

Identification Of Candida Albicans In Saliva Patients With Type 2 Diabetes Mellitus At The Simalingkar Health Center Medan City

Fani Nuryana Manihuruk¹, Atri Gustiana Gultom²

^{1,2}Diploma III Program of Health Analyst, the College of Health Sciences STIKes-Senior Medan

Article Info	ABSTRACT
<p>Keywords: Candida albicans, Type 2 diabetes mellitus, Saliva.</p>	<p>Diabetes mellitus is a predisposing factor for the emergence of oral candidiasis. Increased blood glucose levels can also increase glucose levels in saliva. Excessive blood sugar levels in saliva stimulate the growth of Candida albicans on the mucosal surface, as a result, Candida albicans will be found in the saliva of DM patients. Oral candidiasis can attack mucosal tissues such as the mouth and pharynx and can be a complication of cancer. Purpose of this study was to identify candida albicans in the saliva of patients with type 2 diabetes mellitus at the Simalingkar Health Center, Medan City. Method This study is a descriptive study with a cross-sectional approach. The sampling technique was random. The number of samples identified in this study was 25 respondents, macroscopic and microscopic examinations were carried out, then the data were analyzed descriptively. The result of the descriptive analysis of the examination obtained the results of the study 56% positive Candida albicans, and 44% of respondents were negative for Candida albicans fungus. Conclusion The percentage value shows that most saliva samples from type 2 diabetes mellitus patients contain Candida albicans. These results emphasize the importance of routine monitoring of blood glucose levels to prevent the occurrence of oral candidiasis more effectively in type 2 diabetes melitus.</p>
<p>This is an open access article under the CC BY-NC license</p> 	<p>Corresponding Author: Fani Nuryana Manihuruk Diploma III Program of Health Analyst, the College of Health Sciences STIKes-Senior Medan Jl. Djamin Ginting Km. 8,5 No 13, Mangga, Medan Tuntungan District, Medan City, Sumatera Utara 20141, Indonesia fanimanihuruk27@gmail.com</p>

INTRODUCTION

Diabetes mellitus is a chronic disease that can be suffered for life caused by metabolic disorders. Metabolic disorders that occur in the pancreas are characterized by an increase in high glucose levels in the blood (hyperglycemia). Hyperglycemia is caused by a decrease in the amount of insulin from pancreatic cells (Deanita, 2023).

Based on the International Diabetes Federation (2019) the world's DM case continues to increase every year. In 2011 there were 366 million people, then increased to 382 million in 2013, and also experienced an increase in 2015 and 2017 to 415 million and 425 million and continued to increase to 463 million cases of DM in 2019. In 2030 And 2045 is estimated that the case of diabetes will increase to 578 million to 700 million people diagnosed with diabetes mellitus (Dzaki, I.R., et al. 2023).

Indonesia is ranked seventh among the 10 countries with the highest number of sufferers, amounting to 10.7 million people. Indonesia is the only country in Southeast Asia in the list of 10 countries with the highest number of diabetics in the world, so that Indonesia's contribution can be estimated to the prevalence of diabetes cases in Southeast Asia (Provsu Health Office. 2020).

In Indonesia the prevalence of diabetes mellitus continues to increase as evidenced by the results of Riskesdas in 2018 increased to 8.5% from the previous amount of 6.9% in 2013. In 2019 there were 10.7 million DM cases in 2019 and in the year 2045 is predicted to increase to 16.6 million people.

Based on national studies conducted by IDF regarding the prevalence of DM there are 85-95% of adults around the world suffering from cases of type 2 diabetes mellitus (Dzaki, I.R., Et all, 2023). Increasing the DM case in Indonesia occurred due to an increase in cases in each region, one of which was in North Sumatra Province. Based on the results of data recapitulation from the health profile of North Sumatra Province (2020). The number of people with diabetes mellitus in North Sumatra Province in 2020 is 161,267 sufferers, 144,433 people (or 80-90%) have received health services (Dinkes Provsu. 2020).

