Microbiological Profile in Oral Cavity Infection in Diabetic Rats with Periodontitis

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ABSTRACT

Background: *Diabetes melitus is a chronic metabolic disease due to the inability of the pancreas to produce insulin or the body cannot use insulin effectively.Periodontitis is one of the complications of microvascular disorders that ranks second in oral cavity diseases. One of the aerobic bacteria thought to play a role in the severity of diabetes melitus is Staphylococcus aureus, while the pathogenic anaerobic bacteria in periodontal disease are Treponema, Bacteroides, Porphyromonas, Prevotella, Capnocytophaga, Peptostreptococcus, Fusobacterium, Actinobacillus, and Eikenella. The study aimed to determine the microbiological profile in oral cavity infections of rats with diabetes melitus and periodontitis*

Method: *This study was experimental laboratories on male wistar rats induced by diabetes melitus and periodontitis using wire ligature on mandibular molars, which were divided into 3 treatment groups: group I (control), group II (diabetes melitus) and group III (diabetes melitus and periodontitis). Periodontitis (wire) rats were treated for 7 days and saliva was collected to identify the microbiological profile of the oral cavity*.

Result: *Identification indicated the presence of genus and species of bacteria in the saliva of rats in 3 groups, gram positive: Staphylococcus intermedius, Staphylococcus cohnii subsp.urelyticus. Gram negative: Chromobacterium violaceum, Kleibseilla pneumoniae, Eikenella corrodens, Enterobacter sakazaki and Chryseobacterium meningosepticum.*

Conclusion: *The number of bacterial colonies in the treatment group of diabetes melitus and periodontitis rats was greater than the treatment group of diabetes melitus rats and the control group. Gram staining results found groups of gram positive and gram negative bacteria.*

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INTRODUCTION

Diabetes melitus is a metabolic disorder that occurs in many communities with a total of 171 million people worldwide and is projected to increase to 366 million by 2030. Diabetes melitus is a clinically and genetically heterogeneous group of disorders affecting carbohydrate, lipid and protein metabolism. Diabetes melitus is characterized by hyperglycemia due to disruption of pancreatic hormones in regulating insulin levels in the body.[1,2] Hyperglycemia in diabetes melitus is directly related to a number of complications, both macrovascular and microvascular. Macrovascular abnormalities in the form of atherosclerosis of blood vessels that can cause myocardial infarction and the risk of stroke. Meanwhile, microvascular complications include impaired healing, neuropathy, risk of retinopathy infection, advanced kidney damage and periodontitis. Periodontitis is the 6th complication of diabetes melitus.[3] Several scientific evidences show that diabetes melitus and periodontitis modulate an increase in the host immune system and an increase in pro-inflammatory cytokines in initiating tissue damage.Vascular complications in diabetes melitus are directly related to the formation of ROS (reactive oxygen species) and are mediated by AGEs (advanced glycation product). Research that has been done shows that in diabetics there is an increase in the amount of ROS and a decrease in antioxidants in the gingival tissue which triggers inflammation and destruction of the periodontal tissue. Excessive ROS production causes damage to DNA, lipids, proteins, tissues, apoptosis and triggers further complications of diabetes.[4] Further complications are supported by the role of oral bacteria microorganisms.

Balci et al reported that bacterial inoculation in the periodontal tissues of diabetic rats was shown to increase the severity of periodontal disease. Otherwise,increased blood glucose levels (HbA1c) have been shown to be associated with the number of red complex bacteria, including P. gingivalis which increases systemic inflammation, especially in adipose tissue through the induction of IL-6 which can induce insulin resistance. Based on research conducted by Kang et al showed that P gingivalis infection can increase inflammation in the form of increased mRNA expression of pro-inflammatory cytokines (TNF-a, IL-6, IL-17, and IL-23) and chemokines (CCL2, CCL8, and CXCL10) in serum causing impaired glucose metabolism in mice.[5] Normal flora of the oral cavity with a poor environment can multiply into pathogens. Beside of bacteria, saliva has a role as a medium for bacterial growth. According to Caixeta 2020 that in patients with diabetes melitus there is an increase in salivary glucose levels. Glucose is like a small molecule that can move easily in the vascular membrane, which can exit the blood plasma into the gingival fluid through the gingival sulcus, then reach the saliva. Increased blood glucose levels in diabetic patients can cause glucose levels in saliva to be higher, which results in a loss of homeostasis and a greater susceptibility to diseases in the oral cavity. Glucose is a good medium for the proliferation of aerobic and anaerobic bacteria in the oral cavity.[6,7]Gram-positive and Gram-negative bacteria increase in number due to increased blood glucose levels in patients with diabetes mellitus. According to Pase et al showed that in DM patients there was an increase in the number of S. aureus and Klebseilla peneumoniae bacteria compared to non DM patients. This is supported by previous research that in DM patients there were gram-positive Streptococcus 47%, alpha haemolytic Streptococcus (2%), gram-positive rods 31.4%, gram-positive Staphylococcus 19.6%, and gram-negative rods 2%, gram-negatives including Neisseria and Veillonella, Klebsiella pneumonia (25.7%), Staphylococcus epidermidis (11.5%) and Strepteococcus sanguis (11.1%).[8–10]Several studies suggest there is a relationship between the number of aerobic bacteria and an increase in the number of anaerobic bacteria in the oral cavity which has an impact on the progression of advanced periodontal disease. One of the aerobic bacteria thought to play a role in the

