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Correlation between presence of bacillus species and OHI-s in recurrent aphthous stomatitis patients

Amy Nindia Carabelly¹, Maharani Lallyza Apriasari², Irmamanda Dwipura³, Selviana Rizky Pramitha², Viranda Sutanti⁴, Annisa Kurniasari⁵, Riznasyarielia Nikmatun Nafisah⁵

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INTRODUCTION

Recurrent aphthous stomatitis (RAS) is a recurrent painful ulcerative disorder that occurs in the oral cavity.¹ This lesion is presented in 25% of cases of

total ulcer prevalence (4%) that occur among the worldwide population.² The prevalence of RAS in Indonesia was 2.6% in males and 3% in women.³ The prevalence of RAS at the Djafar Harun General Hospital, Southeast Sulawesi Province- Indonesia

in 2022 was 33,38%.⁴ Based on the Patient's medical records from 2010-2012, RAS was found in 55% of all the oral ulceration cases at Universitas Padjadjaran Dental Hospital, Bandung-Indonesia.⁵ Based on the patient's medical records from 2017-2019, the prevalence of RAS was 76,5% at the Ulin Hospital, Banjarmasin-Indonesia.⁶ In Gusti Hasan Aman Dental Hospital Banjarmasin, it was also reported that the population gets affected with a 45.42% incidence rate.⁷ RAS clinically appears as round, single, or multiple ulcers surrounded by an erythematous halo edge which recurrently manifests in the mucosa of the oral cavity.¹ The etiology of RAS has yet been identified for sure, however several predisposing factors may promote the occurrence of RAS including food, virus, bacteria, dysregulation of the immune system, and poor oral hygiene.^{1,8}

Bacillus sp. is a gram-positive bacteria, rod-shaped, spore-forming, and is aerobic or facultatively anaerobic.⁹ Bacillus sp. can form endospores that can withstand physical, chemical, antiseptic compounds, and extreme weather factors. Bacillus spores can spread through the air, dust, water, and food.¹⁰ Ulcers found in RAS are potentially contaminated with Bacillus sp. This happens because Bacillus sp. is a bacterial pathogen that can enter through inflammation or dermal exposure.⁵ Oral hygiene is affected by debris and calculus. Oral Hygiene Index Simplified (OHI-S) is an index that describes the condition of oral hygiene assessed based on the debris index and calculus index.¹² Poor oral conditions will increase the possibility of bacterial contamination, especially for ulcers found in RAS.¹³ No studies have revealed the existence of Bacillus in RAS patients, therefore this study aimed to analyze correlation between the presence of Bacillus species and OHI-S in the oral ulceration of RAS patients.

METHODS

This was an analytical cross-sectional study conducted among Oral Medicine Department outpatients at Gusti Hasan Aman Dental Hospital, Banjarmasin, Indonesia.

Sample Criteria

The population of this study was individuals

diagnosed with RAS at the Oral Medicine Departement of Gusti Hasan Aman Dental Hospital, Banjarmasin from August to October 2018.

The sample was identified using a consecutive sampling method in which the individual was selected based on inclusion and exclusion criteria. Inclusion criteria comprised an individual's willingness to be a respondent in this study with an age range of 12 to 35 years old. The exclusion criteria comprised individuals who consumed mouthwash and oral antibiotics in the last seven days, individuals with a history of systemic disease, and pregnant women. Based on those standards, 38 subjects attained the inclusion criteria.

Data Collection

Patient name, age, gender, occupation, and address were recorded in addition to history of any systemic disease. All subjects agreed to participate in this study after collecting informed consent, with a 100% response rate. OHI-S was assessed with 0.0 - 1.2 score in a good category; 1.3 - 3.0 score in a fair category and 3.1 - 6.0 score in the poor category.

Smear collection for microbiological investigation

Assisted by a sterilized mirror, smear from the oral ulcer site of the subjects was collected. Cotton swabs were dipped in 0.9% NaCl solution and pressed against the tube wall to drain the solution. Cotton swabs were then applied in trust and rotating movement which was directly inserted into 0.9% NaCl solution.