From the results of Riskesda 2018, the results obtained that the prevalence of diabetes mellitus in Indonesia in the age group of 15 years and over is around 2%. Based on sex, the prevalence of women suffering from diabetes mellitus reaches 1.78% and 1.21% in men. For prevalence based on age groups suffering from diabetes mellitus the highest in the highest age group occurs in the age group 55-64 years with an amount of 6.3% (Rahayu, C, et all, 2020). The condition of diabetes mellitus can cause changes in the oral cavity that is influenced by diabetes. Some changes that occur such as dry mouth, dirty tongue, and a decrease in the acidity of saliva which is a supporting factor for changes in the number of normal flora in the oral cavity (Rahayu, C, 2020).

High blood sugar levels can also increase blood sugar levels in saliva. Excessive blood sugar levels in saliva can stimulate the growth of *Candida Albicans* fungus in mucosal surfaces. In patients with diabetes mellitus salivary disorders are one of the causes of complications in the mouth of diabetes mellitus. Abnormalities in the salivary glands can be caused by several things, one of which is the low pH sovereignty which causes *Candida Albicans* fungus to grow and multiply (Suraini, 2023).

Salivary fluids are composed of residual substances released from the body, which are usually called mixture or total salivary saliva with a water content of around 99.5%. About 0.5% the remaining is composed of other compounds. Human saliva has many functions, one of which is maintaining the pH in the oral cavity to remain stable, as antimicrobial, lubricate the cavity of the oral cavity, and helps produce amylase enzymes that become simple carbohydrates in the form of glucose (Stephen Creanor, 2020).

Oral candidiasis is an infection caused by *Candida Albicans* fungus which generally attacks the oral cavity. *Candida* can be a pathogen if there are predisposing factors from a person to carry out certain health practices which include knowledge, attitudes, work, habits and cultures that change the atmosphere in the oral cavity (Aji.A.K, 2018).

Candidiasis can attack mucosal tissue such as the mouth and pharynx. Oral candidiasis is characterized by red lesions and white lesions caused by *Candida Albicans*. *Candida albicans* infection is an opportunistic infection that does not cause disease in immunocompetent individuals but can only occur in individuals who experience immune system disorders such as HIV disease, diabetes mellitus and so forth. The opportunistic properties of *Candida Albicans* are able to cause thrush, lesions on the skin, vulvovaginitis, candiduria, gastrointestinal candidiasis that can cause stomach ulcers, can even be a complicated cancer (Meylani Vita, 2021).

Candida infection can occur in people with diabetes mellitus due to increased blood glucose levels in the oral cavity which is a supporting factor for faster fungal growth and a decrease in the patient's immune system so that in patients with uncontrolled diabetes there is a decrease Saliva which is a supporting factor for the growth of *Candida Albicans* (Aji.A.K.2018).

Basically the genus *Candida* is one of the normal flora that is in the oral cavity, and becomes pathogen when the body experiences a decrease in the immune system. *Candida* generally develop in mucosa such as the mouth and cause abnormalities in the mucosa such as candidiasis. Oral candidiasis does not only occur in patients who suffer from diabetes mellitus but also occur in healthy people who experience a decrease in immune response (Sophia.A, 2023).

Candida Albicans has characteristics developing into yeast, and hyphae. This form of hyphae plays an important role in causing disease by entering the epithelial cell and then causing damage to the tissue. In the case of candidiasis there are many fungi in the oral cavity that causes candidiasis (Sophia.a, 2023). Based on the background above, the researcher wants to conduct research on "Identification Of *Candida Albicans* On Saliva Patients With Type 2 Diabetes Mellitus At The Simalingkar Puskesmas Medan City

RESEARCH METHODS

Instrument

1. Incubator
2. Sputum Pot
3. Petri cup
4. Erlenmeyer
5. Pipette drops
6. Deck Glass
7. Glass object
8. Measuring cup
9. Three foot + asbestos
10. Bunsen
11. Test tube
12. Stirring stalk
13. Cotton
14. Autoclave

15. match
16. Tube shelf
17. ose
18. Wrapping paper

Research material

The inspection material used for this research is morning saliva.