severity of diabetes melitus is *S. aureus*. The colonization is a risk factor for infection. Among the virulence factors of S. aureus, superantigens (SAgs) are important for pathogenicity. The long-term effect of superantigen toxin-1 (TSST-1) toxic shock syndrome is glucose intolerance. This toxin also causes systemic inflammation as a result of an increase in the concentration of exotoxins in the blood and is a contributing factor to diabetes. The results of research conducted by Klekotka et al stated that there was an increase in *S. aureus* colonization in the nasal cavity of people with diabetes melitus compared to non-diabetics.[11] While the dominant bacteria in the periodontitis is the etiology of periodontitis.

Gram negative anaerobic bacteria in the oral cavity include the black pigmented bacteria (*Porphyromonas, Prevotella Treponema, Bacteroides, Capnocytophaga, Peptostreptococcus, Fusobacterium, Actinobacillus,* and *Eikenella*. These bacteria are found in saliva, gingival epithelium and other internal surfaces of the oral cavity and are concentrated in dental plaque. In mature plaque microbial dysbiosis occurs, which causes a progressive from Gram-positive to Gram-negative dominant anaerobic species.[12] The existence of aerobic and anaerobic bacteria in the oral cavity needs to be studied further, this is because there is an interaction of metabolic nutrients between bacterial species. Aerobic bacteria in dental plaque such as *Streptococcus* produce metabolic products. *Streptococcus* aerobic bacteria produce metabolic products such as lactate, formate, H2O2, succinate and CO2. Lactate is a nutrient for *Veilonella*, while formate is needed by *Woilella campyrobacter* to grow. *Veilonella* produces vitamin K products, while *Woilella campyrobacter* produces protohaem. Vitamin K and protohaem are nutrients needed by black pigmented bacteria such as *P. gingivalis* and *Prevotella intermedia*. Therefore, there is a need for further research on the identification of aerobic and anaerobic bacteria in the oral cavity in order to explain the interaction or relationship of mutually beneficial nutritional needs in the growth of pathogenic bacterial species.[13] Based on the above background, the researchers wanted to identify and characterize oral bacteriopathogens (aerobic and anaerobic) in a rat model of diabetes melitus with periodontitis, so that this study can describe the relationship between the presence of aerobic and anaerobic bacteria that are interrelated in causing periodontal and systemic tissue damage. Furthermore. This research is based on the research roadmap, which is to identify microorganisms as triggers for systemic disease by identifying oral bacteria such as *S. aureus* that plays a role in the occurrence of diabetes melitus and oral periodontopathogenic bacteria.

RESEARCH METHOD

The Animals study will be carried out on experimental male wistar rats (Rattus novergicus) with predetermined criteria. The research was conducted based on ethical clearance at the Research Ethics Commission of the Faculty of Dentistry, Jember University No. 1268/UN25.8/KEPK/DL/2021. Animals were acclimatized for one week before being treated for adaptation of rats in cages and given standard feed and drink.

The Animal Groups

Experimental animals that have been acclimatized are divided into 3 groups as follows:

a. Group I is a control/normal group that is not treated

b. Group II is a group of diabetes melitus

c. Group III is a group of diabetes melitus with periodontitis using a wire ligature

Diabetes melitus Rats

Diabetic rats were obtained by injecting streptozotocin (bioWORLD, USA) using a single dose of 45 mg/Kg BW intraperitoneally. Rats were given standard food and drinking ad libitum. After the streptozotocin was injected, the rats' blood sugar levels were checked by taking peripheral blood from the tail vein using the Glucose Stick Test. The method used is enzymatic.

