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THE EFFECT OF STREPTOMYCIN AND CLINDAMYCIN ON DNASE I ACTIVITY ON THE QUALITY OF DNA OF SALIVARY ORIGIN

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Clindamycin, DNase, salivary DNA, Streptomycin, Preservation

ABSTRACT
Deoxyribonucleic acid (DNA) contains genetic material with all an individual's information. Saliva has the potential to be a good source of human DNA. When compared to blood sampling, saliva is easier to collect and the collection process is non-invasive. However, human DNA collected from saliva has the potential to be degraded due to deoxyribonuclease (DNase) activity in saliva. DNase activity can be inhibited by antibiotics, such as aminoglycoside antibiotics and lincomycin derivatives. This study aimed to determine the effect of streptomycin and clindamycin on DNAse I activity against the quality of human genomic DNA from saliva. The sampling technique was that samples were collected from 9 subjects with each subject flowing into a saliva pot. Before saliva collection, subjects were asked to rinse their mouth with a solution of chlorhexidine gargle (Listerin™) for 30 seconds, then the samples were divided into four groups. The average concentration of DNA extracted from the spin-column method was 32.91 g/mL (7.10-99.45 g/mL) while the average purity was 1.813 g/mL (1.639-2.043 g/mL). With clindamycin treatment, PCR was able to amplify the human NOTCH2 gene (~704 bp) while various concentrations of streptomycin produced multiple bands of ~100 bp. The concentration of 3.2 mM clindamycin effectively inhibits DNAse I activity and can amplify the human NOTCH2 gene while streptomycin cannot protect.

INTRODUCTION

Deoxyribonucleic acid (DNA) contains genetic material that contains all the identifying information of an individual (Syaifiatul, 2017). DNA resides in the nucleus of eukaryote cells and is found in almost all individual cells. Human body parts such as saliva, blood, sperm, skin cells, hair, urine, sweat, etc. can be a source of DNA. (Putri & Yudianto, 2016), (Wahab et al., 2017).

Saliva has the potential to be a good source of human DNA. When compared to blood sampling, saliva is easy to collect and its collection is non-invasive. (Takeshita et al., 2016), (Garbieri et al., 2017) Saliva is produced and secreted by the salivary glands as a complex fluid with 99% saliva consisting of water, organic, and inorganic substances. (Wahab et al., 2017) In addition, saliva also contains normal flora, enzymes, cells, electrolyte fluids, microorganisms, epithelial cells, hormones, immunoglobulins and so on. (Tiwari, 2011), (Bibi et al., 2021), (Aas et al., 2005) Epithelial cells in the oral mucosa that are released along with

saliva have the potential to be a source of DNA for diagnostic purposes. However, contaminants in saliva can damage DNA (Garbieri et al., 2017).

Human DNA collected from saliva is potentially damaged due to deoxyribonuclease (DNase) activity in saliva (Garbieri et al., 2017). DNase is an enzyme capable of breaking down nucleic acids (Rodwell et al., 2015). Belonging to a heterogeneous class of enzymes, DNase catalyzes the hydrolysis of DNA and eventually degrades it. The two main types of DNase are DNase I and DNase II (Kolarevic et al., 2014). DNase II is known as acidic DNase because its optimal activity is in the low pH environment of lysosomes (Varela-Ramirez et al., 2017).

Activity DNase can be inhibited by antibiotics, such as aminoglycoside antibiotics and lincomycin derivatives (lincosamides) (Kolarevic et al., 2014), (Spížek & Řezanka, 2017). Aminoglycosides are antibacterial produced by Streptomyces or other fungi. Since 1943, various aminoglycoside derivatives have been developed. Examples of aminoglycoside drugs are streptomycin, neomycin, kanamycin, gentamicin, amikacin, and tobramycin (Rohmah et al., 2019). Aminoglycosides with the suffix "mycin" (streptomycin, neomycin, kanamycin, paromomycin, and tobramycin) are derived from Streptomyces; aminoglycosides ending in "main" (gentamicin, netilmicin, and amikacin) are derived from Micromonospores (Fransiska, 2019).

The term lincomycin originated in Lincoln, Nebraska (United States), the area where this type of antibiotic was first isolated from Streptomyces lincolnensis in soil samples (Spížek & Řezanka, 2017). Lincosamide is a relatively small class of antibiotics with a chemical structure consisting of amino acids and sugars. Members of the group of antibiotics that form naturally from lincosamide are lincomycin and celesticetin. Many semisynthetic lincomycin derivatives have been synthesized. Of these types, only clindamycin is effective. Lincosamide is produced by several species of Streptomyces especially S. lincolnensis, S. Roseland, S. caelestetis, and Micromonospora halophytica. Lincosamide is widely active against anaerobic bacteria (Spížek & Řezanka, 2017).