Reagents

1. Violet crystals
2. Lugol
3. Alcohol 70%
4. Safranin
5. Immersion Oil

Media

Sabouroud Dextrose Agar (SDA) or Potato Dextrose Agar (PDA) media

Working Procedure

Pre-Analytic

- a. Patient Preparation

The main preparation that needs to be considered before conducting research is the time and sample requirements. In this examination, the saliva used is morning saliva with a time span of 8-10 am. Before taking saliva samples, patients are expected to rinse their mouths with water to clean the mucosal area. The method of taking saliva is done by asking the patient to collect their saliva in their mouths, after the saliva is collected, the patient is asked to put the saliva into a sterile tube provided by the researcher.

- b. Sterilization of equipment

Before the research process is carried out, Erlenmeyer flasks, petri dishes, droppers, measuring cups are sterilized first using an autoclave at a temperature of 121°C for 15 minutes.

- c. Making SDA media

The media used for the mushroom cultivation process is Sabouraud dextrose agar media, where the composition of this media is that each liter of Sabouraud contains 10 grams of mycological peptone, 40 grams of glucose, 15 grams of agar, 1000 ml of aqadest (Farizal.J, 2017). The need for media used for mushroom culture based on the number of 48 samples in the study is determined by the formula:

$$\frac{V1}{W1} = \frac{V2}{W2}$$

Description:

W1: Standard weight of the media to be used

V1: Solvent required (according to media production rules)

W2: Weight of the media to be weighed

V2: Volume of solvent required

Given

$$V1 = 1000$$

$$W1 = 65.0$$

$$V2 = 25 \times 15 \text{ mL} = 375 \text{ mL}$$

$$W2 = \dots\dots?$$

Calculation of SDA media requirements

$$V1/W1 = V2/W2$$

$$1000/65.0 = 375/W2$$

$$1000 \times W2 = 375 \times 65.0$$

$$W2 = 24,375/1000$$

$W2 = 24.7$ grams of SDA media with a volume of 375 mL of Aquades.

Media Preparation

SDA Media.....24.7grams
Aquadest.....375 mL

1. Prepare research tools and materials
2. Dissolve 24.7 grams of SDA media with 375 mL of aquadest, then boil over a Bunsen burner.
3. After boiling, the agar solution is first sterilized in an autoclave at a temperature of 121°C for 15 minutes.
4. After being sterilized, the media is put into a petri dish.

Analytical

Macroscopic Examination

1. Using a sterile cotton swab, a saliva sample is taken that has been allowed to absorb on the cotton swab for 1-5 minutes.
2. Inoculate in a Sabouraud dextrose agar (SDA) slant test tube evenly.
3. Incubate the media in an incubator at a temperature of 37°C for 48 hours and *Candida albicans* fungal colonies can be observed on the media (Farizal.J, 2017).

Microscopic examination with gram staining

1. Fix the glass object over the Bunsen flame then take the fungal colony with a sterile ose.
2. Make a preparation, let it dry at room temperature then fix it over the Bunsen flame 2-3 times.
3. Do the coloring, flood the preparation with Crystal violet solution for 1 minute, then rinse with running water.
4. Flood the preparation again with iodine solution for 1 minute, then rinse the preparation with running water.
5. Decolorize for approximately 30 seconds, until the color on the preparation fades.
6. Pour safranin on the preparation, wait for 30 seconds, then wash with running water, then dry and observe under a microscope at 10X and 100X magnification (Sri Indrayati, 2018).

Microscopic examination with 10% KOH

1. Drop 10% KOH solution on the glass object

2. Moisten the end of the ose using 10% KOH solution and take the colony using the end of the ose.
3. Place the colony on the 10% KOH drop and cover it using a deck glass and avoid air bubbles.
4. Pass the preparation several times over the flame
5. Observe under a microscope, initially with 10x magnification and 40x magnification.
6. Observe and record the results obtained (Farizal, et al, 2017).