Measurement of Blood Sugar Levels

Examination of blood sugar levels was carried out before induction and 1 week after streptozotocin induction to determine whether the rats had diabetes. The KGD examination is done by placing one drop of blood from the tail vein on the Glucose Stick Test tool (GlucoDr, Germany). The tool will automatically bring up the level of sugar contained in the blood. If the KGD > 200mg/dl the mice were classified as having diabetes.

Periodontitis rat models (wire ligature)

After streptozotocin induction and the rats were declared to have diabetes, the rats were treated with periodontitis using a wire ligature (Dentsply, USA). Wire ligature made of wire (stainless steel wire) measuring 0.5 mm in diameter was attached to the cervix of the lower left molar. The wire ligature is shaped like a U using coil pliers and is attached to the mesial hug of the rat molar, then the wire ligature is carefully pressed down so that it is right on the cervical tooth, the final position of the wire ligature is above the gingival sulcus so as not to cause irritation. Installation of this wire ligature serves for mechanical stimulation and as retention of dental plaque, thereby accelerating the occurrence of periodontitis. Wire ligature was installed for 7 days to get periodontitis condition.

Identification rat saliva

The rat saliva was collected after the rat was declared to have diabetes melitus and periodontitis. Before sampling, the rats were anesthetized using ketamine, sterile paper points were inserted into the oral cavity of the rats in the area of the mandibular first molar which had been treated with wire ligature for 10 seconds. A total of 3 paper points were inserted alternately in the mandibular first molar area to absorb saliva. Two paper points in the lingual area and one paper point in the buccal area. The aim is to obtain an adequate amount of saliva so that sufficient bacteria can be identified and counted. Then the paper points were taken and put into an Eppendorf tube containing 3 ml of PBS and labeled according to the treatment group.

a**. Inoculation on culture media**

The sample in an eppendorf tube containing 3 paper points in PBS solution was centrifuged (10,000 rpm) for 10 seconds, then 1 ml of the sample was diluted with 9 ml of aquadest solution and diluted until it reached 10^{-5}. This dilution was carried out in laminar flow to prevent contamination. The diluted sample solution was taken 0.1 ml and inoculated on MSA (Mannitol Salt Agar) and brucella agar media. Inoculation was done by streaking technique using a sterile loop on petridish culture media. Inoculation was carried out in laminar flow to prevent contamination.

b. Identification and Characterization of microbiological profile

The medium that has been inoculated with saliva is incubated for 24 hours to see the growth of bacterial colonies. On brucella agar media, after growing bacterial colonies, incubation is carried out again for 4-8 days to see the growth of black pigmented bacteria (black colonies) and colony counts are carried out using a colony counter with CFU/ml (colony forming units/ml) units. Bacterial identification was carried out by taking different colonies in each treatment group for Gram staining. This painting aims to identify gram-positive and gram-

negative bacteria as well as the morphology of coccus, bacillus or coccobacillus bacterial cells. Bacterial characterization can be done by looking at the colony morphology, color and structure of bacterial cells. To determine the bacterial species, biochemical tests were carried out on both MSA and *brucella* agar media bacteria. Based on this biochemical test, it is expected to identify the presence of *Staphylococcus aureus* and black pigmented bacteria.

RESULTS

This study aims to determine the profile of oral bacteriopathogens in a rat model of diabetes melitus with periodontitis. Rats were declared to have diabetes melitus if their blood glucose levels reached > 200 mg/dl. Mice were declared periodontitis if there was periodontal tissue damage which was characterized by a reddish color on the gingiva, alveolar bone resorption, periodontal ligament damage and gingival recession.

