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

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ABSTRACT

Introduction: The approximate prevalence of Recurrent Aphthous Stomatitis (RAS) was 45.42% at Gusti Hasan Aman Dental Hospital Banjarmasin. Bacillus sp. is a bacterium that can form spores that can be transmitted through air, dust, water and food. Poor oral conditions will increase the possibility of bacterial contamination, especially for ulcers found in RAS. This study aimed to analyzed 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. A total of 38 subjects were examined to assess Oral Hygiene Index Simplified and obtained bacteria samples using the swabs technique. Bacteria were then inoculated in blood agar base media and concurrently presented with gram staining. Bacteria were then identified using the catalase test, urease test, and oxidase test. **Results**: Bacillus species were predominantly identified in oral ulceration of RAS patients at Gusti Hasan Aman Dental Hospital Banjarmasin. Bacillus sp. (60,5%), Streptococcus sp. (34,2%), Neisseria sp. (2,6%), and Lactobacillus sp. (2,6%) were found in ulcerated RAS lesions. Spearman test resulted in p = 0.862 ($p > 0.05$), where no significant difference was obtained between presence of bacillus species and OHI-s in recurrent aphthous stomatitis patients. **Conclusion**: Bacillus species were predominantly identified in oral ulceration of RAS patients a. However, the presence of Bacillus sp. was not associated with OHI-S in RAS patients.

Keywords: bacillus sp.; oral hygiene index simplified; recurrent aphthous stomatitis

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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

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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.[11] 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 analyzed 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			
Bacillus sp.	round	rough	Unhemolysis	white	rod	chain	+	+	+	+
Streptococcus sp.	round	smooth	Hemo Lysis	white-pale creamy	coc-cus	chain	+	-	+	+
Lactobacillus sp.	round	smooth	Unhemolysis	white-plae	coc-cus	single	+	-	+	+

Table 2. The frequency of bacteria detected in RAS patients

Types of RAS Patient Bacteria	Frequency	%
Bacillus sp	23	60.5
Streptococcus sp	13	34.2
Neisseria sp	1	2.6
Lactobacillus 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
Bacillus	Good	7	58.3	
	Fair	12	63.2	
	Poor	4	57.1	
Streptococcus	Good	4	33.3	
	Fair	6	31.6	
	Poor	3	42.9	
Neisseria	Good	0	0	0.862, *n.s*
	Fair	1	5.3	
	Poor	0	0	
Lactobacillus	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. are 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, difficidin, macrolatin, bacilysin, surfactin, difficidin, 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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