Bacterial examination procedure

Swab samples were inoculated on Brain Heart Infusion (BHI) agar and grown at 37°C for 24 h under aerobic conditions, then inoculated in blood agar using the four-quadrants technique. Swab sample sticks were applied for bacterial inoculation in quadrant 1 followed by inoculation loop in quadrants 2 to 4. The media were then kept in an anaerobic jar and incubated at 35-37°C for 48 hours. Colony observations revealed rod, gray, opaque, and uneven layer surfaces. Rough and spread colonies were identified using the Gram staining method and observed under 10x100 magnifications using a microscope (Olympus

CX 22). Bacteria were then identified using the catalase test, urease test, and oxidase test.

Statistical analysis

The data was then analyzed in the SPSS program using the Spearman statistic test.

Research authorization letter No 075/KEPKG-FKGULM/EC/VIII/2018 was obtained from the Research Ethics Review Committee, Faculty of Dentistry Lambung Mangkurat University, Banjarmasin.

RESULTS

The colonies contained in the blood agar plate were identified first. The identification of bacteria is shown in table 1. After the bacterial identification process was carried out, 4 types of bacteria were found in the ulcer lesions of RAS patients.

Table 2 demonstrates the frequency of bacteria detected in RAS patients. The most dominant bacterium was *Bacillus* sp., followed by *Streptococcus* sp., *Neisseria* sp., *Lactobacillus* sp.

Table 1. The identification of bacteria in the oral ulceration of RAS patients

	Colonies in Blood Agar Plate				Gram stain			catalase test	Urease test	Oxidase test
	shape	Elevation	Characteristic	Color	Shape	Structure	Gram test			
<i>Bacillus</i> sp.	round	rough	Unhemolysis	white	rod	chain	+	+	+	+
<i>Streptococcus</i> sp.	round	smooth	Hemo Lysis	white-pale creamy	coccus	chain	+	-	+	+
<i>Lactobacillus</i> sp.	round	smooth	Unhemolysis	white-plae	coccus	single	+	-	+	+

Table 2. The frequency of bacteria detected in RAS patients

Types of RAS Patient Bacteria	Frequency	%
<i>Bacillus</i> sp	23	60.5
<i>Streptococcus</i> sp	13	34.2
<i>Neisseria</i> sp	1	2.6
<i>Lactobacillus</i> sp	1	2.6
Total	38	100

Table 3 presents the species of bacteria associated with *oral hygiene index simplified* (OHI-S) status in RAS patients. All species were

detected in all oral hygiene categories. Spearman test resulted in $p = 0.862$ where no significant correlation was obtained.

Table 3. Oral hygiene index simplified and bacteria species

Type of bacteria	OHI-S	Frequency	%	P value
<i>Bacillus</i>	Good	7	58.3	0.862, n.s
	Fair	12	63.2	
	Poor	4	57.1	
<i>Streptococcus</i>	Good	4	33.3	
	Fair	6	31.6	
	Poor	3	42.9	
<i>Neisseria</i>	Good	0	0	
	Fair	1	5.3	
	Poor	0	0	
<i>Lactobacillus</i>	Good	1	8.3	
	Fair	0	0	
	Poor	0	0	