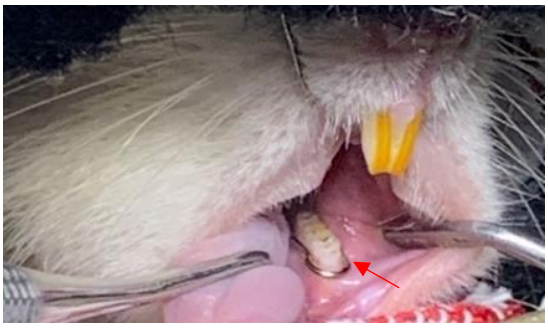

Figure 1. Wire ligature in the rat's left lower molar region (red arrow)

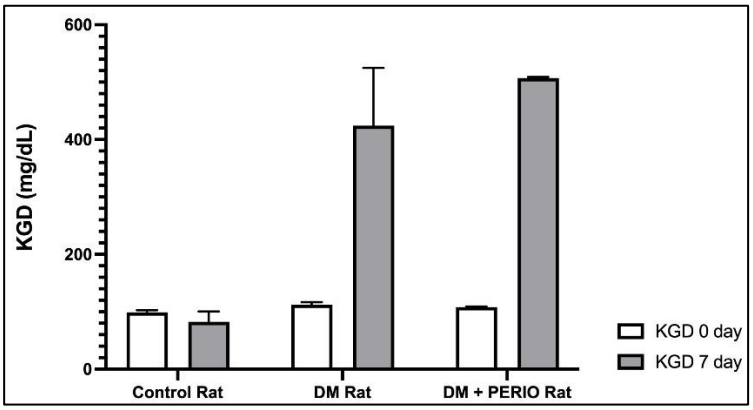

Figure 2. Blood glucose levels in normal rats, rats with diabetes melitus, rats with diabetes melitus and periodontitis before and 7 days after STZ injection

a. Blood Glucose Level

Based on the table above, it can be seen that in the early stages before STZ injection, the average KGD was normal, which was 106 mg/dL. In group 2, there was an increase in KGD from 112mg/dL to 424.3 mg/dL, while in group 3 there was an increase in KGD from 108mg/dL to 507mg/dl. Based on the table above, it can be seen that there was a significant increase in KGD ($p<0.05$) in DM rats with periodontitis by administering a wire ligature. After the treatment was carried out, then the identification and counting of the number of bacterial colonies of rat saliva was carried out according to the treatment group.

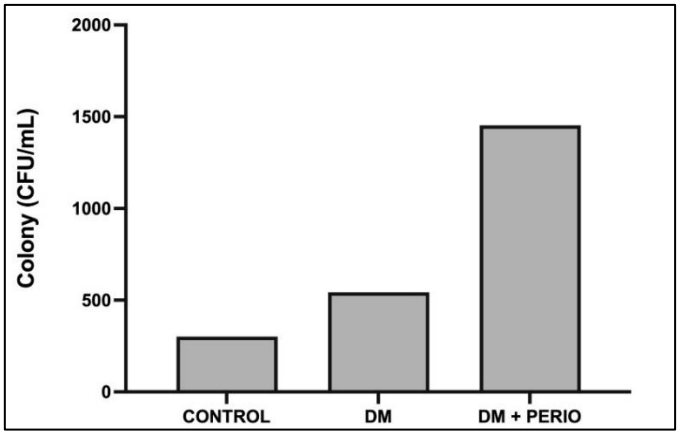

Figure 3. The number of bacterial colonies CFU/ml

b. Counting the number of bacterial colonies

Figure 3 shows that there was an increase in the number of bacterial colonies in the treatment group compared to the control group. In the control group it was 3.01 x 102 CFU/ml, in the group with diabetes melitus it was 5.43 x 102 CFU/ml and in the diabetes treatment group with periodontitis it was 1.453 x 103 CFU/ml.

c. Identification of rat's saliva bacteria

Bacterial identification was based on Gram staining of bacterial colonies cultured on MSA media and Brucella agar media. MSA was used to identify gram-positive bacteria, while Brucella agar was used to identify gram-negative bacteria. To identify the shape of bacterial cells using a 1000x magnification microscope. Identification was taken from different colonies to determine the species of bacteria found in rat saliva.

