description of salivary secretions and the number of facultative anaerobic bacterial colonies in female smokers.

Materials and methods

This research is descriptive, that will provide an overview of salivary secretions and the number of facultative anaerobic bacterial colonies in female smokers. The population in this study were female smokers aged 19-25 years, students of the Faculty of Social and politic at the University of Padjadjaran with 30 samples of female smokers. Saliva samples were taken as much as 1mL using a sterile suction pipette and then inserted into a reaction tube containing a 9 mL bullion. Saliva and bullion in the test tube were homogenized using vortex and then diluted to 0.5 Mc Farland concentration, equal to the bacterial count of 1.5×10^8 CFU / mL. The solution mixture was diluted up to four times to reach the dilution level of 10^4 . Dilution results were taken as much as 0.1 mL and then inoculated on Blood agar plate in duplicate and distributed evenly using oase. Blood agar plate was incubated in an excicator that has been cleaned with 70% alcohol and given a lit candle and water-soaked cotton to get an anaerobic facultative atmosphere. The excicator containing Blood agar plate was incubated at 37 ° C for 18-24 hours and then counted the number of the anaerobic facultative bacterial colony. The data obtained from this

study are display in diagrams and then calculated the average salivary flow rate and the number of an anaerobic facultative bacterial colony in female smokers.

Results

The characteristic of female smokers in this study were grouped based on age and duration of smoking. The subjects of this study were female with an age range of 19-25 years. In Figure 1 it can be seen that the highest percentage of female smokers is 21 and 22 years at 30%. The lowest percentage of female smokers is 3% at the age of 24 years. The 19-year-old smoker is 17%. Smoker female aged 20 and 23 years respectively as much as 10%.

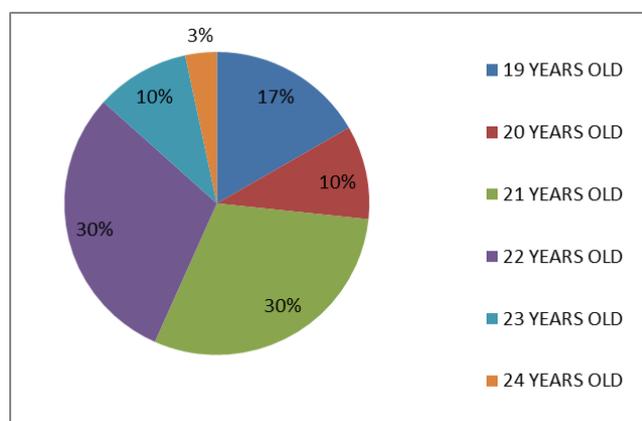


Figure 1. Age percentage of female smokers.

The duration of smoking is divided into three groups, there is a smoking period of 1-2 years, 2-5 years and more than 5 years. On average, the female who smokes has smoked for 2-5 years. Smokers who have smoked for 2-5 years are 19 people. Smokers who have smoked for more than 5 years are 6 people. There are 5 smokers with a smoking period of 1-2 years or the lowest number of groups in this study (Figure 2).

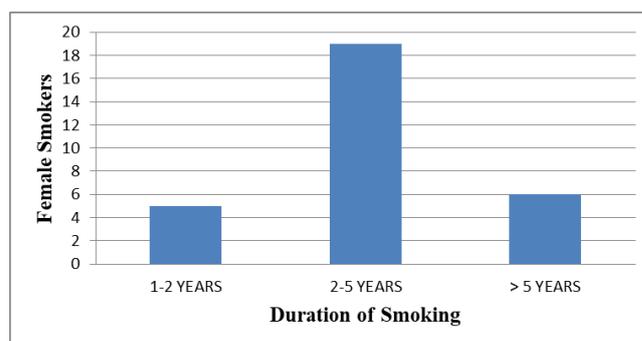


Figure 2. Grouping of female smokers based on

duration of smoking.

In Figure 3 it appears that cigarette consumption in females who smoke longer. Two out of five female smokers who have smoked for 1-2 years consume 1-4 cigarettes per day, and three of them consume cigarettes as much as 5-14 cigarettes/day. Female who have smoked for 2-5 years on average consume cigarettes as much as 5-14 cigarettes/day. Female who have smoked for more than five years consume cigarettes as much as 5-14 cigarettes/day and more than 14 cigarettes/day.