Interpretation of Results

The results of macroscopic observations of the growth of *Candida albicans* fungus on Sabouroud Dextrose Agar (SDA) media after incubation at a temperature of 37°C, the colonies that grew after 24 to 48 hours, were yeast-like colonies that appeared round, soft consistency, yellowish white or cream in color, smooth colony surface and had a distinctive yeast or sour odor (Suraini, Et al, 2023). Microscopic examination using gram staining found *Candida albicans* to be round or oval in shape with a purple color (Suraini, Et al, 2023).

RESULTS AND DISCUSSION

Based on the research sample criteria, 25 respondents met the research sample criteria. This study aims to identify *Candida albicans* fungus in the saliva of type 2 diabetes mellitus patients at the Simalingkar Health Center. The *Candida albicans* identification research process was carried out by macroscopic examination and microscopic examination (10% KOH solution and gram staining). No Sample Age Gender Culture on SDA Media Gram Staining

Table 1. Research Result Data

No Sample	Age	Gender	Culture on SDA Media	Gram Staining	<i>Candida albicans</i>
Sample 1	60	Female	(+)	Positive	Positive
Sample 2	55	Male	(+)	Positive	Positive
Sample 3	49	Female	(+)	Positive	Positive
Sample 4	55	Female	(-)	Negative	Negative
Sample 5	57	Male	(+)	Positive	Positive
Sample 6	51	Female	(-)	Negative	Negative
Sample 7	40	Male	(-)	Negative	Negative
Sample 8	62	Female	(+)	Positive	Positive
Sample 9	61	Female	(+)	Positive	Positive
Sample 10	52	Male	(+)	Positive	Positive
Sample 11	59	Female	(+)	Positive	Positive
Sample 12	53	Female	(-)	Negative	Negative
Sample 13	49	Female	(-)	Negative	Negative
Sample 14	43	Male	(-)	Negative	Negative
Sample 15	48	Female	(+)	Positive	Positive
Sample 16	40	Female	(+)	Positive	Positive
Sample 17	47	Male	(+)	Positive	Positive
Sample 18	45	Female	(-)	Negative	Positive

No Sample	Age	Gender	Culture on SDA Media	Gram Staining	<i>Candida albicans</i>
Sample 19	41	Male	(-)	Negative	Positive
Sample 20	50	Female	(-)	Negative	Negative
Sample 21	52	Male	(+)	Positive	Positive
Sample 22	64	Female	(+)	Positive	Positive
Sample 23	51	Female	(-)	Negative	Negative
Sample 24	49	Male	(-)	Negative	Negative
Sample 25	54	Male	(-)	Negative	Negative

Table 2. Results of Macroscopic Identification of *Candida albicans*

Macroscopic examination	Frequency	Percentage (%)
Positive	14	56%
Negative	11	44%
Total	25	100%

Based on table 2, there were 14 respondents (56%) who showed positive results in macroscopic examination of *Candida albicans*, while 11 respondents (44%) showed negative results, no *Candida albicans* was found in the respondents' saliva.



Figure 1. Colony form of *Candida albicans* on SDA media



Figure 2 *Candida albicans* fungal threads on SDA media

Table 3. Results of *Candida albicans* Microscopic Test (Gram Staining)

Gram Staining	Frequency	Percentage (%)
Gram Positive	14	56%
Gram Negative	11	44%
Total	25	100%

Based on table 3, 14 respondents (56%) found results with gram-positive fungi, while 11 respondents (44%) showed gram-negative results.