Significant at least at $p < .05$; n.s.: not significant

DISCUSSION

This study unveils the presence of *Bacillus* species as one of the prevailing bacteria in RAS lesions, followed by *Streptococcus* sp., *Neisseria* sp., and *Lactobacillus* sp. In this study, *Bacillus* sp. was the most common bacteria found in ulcers of RAS patients. *Bacillus* sp. was detected in all oral hygiene categories. According to the test listed in table 1, *Bacillus* sp. is a rod-shaped gram-positive bacteria.^{9,14} *Bacillus* sp. gave positive results on the gram, catalase, and oxidase tests.^{15,16} *Bacillus* sp. on blood agar, the species exhibit various degrees of hemolysis.¹⁷ Other bacteria found in RAS patients in this study were *Streptococcus* sp., *Neisseria* sp., and *Lactobacillus* sp. According to the test listed in table 1, *Streptococci* sp. Gram-positive cocci often occurring in chains. On blood agar, the species exhibit various degrees of hemolysis.¹⁷ *Neisseria* sp. are gram-negative cocci, often arranged as diplococci.¹⁸ *Lactobacillus* sp. are gram-positive, rod-shaped coccobacilli, occurring in chains, and catalase negative.¹⁹

Bacillus species were predominantly identified in oral ulceration of RAS patients at Gusti Hasan Aman Dental Hospital Banjarmasin. According to the test listed in table 2, bacteria found in RAS patients in this study were *Bacillus* sp. by 60,5%, *Streptococcus* sp. by 34,2%, *Neisseria* sp. by 2,6%, and *Lactobacillus* sp. by 6,2%. *Streptococcus* sp. and *Neisseria* sp. were predominantly detected in RAS lesions.²⁰

The unprecedented result contradicts with the previous study where Alpha-hemolytic *Streptococci*, Coagulase-negative *Staphylococci*, *Streptococcus viridians*, and *Neisseria* were predominantly detected in RAS lesions.²⁰ This indicates that *Bacillus* sp. may play a role in the contamination of RAS. The presence of *Bacillus* sp. in RAS lesions is generated as the result of their endospores' resistance to heat, radiation, disinfectant, and dehydration as well as the adherence characteristics of the spore that cause the bacteria to attach.²¹ Several *Bacillus* genus are pathogenic for humans. *Bacillus anthracis* can cause anthrax, and *Bacillus cereus* can contaminate food.²² However, *Bacillus subtilis* acts as an antibiotic.⁹ *Bacillus* sp. is also known to be able to produce antimicrobial compounds such as bacillibactin, teichuronic, diffidin, macrolatin,

bacilysin, surfactin, diffidin, fengycin, iturin, bacillomycin as antifungal compounds.²³ Antimicrobial and antifungal properties of *Bacillus* sp. can inhibit protein synthesis of other bacteria to inhibit the growth of other bacteria. *Bacillus* sp. also can grow faster which can cause the metabolism of other pathogenic bacteria to be disrupted.¹⁵ Interestingly that further research can be carried out, on whether *Bacillus* sp. which is present in ulcers of RAS patients acts as a bacterial pathogen or can accelerate the healing of RAS.

The results of this study utter no difference in OHI-S and bacteria species in RAS patients. Spearman test resulted in $p = 0.862$ ($p > 0.05$) where no significant difference was obtained (Table 3). Oral hygiene is influenced by the frequency of toothbrushing. Infrequent toothbrushing will cause accumulation of bacteria in the oral cavity which can be a dispositional factor for RAS.¹³ However, the frequency of toothbrushing only causing the progression of RAS by 21.43%.¹³

The results of this study also support a research by Widyastutik and Permadi, which stated that there is no relationship between tooth brushing frequency and the occurrence of RAS.²⁴ The occurrence of RAS is more related to genetics, trauma when brushing the teeth, lack of drinking water, high socioeconomic status, allergies, and the use of detergent-containing toothpaste.²⁴ Oral hygiene status is significantly determined by the patient's health behavior and attitudes. Health behavior is influenced by internal factors including knowledge, perception, emotion, and motivation, while external factors include both physical and non-physical environment.²⁵ Other factors that can interfere with access to oral healthcare include language and psychosocial, structural, and cultural barriers.²⁶ The limitation of this study is that respondents' behavior regarding food consumption and oral health behavior was not assessed. The bacterial examination was also limited only to the identification of genus.

CONCLUSION

Bacillus species were predominantly identified in oral ulceration of RAS patients. However, the presence of *Bacillus* sp. was not correlated with OHI-S in RAS patients.

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