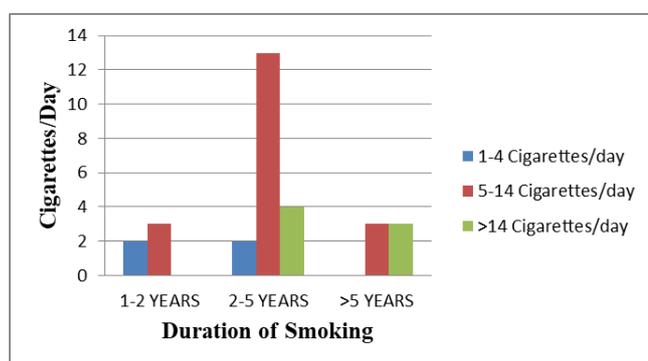


Figure 3. Duration of smoking to number of cigarette consumption / day.

The results of this study indicate that the flow rate in female smokers is different. In Figure 4 it can be seen that 3 female smokers had a low salivary flow rate of less than 0.2 mL/minute, as many as 19 smokers had a salivary flow rate of 0.2-0.3 mL/minute and 8 other smokers had a salivary flow rate 0.3-0.6 mL/minute. The average salivary flow rate in 30 samples of smokers is 0.289 mL/minute.

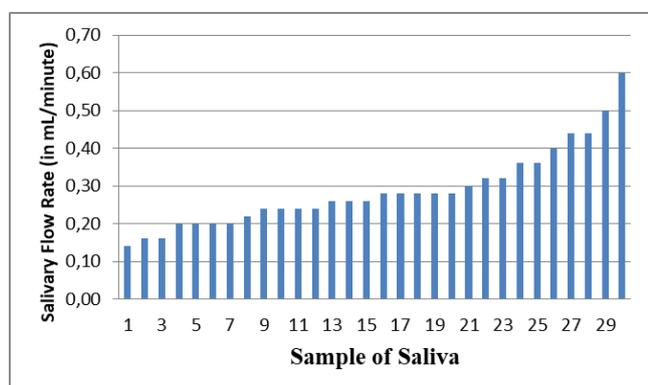


Figure 4. Salivary flow rate in female smokers.

Figure 5 shows the rate of salivary flow seen from the duration of smoking. Females who have smoked for 1-2 years have lower salivary

flow rates than females who have smoked for 2-5 years and more than 5 years. The average salivary flow rate in females who have smoked 1-2 years is 0.236 mL/minute. Female who have smoked for 2-5 years have an average salivary flow rate of 0.286 mL/minute. The average salivary flow rate in females who have smoked for more than five years is 0.287 mL/minute.

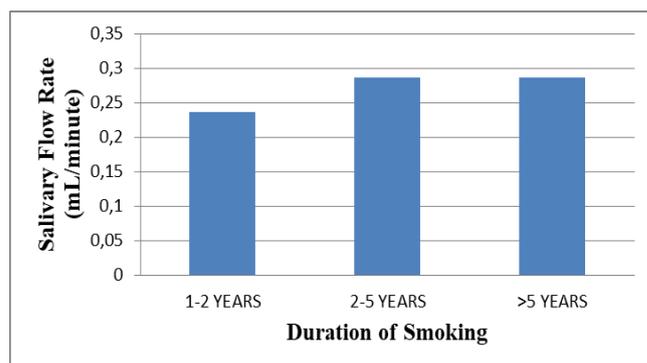


Figure 5. Relation of salivary flow rate and duration of smoking.

The results of inoculation saliva samples of female smokers in the blood plates agar can be seen in Figure 6. Bacterial colonies were grown on the blood agar plate are round with different diameter sizes. Description of faculties of facultative anaerobic bacteria that were grown on the plates so that the blood is mostly greenish-white around the colony, in addition to colonies measuring 1-2 mm in diameter which are white and yellowish-white.



Figure 6. Description of facultative anaerobic bacteria colony of female smokers in blood agar

plate.

The results of the calculation of the number of anaerobic facultative bacterial colonies in female smokers obtained by the average number of bacterial colonies from 30 smokers samples of female smokers were $(167.63 \pm 43.16) \times 10^5$ CFU / mL.