Table 4. Results of the *Candida albicans* microscopic test (10% KOH)

10% KOH examination	Frequency	Percentage (%)
Positive	14	56%
Negative	11	44%
Total	25	100%

Based on table 4 of 25 respondents who met the research sample criteria and were examined microscopically with 10% KOH solution, there were 14 respondents (56%) who were positive for blastospores in their saliva, while 11 respondents (44%) did not find blastospores in the respondents' saliva. *Candida albicans* is a type of microorganism that lives as normal flora in the oral cavity, digestive tract and genitals. Infection can occur when the number of *Candida albicans* microorganisms is higher than the normal number. Where the normal number of *Candida albicans* in the mucosa is less than 200 cells/mL of saliva (Wayan.N.D.B, et al, 2020).

Diabetes mellitus is a disorder of the metabolic system that is a supporting factor for someone to get a fungal infection when the patient's immune system decreases. People with diabetes mellitus experience increased blood glucose levels, where this condition can increase glucose levels in saliva. The high glucose content in saliva is a supporting factor for the growth of *Candida albicans* fungi in the mucosal area (Suraini, 2023).

Based on the results of the study, respondents who suffered more from diabetes mellitus were women, this is in accordance with previous studies which stated that as age increases, vital organs of the body will experience decreased function, for example sensitivity to insulin and women who experience menopause also experience decreased sensitivity to insulin, leading to diabetes mellitus (Nasution. F, 2021).

Type 2 diabetes mellitus occurs due to the inability of insulin to work optimally and abnormalities in the function of pancreatic beta cells so that insulin secretion is disrupted. The incidence of type 2 diabetes mellitus occurs more often because there are causal factors that support the occurrence of type 2 diabetes mellitus, including age, obesity, unhealthy lifestyles and patterns, and the influence of race (Hardianto, D, 2020). From table 3, there are 14 respondents (56%) who have positive results in macroscopic *Candida albicans* examination, while 11 respondents (44%) show negative results for *Candida albicans* in the respondent's saliva. This is in accordance with the characteristics of the results of macroscopic observations from previous studies.

After culturing in an autoclave with an incubation temperature of 37°C for 24-48 hours, after 3 days a *Candida albicans* colony the size of a pinhead appeared, one to two days later the growth of the colony can be clearly observed. The *Candida albicans* colony will appear yellowish white or cream, appearing on the surface of the media, with a surface that is initially smooth and slippery and can be slightly wrinkled with a distinctive odor like the smell of yeast (Keumala, V. M, 2016).

Based on the results of the study by J. Farizal and Abdul R. Serbasa Dewa (2017), it is known that of the 27 samples identified, the results obtained were that most respondents (52%) were positive for *Candida albicans* fungus in saliva, and some respondents (48%) were

negative for *Candida albicans* fungus in the saliva of diabetes mellitus patients at RSUD dr. M. Yunus Bengkulu in 2017.

Based on the research results of Suraini and Anggun Sophia (2023) the results of the study that had been conducted on 10 samples of diabetes mellitus patients obtained the results of 6 saliva samples (60%) positive for *Candida albicans* fungus and 4 saliva samples (40%) did not contain *Candida albicans* fungus in the saliva of Diabetes mellitus patients at the Mohammad Natsir Hospital, Solok City.

Based on the research results of Deanita Rabiatul. Z.P, et all (2023) the results obtained from 33 saliva samples of Type 2 diabetes mellitus patients at the Harapan Baru Samarinda Health Center. The number of respondents based on the age of DM patients, the highest percentage of positive *Candida albicans* fungus was in the age range of 45-59 years as many as 12 respondents (52%), in the age range of 60-70 years, as many as 5 respondents (50%). Based on gender, diabetes mellitus sufferers with the highest percentage of positive *Candida albicans* fungus occurred in women, namely 15 respondents (58%), while men, as many as 2 respondents (29%) were positive for *Candida albicans* fungus.

Based on the results of previous studies, it shows that the positive results of *Candida albicans* are higher than the percentage of negative results of *Candida albicans*, while if the results of the study obtained show that negative results are higher than positive results, this is in accordance with previous researchers where DM patients are very susceptible to fungal and bacterial infections, it is possible that there are false negative results caused by the pre-analytical and analytical stages, namely the inaccurate saliva sampling stage and the poor sterilization stage during sample culture.