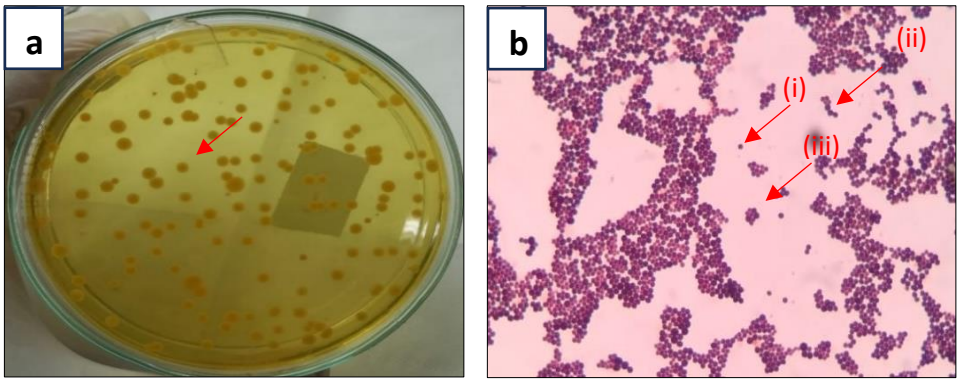

Figure 4. (a) Yellow bacterial colonies on MSA group 1, (b) identification of bacteria by Gram staining and using a 1000x magnification microscope showing Gram positive cocci in (i) solitary arrangement, in rows (ii) and in clusters (iii)

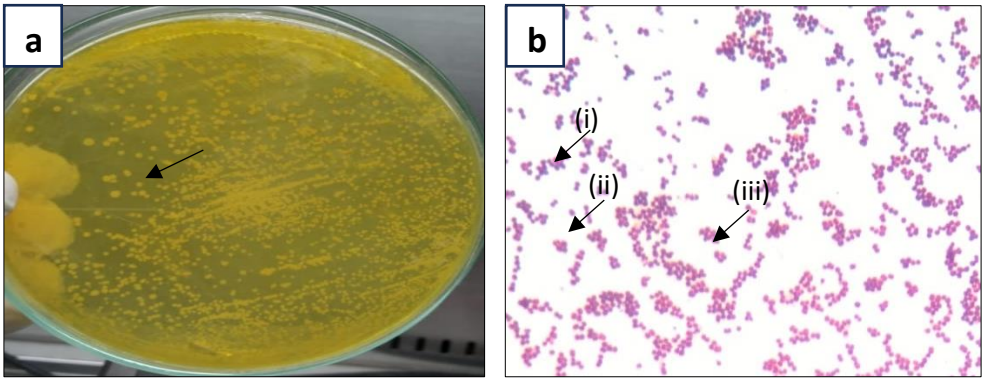

Figure 5. (a) Yellow bacterial colonies on MSA group 2, (b) identification of bacteria by Gram staining and using a 1000x magnification microscope showing Gram positive cocci in solitary arrangement (i), in rows (ii) and in clusters (iii)

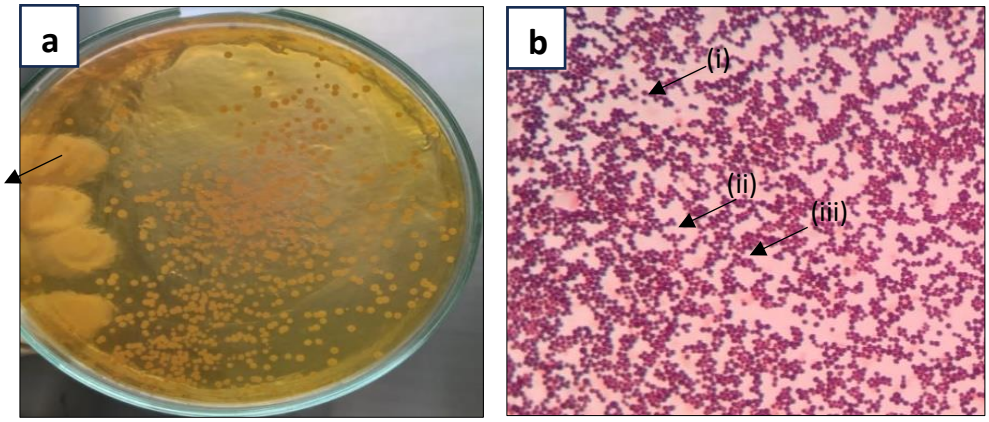

Figure 6. (a) Yellow bacterial colonies on MSA group 3 (b) identification of bacteria by Gram stain and using a 1000x magnification microscope showing Gram positive cocci in (i) solitary arrangement, in rows (ii) and in clusters (iii)