The potential of aminoglycoside antibiotics on DNase activity appears to vary. Clindamycin, a derivative of lincomycin produced by Streptomyces lincolnensis, shows activity against bacteria, especially Gram-positive bacteria, and protozoa. These antibiotics are bacteriostatic, inhibit protein synthesis in sensitive bacteria, and generally at higher concentrations can be bactericidal. Clindamycin is more effective than lincomycin in the treatment of bacterial infections, in particular those caused by anaerobic species. In addition, clindamycin effectively inhibited DNase activity in mouse lesions by necrotizing fasciitis at a high dose of 100 μg/rat twice daily intraperitoneally, although its role in inhibiting DNA degradation is unclear (Andreoni et al., 2017), (Bertram, 1998), (Spížek & Řezanka, 2017).

Preservation of human DNA from saliva can be done using aminoglycoside antibiotics and lincomycin derivatsives. Streptomycin and clindamycin, in addition to inhibiting DNase activity, are also easily obtained at low prices. The assessment of the successful preservation of human DNA of salivary origin rests on the inhibition of DNase activity and can be assessed in quality and quantity. This study aims to assess the effect of streptomycin and clindamycin on DNAse I activity on the DNA quality of the human genome of salivary origin.

METHOD RESEARCH

This type of research is using purely experimental research with a pretest-posttest design. This research was carried out at the Integrated Laboratory of the Faculty of Medicine, University of North Sumatra (USU). This study took place in December 2021-January 2022. In this study, saliva was collected from each subject. Before saliva collection, subjects were asked to gargle with a solution of chlorhexidine gargle (Listerin™) for 30 seconds and sterile equates. Inclusion and exclusion criteria are not required. A total of 15 mL of clean saliva was collected from each subject with each subject draining into a clean saliva pot.

Saliva samples from each subject were immediately divided into 4 groups: negative control (K1), positive control (K2), Streptomycin (K3), and Clindamycin (K4). Data analysis was carried out with univariate mean and standard deviation for the concentration and purity of extracted DNA. DNase I activity on DNA extraction from saliva was qualitatively assessed through observation of the electrophoresis result of 1% agarose gel. Assessment of PCR results for human NOTCH2 genes from DNase I inhibition results by antibiotics, was carried out using a descriptive approach with observations on the results of 1% agarose gel electrophoresis. The expected result on 1% agarose gel electrophoresis is the presence of an expected amplicon length (~704 bp).

RESULT AND DISCUSSION

After conducting research, the data that has been collected is that nine DNA samples obtained have been successfully extracted by the spin column method. The concentration and purity of DNA obtained varied from sample to sample (Table 4.1). The mean DNA concentration was 32.91 µg/mL in the range of 7.10-99.45 µg/mL. The average purity of the extraction yield was 1.813 µg/mL in the range of 1.639-2.043 µg/mL.

Table 1
Purity and Concentration of DNA of Salivary Origin

Sample	Concentration (ng/uL)	Purity
1	13,25	1,791
2	7,10	2,043
3	20,05	1,759
4	20,10	1,803
5	7,95	1,639
6	10,60	1,927
7	33,45	1,848
8	99,45	1,742
9	84,25	1,761
Average	32,91	1,813
Range (Minimum-Maximum)	7,10-99,45	1,639-2,043

DNA degradation testing was performed to determine the activity of DNAse I against DNA extracts of salivary origin and visualized by electrophoresis on 1% agarose gel. DNase I degrades a sample of salivary-origin DNA (Figure 1).

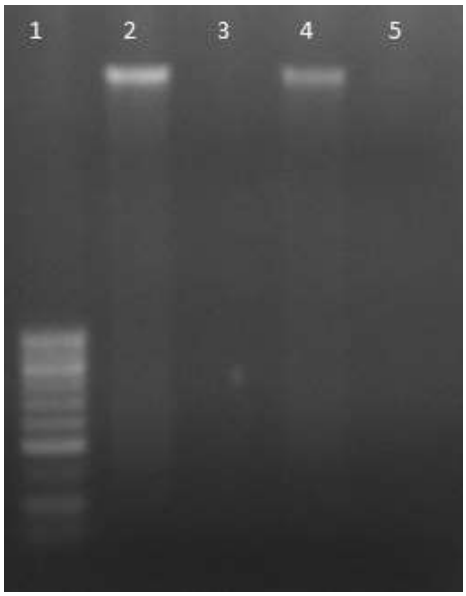

Figure 1
DNA degradation assay

Lane 2 and lane 4 (10µL and 5µL salivary DNA extract) as positive control DNA degradation assays, showing intact salivary origin DNA. The addition of DNAse I to salivary origin DNA samples (lanes 3 and 5 (10µL and 5µL salivary DNA extract) showed empty lanes 3 and 5 and showed no presence of DNA. There is no DNA smear or DNA fragmentation in lanes 3 and 5.