Based on table 3. the results showed that 14 respondents (56%) had gram-positive *Candida albicans* examination results, while 11 respondents (44%) showed gram-negative results. According to Ayu, I.P, et al, 2023, the results of microscopic examination through gram staining of *Candida albicans* fungi were gram-positive in an oval shape, with a diameter of approximately 5 micrometers, and were able to reproduce by forming budding.

Based on table 4 of 25 respondents who met the research sample criteria and were examined microscopically with 10% KOH solution, there were 14 respondents (56%) who were positive for blastospores in their saliva, while 11 respondents (44%) did not find blastospores in the respondents' saliva.

In positive *Candida albicans* samples, it can be seen from the presence of round or oval *Candida albicans* colonies with a smooth surface, yellowish white in color with a distinctive odor or yeast odor on SDA agar media (Sabouroud Dextrose Agar). In microscopic observation of *Candida albicans*, blastospores and pseudohyphae were found in microscopic preparations using 10% KOH (Potassium Hydroxide). In negative *Candida albicans* samples, no *Candida albicans* colonies were found in macroscopic observations using SDA slant agar media (Jon Farizal, et al, 2017).

Candidiasis is influenced by endogenous factors where increased glucose levels in urine, saliva and blood increase, stimulating faster growth of *Candida albicans* fungi. Obesity also plays a role in causing candidiasis. This obesity causes a lot of sweat so that skin maceration occurs and this helps facilitate the invasion of *Candida albicans* and other

diseases. Then for exogenous factors include weather and humidity factors that affect the growth of *Candida albicans*, namely weather with a hot climate causes a lot of sweat so that the skin experiences maceration and this facilitates the invasion of *Candida albicans*. then work related to personal hygiene is very necessary to minimize *Candida albicans* infection, especially the cause of oral candidiasis (Jon, Farizal, 2017).

Excessive blood sugar levels in saliva can stimulate the growth of *Candida albicans* fungus on the mucosal surface. in patients with Diabetes mellitus, salivary abnormalities are one of the causes of complications of disorders in the mouth of patients with diabetes mellitus. Abnormalities in the salivary glands can be caused by several things, one of which is a low pH condition that causes the *Candida albicans* fungus to grow and reproduce (Suraini, 2023).

Candida albicans infection is an opportunistic infection that can cause oral candidiasis infection which is characterized by the presence of confluent white patches that adhere to the surface of the oral mucosa and pharynx, especially in the mouth and tongue. The opportunistic nature of *Candida albicans* can cause canker sores, skin lesions, vulvovaginitis, candiduria, gastrointestinal candidiasis which can cause gastric ulcers, and can even become a complication of cancer (Meylani Vita, 2021).

Prevention of oral candidiasis is influenced by decreased saliva levels and xerostomia, so prevention efforts are needed to get maximum results for people with diabetes mellitus. Maintaining a healthy lifestyle is a factor that can control blood sugar levels so that controlled sugar levels can reduce the source of nutritional intake for the growth of *Candida albicans* (Jon, Farizal, 2017). Local treatment for oral candidiasis can be given antifungals such as nisfatin, gentian violet, amphotericin B, Ketoconazole, miconazole and clotrimazole, ketoconazole can cause side effects such as nausea, vomiting, diarrhea and headaches (Jon, Farizal. 2017).

CONCLUSION

In macroscopic examination, there were 14 respondents (56%) positive for *Candida albicans* fungal colonies in the saliva of DM patients on SDA (Sabouraud Dextrose Agar) media, and there were 11 respondents (44%) negative for *Candida albicans*. In microscopic examination with gram staining, there were 14 respondents (56%) found gram positive in the respondents' saliva samples, and 11 respondents (44%) with gram negative results. In examination using 10% KOH solution, there were 14 respondents (56%) positive for pseudohyphae found in the samples examined, and 11 respondents (44%) with negative results for *Candida albicans* blastospores.

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