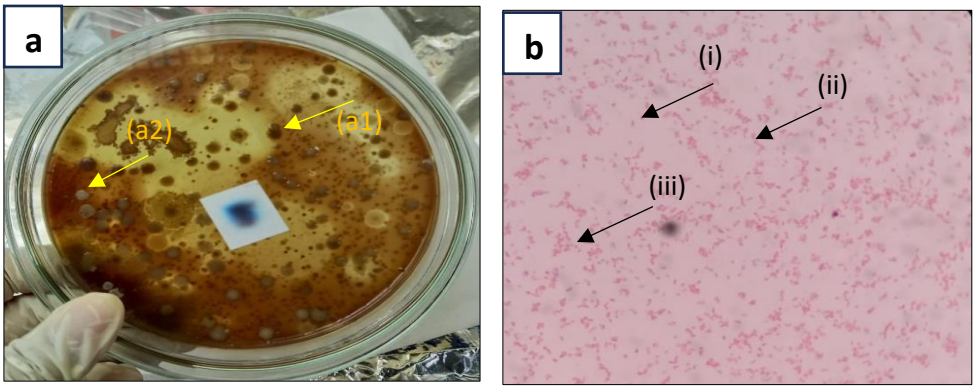

Figure 7. (a1) black colonies, (a2) gray colonies on Brucella agar group 2, (b) identification of bacteria by Gram staining and using a 1000x magnification microscope showing Gram negative cocobacillus in (i) solitary arrangement, in rows (ii) and clustered (iii)

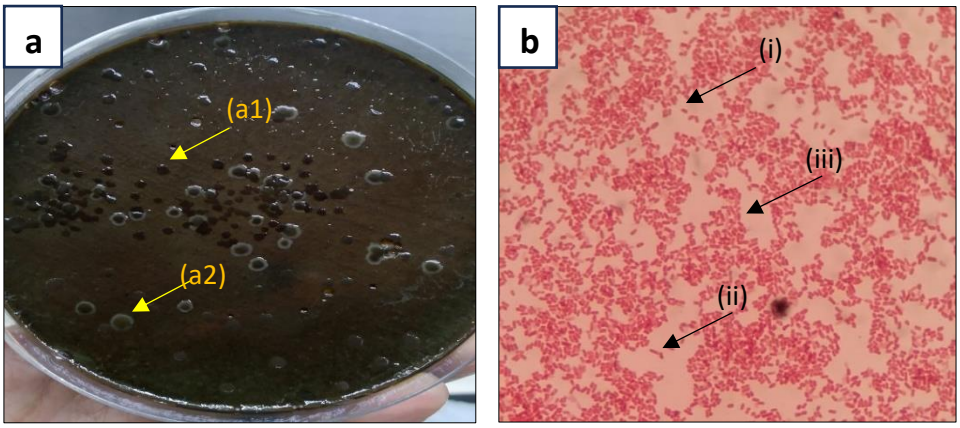

Figure 8. (a1) black colonies, (a2) gray colonies on Brucella agar class 2 media, (b) identification of bacteria by Gram staining and using a 1000x magnification microscope showing Gram negative cocobacillus in (i) solitary arrangement, in rows (ii) and clustered (iii)

After identification of bacteria and morphology of bacterial cells, the next step is to determine the genus and species of salivary bacteria in the 3 treatment groups using biochemical tests and bacterial species identification tests. Bacterial species identification test using MINDRAY TDR-300 PLUS, this tool also plays a role in determining the type of antibiotic that fits the bacterial species correctly.

Table 1. Bacterial Identification

No	Identification	Genus	Spesies	Persentage
1	Gram positif bacteria			
	Yellow colony	*Staphylococcus*	*Staphylococcus intermedius*	99,69%
	Yellow colony	*Staphylococcus*	*Staphylococcus cohnii subsp.urelyticus*	99,39%
	White colony	*Staphylococcus*	*Staphylococcus cohnii subsp.urelyticus*	93,67%
2	Gram negatif bacteria			
	Black colony	*Chromobacterium*	*Chromobacterium violaceum*	92,01%
	Mucoid colony	*Kleibseilla*	*Klebsiella pneumoniae*	94,11%
	Small black colony	*Chromobacterium*	*Chromobacterium violaceum*	92,36%
	Transparant white colony	*Eikenella*	*Eikenella corrodens*	89,69%
	Black colony	*Enterobacter*	*Enterobacter sakazakii*	99,39%
	Black colony	*Chryseobacterium*	*Chryseobacterium meningosepticum*	98,64%