Discussion

Female smokers have a salivary flow rate of 0.2-0.29 ml/minute. Salivary secretion in normal conditions reaches 0.4 - 0.5 mL/minute. Salivary secretions as much as 0.2 - 0.4 mL/min indicated oligosialia or reduced salivary secretions. Someone indicated hyposalivation when salivary secretion was less than 0.2 ml/minute.⁷ Haroen, 2002 states that the salivary flow rate in rest position is 0.538 mL/minute. These data show that the average salivary flow rate in female smokers is lower than that of non-smokers.

Long-term consumption of cigarettes is thought can cause a decrease in taste sensitivity associated with salivary secretion reflexes.⁸ The nicotine content in cigarette smoke will be absorbed through the lungs and mucous membranes then flowed through the bloodstream to the brain and all body tissues within 10- 20 seconds. Nicotine will work on certain cholinergic receptors in the brain which can affect central nervous system activity resulting in changes in salivary secretion and taste sensitivity. Blood flow that carries nicotine can also affect blood vascularization in the salivary glands, resulting in changes in gland function and morphology.⁹ These changes can disrupt the saliva flow rate.⁹ Nicotine and other tobacco contents that flow through blood vessels to the brain are thought to inhibit aromatase activity in the brain so that it can cause a decrease in the secretion of estriol, estradiol, and estron resulting in a decrease in plasma estrogen in female smokers. Decreased estrogen levels caused by smoking can affect the maturation process of the oral cavity into the thin and atrophic cavity, causing changes in gland function and salivary flow rate.¹⁰

Changes in salivary flow rates due to smoking can cause a decrease in the antibacterial content of saliva such as IgA which functions as antibodies to bacterial antigens and can inhibit the attachment of bacteria to the oral

cavity. In smokers, there is also a decrease in bicarbonate content in saliva which is associated with a decrease in the pH of the oral cavity. The results of Grover et al . 's study showed that the pH in smokers was 6.75 while the pH of non-smokers was 7.¹¹ This was caused by a decrease in the buffer system function in saliva which serves to inhibit colonization of pathogenic microorganisms by preventing bacteria from utilizing environmental conditions that support growth.^{12,13}

The results of this study showed the number of anaerobic facultative bacteria in 30 smoker samples of smoker female obtained an average of 167.63×10^4 . In female non-smokers, the average number of anaerobic facultative bacterial colonies was 119.750×10^5 ¹². These results showed that the average number of facultative anaerobic bacterial colonies in female smokers was higher than that of non-smokers. When smoking, there is a decrease in oxygen levels and an increase in carbon dioxide in the oral cavity caused by cigarette smoke. The results of the analysis of Jing Wu et al., Stated that cigarette smoke can make the oral environment tend to be facultative anaerobic. The anaerobic facultative environment can support the growth of facultative anaerobic bacteria in the oral cavity.¹³

Previous research on oral bacteria found the presence of the bacteria *Streptococcus sobrinus* and Eubacterium branched in smoker's saliva. This study shows facultative anaerobic colonies that grow on greenish-white blood agar around the colony as oral *Streptococcus* (*S.viridans*) which indicate the presence of α -hemolysis on blood agar or greenish color in colonies.⁵ Increased growth of *Streptococcus* in female smokers shows an increase of facultative anaerobic bacteria.^{14,13} That caused by the nicotine content in the bloodstream which can affect the function of neutrophils in killing bacteria. Neutrophils have bactericidal activity against oral pathogens by producing microbiocidal reactive oxygen species (radical oxygen). The effect of nicotine in inhibiting the antimicrobial aerobic function of neutrophils can change the ecology of microorganisms in the oral cavity, resulting in a decrease in oral cavity health in smokers.¹⁵

However, data processing by statistician and further research needs to be done in this study to determine differences in

salivary flow rate and the number of facultative bacterial colonies in smokers and non-smokers.

Conclusions

Based on the results of research that has been done can be concluded that in the oral cavity of female smokers obtained salivary flow rates indicated oligosialia and the number of facultative anaerobic bacteria as much $(167.63 \pm 43.16) \times 10^5$ CFU/mL.

Declaration of Interest

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