Electrophoresis of 1% agarose gel was performed for DNA visualization after the addition of various concentrations of streptomycin as an inhibitor of DNAse I. Although at a concentration of 0.1 M streptomycin was not able to inhibit, DNAse I activity appeared to be inhibited with the addition of streptomycin concentrations (Figure 4.2). Streptomycin concentration of 0.2 mM can maintain the integrity of 1 µg DNA from 2.5 mg/mL DNase I activity (Figure 2).

The PCR approach could not show the amplicon length of the NOTCH2 gene as expected (~704 bp). Instead, the amplicon length formed measures about 100 bp (Figure 3).

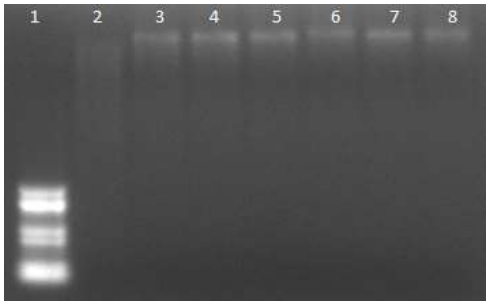

Figure 2
Hasil electrophoresis Streptomycin-DNase I protection assay.

There is protection from DNA degradation by DNase I in lanes 3, 4, 5, 6, 7, and 8. At a streptomycin concentration of 0.1 mM (lane 2), there is a DNA smear. Increased streptom concentrations to 0.2 mM (lane 3), 0.4 mM (lane 4), 0.8 mM (lane 5), 1.6 mM (lane 6), 2.0 mM (lane 7), 3.2 mM (lane 8) showed streptomycin could maintain whole DNA bands of DNase I activity.

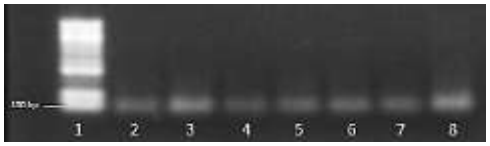

Figure 3
Electrophoresis results from human NOTCH2 gene PCR

PCR results of the human NOTCH2 gene from salivary DNA samples on agarose gel 1% after administration of streptomycin, 2.5 mg/mL DNase I added to salivary DNA showed the presence of bands measuring 100 bp in all lanes with streptomycin concentrations of 0.1-3.2 mM.

Clindamycin with different concentrations, affects the preservation of salivary origin DNA (Figure 4.4). The higher the concentration of clindamycin, the higher its preservation ability to salivary DNA that undergoes DNA degradation assay. DNA bands appear more pronounced at concentrations of 3.2 mM (Figure 4.4). DNA smears still appear at 0.8 mM clindamycin, showing the partial ability of clindamycin to prevent salivary DNA degradation by 2.5 mg/mL DNase I.

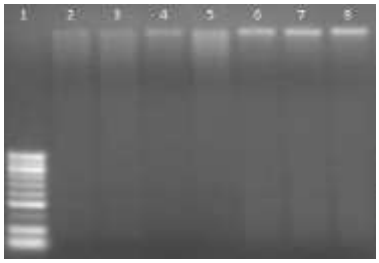

Figure 4
The effect of clindamycin on DNAse I activity.

Clindamycin inhibits DNase I activity at different concentrations with an effect that gets stronger with increasing concentration. Clindamycin concentrations are 0.1 mM (lane 2), 0.2 mM (lane 3), 0.4 mM (lane 4), 0.8 mM (lane 5), 1.6 mM (lane 6), 2.0 mM (lane 7), and 3.2 mM (lane 8). DNA smear (lanes 2, 3, 4, 5) becomes DNA without it (lanes 6, 7, 8). Lane 8 shows very clear and thick intact DNA with the highest concentration of clindamycin (3.2 mM).

PCR electrophoresis results from the human NOTCH2 gene that had been given varying concentrations of clindamycin (0.1-3.8 mM) and 2.5 mg/dL showed a single band at ~704 bp. Expected bands (~704 bp) of the human NOTCH2 gene were seen at all concentrations of clindamycin (Figure 5).