DISCUSSION

The study was conducted on experimental animals wistar rats induced by diabetes melitus and periodontitis by using a wire ligature inducer. Diabetes melitus is a disease characterized by chronically elevated blood glucose concentrations compared to normal conditions. Several studies have used experimental rats to make diabetes melitus, this is because it resembles the human condition to study the pathogenesis and mechanism of the disease. Research on diabetes melitus in experimental rats using

streptozotocin has been shown to cause damage to pancreatic beta langerhans cells which causes an increase in blood glucose levels in rats compared to control (without treatment).[14] In control rats, the average fasting blood glucose level was 108 mg/dL, whereas after streptozotocin induction there was a significant increase of 465 mg/dL. These results are supported by research conducted by Saputra et al that streptozotocin induction can cause mild, moderate and severe increases in blood glucose levels. Streptozotocin is effective in increasing blood glucose levels.[15]

Increased blood glucose levels associated with complications of diabetes melitus accelerate the formation of AGEs. The binding of AGEs to the RAGE receptor triggers the release of proinflammatory mediators such as TNF alpha, IL-1β, IL-6 and MMPs. Elevated blood glucose levels worsen the condition of the periodontal tissue and some opinions state that periodontitis is the 6th complication of diabetes melitus. Research has shown an association between diabetes and an increase in proinflammatory mediators, osteoclastogenic factors and matrix metalloproteinases. Several researchers have shown that diabetes melitus interferes with host defense including chemotaxis, adherence, phagocytosis and apoptosis that trigger tissue damage. Mice were declared periodontitis because there were clinical signs of inflammation in the periodontal tissues which included redness of the gingiva due to increased vascularity, enlargement of the gingival margin due to hyperemia and tooth mobility. If an X-ray is performed, alveolar bone resorption will be found.[16]

Bacterial identification was carried out in 3 groups, namely control, diabetes melitus group and diabetes melitus group with periodontitis. Bacterial identification was taken from rat saliva in the right lower molar region using paper points and bacterial growth was carried out on MSA and Brucella agar media. Figure 3 shows that the highest number of bacterial colonies was found in group 3, namely rats with induced diabetes melitus with periodontitis at 1,453 x 103 CFU/ml. One of the identifications of bacterial species is based on the colonies growing on the media. A colony is defined as a visible mass of all microorganisms and originates from a single cell, so a colony is a clone of all the same genetics. Colony characteristics can be useful in the early identification of bacterial species. Colonies with different characteristics, when grown on the same medium, can be assumed to contain different bacterial species.[17]

Bacterial colony morphology can be distinguished based on size, shape, elevation, margin, color, surface, density and consistency. Based on the research data, it was found that the color variations of bacterial colonies in both MSA and Brucella agar media were found, namely white, yellow, gray and black colonies. Other studies have stated that the color of bacterial colonies varies, consisting of white, yellow, black or orange pigments.[18] Pigmentation is important as a taxonomic tool for bacterial identification. These pigments play an important role in physiological and molecular processes of microorganisms such as photosynthesis, resistance to oxidative damage and resistance to ultraviolet (UV) radiation. The color variation of bacterial colonies is due to differences in the composition of the chemical structure and the presence of certain chromatophores. Color expression can be influenced by a number of environmental factors such as oxygen availability, nutrients, temperature and colony age.

In most nonchromogenic microorganisms, cream, white or gray colored colonies will appear, while in some bacteria such as *Micrococci, Mycobacteria, Corynebacterium, Streptococcus faecium, Staphylococcus aureus* have carotenoid pigments so that they appear on yellow, orange, or reddish orange media. From the results of chromatographic analysis that this pigment is related to the presence of hydroxylates, hydrocarbons and ketone components in carotenoid pigments. The presence of these pigments is intended as a marker or defense when

conditions do not support bacterial growth.[19] This statement supports the results of the research carried out, namely in Figures 4, 5 and 6, the colonies are yellow and reddish orange and based on the results of the identification of bacteria that in these colonies belongs to the genus *Staphylococcus*.

The results of the growth of bacterial colonies were continued with the identification of bacteria to determine the genus and species of bacteria. Based on the data obtained from the results of Gram staining, two groups were obtained, namely gram-positive bacteria and gram-negative bacteria. In the group of gram-positive bacteria were obtained from the growth media of mannitol salt agar (MSA), while the gram-negative bacteria were obtained from the growth media of brucella agar. Furthermore, the results of the Gram stain were followed by identification of the genus and species of bacteria using the MINDRAY TDR-300 PLUS tool. This tool can detect the genus and species of bacteria based on the biochemical tests contained in the tool. In the gram-positive group, the genus *Staphylococcus* was found with several species including *Staphylococcus intermedius, Staphylococcus cohnii subsp.urelyticus*. *S. intermedius* is a group of *S intermedius* (SIG) including *S. intermedius, S. pseudintermedius, and S. delphini*. Several studies have shown that *S. intermedius* is a secondary bacterium to *S. aureus* that is found in patients with medical compromise, namely diabetes melitus and this bacterium is found in areas of soft tissue infection.[20–22]

Based on research data on gram negative bacteria, several genera and species of bacteria were obtained, namely *Chromobacterium violaceum*, *Kleibseilla pneumoniae, Eikenella corrodens, Enterobacter sakazaki* and *Chryseobacterium meningosepticum*. *Eikenella corrodens* found in the treatment group of periodontitis and diabetic rats with periodontitis is in line with previous studies. According to Yacoubi stated that microbiological examination in periodontitis patients with diabetes melitus found the presence of *Eikenella corrodens* bacteria. These bacteria play a role in the occurrence of periodontal tissue damage in patients with periodontitis and diabetes melitus. *Eikenella corrodens* is commonly found in dental plaque and saliva from patients with periodontitis.[23–25]

In the examination of biochemical tests in the group of rats with diabetes melitus with periodontitis, *Kleibseilla pneumoniae* species were also found, this is supported by previous research, namely that *Kleibseilla pneumoniae* is a pathogenic bacterium that plays a role in the severity of periodontal disease and is found in patients with medical compromises such as diabetes melitus.[26],[27] Based on research conducted on the treatment group of diabetic rats and the group of diabetic rats with periodontitis, several genera and species of gram positive bacteria were found, but in this study, the dominant species of *S. aureus* was not found in patients with diabetes melitus. It is possible that the *S. aureus* species was only found in human saliva, while in the experimental rats *S. intermedius* was found which is a derivative of *S. aureus* which belongs to the group of *S intermedius* (SIG). In the group of gram negative bacteria with black pigment colonies, only *Eikenella corrodens* was found for periodontal bacteria, while for other periodontopathogenic bacteria they had not been found. So that further research is needed to identify bacterial species with different samples. Bacterial identification research in periodontitis rats with diabetes mellitus is very important to find out that the more colonies of pathogenic bacteria in the oral cavity, it plays a role in increasing inflammation which is characterised by an increase in pro-inflammatory mediators which cause impaired glucose metabolism in the blood which results in the severity of diabetes melitus.[5] Based on research by Bisong et al mentioned that aerobic Gram-negative bacteria play an important role in dental disease in diabetic patients.[28] Therefore, by identifying bacteria in the oral cavity it is important to provide the right antibiotic therapy target for periodontitis patients with diabetes

mellitus. This study was conducted to identify the bacterial profile in diabetic rats with periodontitis. It aims to show the causal of periodontitis as a risk factor for diabetes. Several studies have shown the role of bacteria in the development of diabetes and impaired blood glucose metabolism. P gingivalis has an impact on increasing serum inflammatory cytokines, chemokines, and glucose-related gene expression levels in the liver and adipose tissue, resulting in impaired glucose metabolism.[29] This is in line with Narayan's 2016 study showing that C. volaceum is associated with bacteremia in medically compromised patients with diabetes mellitus. A number of clinical studies have shown that bacteria can induce pro-inflammatory mediators such as TNF-α, IL-1ß, IL-6, and IL-18 to increase in diabetic patients, contributing to insulin resistance through JNK and IKKb/NFkB pathways. IL-1β also induces pancreatic β-cell apoptosis. Increased production of inflammatory cytokines contributes to insulin resistance and destruction of pancreatic beta cells, which significantly facilitates the onset of diabetes. Therefore, it is important to know the profile of both gram-positive and negative bacteria that contribute to the increase in blood glucose levels and precipitate diabetes

CONCLUSION

Based on the results of the study, it can be concluded that the number of bacterial colonies in the treatment group of diabetes melitus and periodontitis rats was greater than the treatment group of diabetes melitus rats and the control group. Gram staining results found groups of gram positive and gram negative bacteria. Identification indicated the presence of genus and species of bacteria in the saliva of rats in 3 groups, namely gram positive: *Staphylococcus intermedius, Staphylococcus cohnii subsp.urelyticus*. Gram negative: *Chromobacterium violaceum, Kleibseilla pneumoniae, Eikenella corrodens, Enterobacter sakazaki and Chryseobacterium meningosepticum*.

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