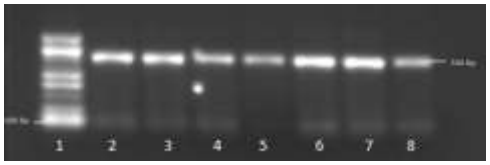

Figure 5
Human NOTCH2 gene PCR Electrophoresis Results

The expected band of the human NOTCH2 gene (~704 bp) is in the lane (2, 3, 4, 5, 6, 7, 8). PCR can amplify human NOTCH2 genes from DNA given clindamycin in concentrations of 0.1-3.2 mM and 2.5 mg/dL DNase I. Clindamycin concentrations are 0.1 mM (lane 2), 0.2 mM (lane 3), 0.4 mM (lane 4), 0.8 mM (lane 5), 1.6 mM (lane 6), 2.0 mM (lane 7), 3.2 mM (lane 8).

Salivary DNA obtained from extraction needs to be observed in terms of quantity and quality. DNA quantity analysis is performed to determine the purity and concentration of DNA. DNA purity can be determined through spectrophotometric rules with a ratio of A260 nm and A280 nm.

The results of this study are by previous studies, where the results of DNA extraction are said to be pure if the ratio of A260 nm and A280 nm reaches 1.8-2.027 or the extraction results are also considered pure if the absorbance ratio is between 1.6 and 2.0.5 The range of values indicates that the amount of DNA in the sample is more than that of proteins. If the ratio of A260/A280 is < 1.8 or > 2.0, it is indicated that DNA is still contaminated with RNA and proteins.[27] In other studies, the average purity of salivary DNA ranged from 1.8 to 2.0.3 If the sample has a ratio of about 1.8 or close to 1.8, then the DNA sample can be said to be relatively pure (Garbieri et al., 2017).

The DNA concentration in this study has a range of 7.10–99.45 µg/mL with a salivary volume of 2 mL and the spin column method. Another study with a volume of 1 mL using the Oragene™ kit showed a lower DNA concentration of 10 ng/µL.5 This can occur due to factors that affect DNA concentration. Factors that can affect DNA concentration such as incubation temperature factors, types of chemicals, and technical factors during DNA extraction work (Khare et al., 2014).

The results of this study, the administration of streptomycin did show the ability of streptomycin to maintain DNA measuring > 1000 bp (Figure 2.). However, this does not guarantee that the DNA is intact. PCR on NOTCH2 showed the ~100 bp band was not as expected ~704 bp. The NOTCH2 gene may have also been degraded because streptomycin is unable to inhibit DNase activity (WOEGERBAUER et al., 2000). Indeed, forward and reverse primers work in PCR reactions to multiply the remaining (pieces) of the NOTCH2 gene while producing complementary sequences from the reverse and forward directions of the primer.

While clindamycin as a DNase inhibitor, can preserve whole DNA (Figure 4). The results of this study were that 3.2 mM levels effectively inhibited DNAse I activity in human genomic DNA of salivary origin and were able to maintain genes by amplification of human NOTCH2 genes such as the estimated length of amplicon (~704 bp) (Figure 5), while streptomycin was not able to do the same (Figure 3). The results of this study are in line with previous studies that clindamycin can inhibit DNase activity in vivo 100 µg/mL.15 In human tissue, DNase activity is completely lost after two days of additional treatment with clindamycin although high bacterial concentrations remain (Andreoni et al., 2017).

The results of this study are also by Liu Yawen and colleagues' research on DNA preservation that salivary DNA can be damaged due to DNase activity in saliva, causing a decrease in DNA quality and quantity.5,29 Therefore DNA preservation or protection is necessary to maintain DNA quality and integrity.29 As a type of enzyme, DNase activity can be affected or even eliminated by regulating pH[30], cofactor30a, and temperature (Huque et al., 2020). In another study, it was said that DNAse activity decreased drastically when it was at temperatures above or below the optimum temperature range (20-70 °C) (Huque et al., 2020).

One strategy of DNA preservation from saliva is to protect DNA from DNase activity by administering DNase inhibitors. Aminoglycoside antibiotics such as clindamycin, gentamycin, and neomycin can work to inhibit DNase activity.10,15,31 Other types of DNase inhibitors are available as synthetic and natural ingredients (Kolarevic et al., 2014).

CONCLUSION

Clindamycin can inhibit DNAse I activity so that it can maintain the quality of human genome DNA from saliva. Streptomycin cannot inhibit DNAse I activity so it cannot maintain the DNA quality of the human genome of salivary origin. Clindamycin 3.2 mM effectively inhibits DNAse I activity in human genome DNA of salivary origin while streptomycin does not inhibit The quality of human genes of salivary origin and can be protected with clindamycin 0.1 mM with PCR can amplify human NOTCH2 genes while streptomycin cannot protect.

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