ORIGINAL ARTICLE

DNA Purity and Concentration Analysis From Toothpick as the Evidence for Forensic Examination

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ABSTRACT

Introduction: : DNA evidence has been set as the forensic standard for investigating and resolving various crimes such as murder, mutilation, and other major crimes. The orofacial region is a good source for DNA analysis. Toothpicks are recommended by the Ministry of Health of the Republic of Indonesia to clean teeth gaps. Objects at the crime scene and their surroundings have a meaning in the examination of forensic identification, especially those which are used by the perpetrator or victim for the last time. This study aims to analyze DNA purity and concentration in toothpicks. **Methods:** Samples were obtained from eight female volunteers who still have complete permanent teeth. Volunteers were instructed to eat fibrous food and drink water before cleaning the gaps between the second premolar and first molar in all teeth region with a toothpick. DNA in the toothpicks are extracted to obtained DNA purity and concentration. **Results:** The lowest DNA purity was 1.069 µg/µl and the highest was 1.265 µg/µl. The average level of DNA purity in toothpicks was 1.164 µg/µl. The lowest DNA concentration value was 150.5 µg/µl while the highest value was 381.5 µg/µl. The average value of DNA concentration in toothpicks was 245.87 µg/µl. **Conclusion:** The number of DNA purity and concentration of toothpicks is qualified for a stage PCR, where PCR amplification technology is an ideal method in the analysis of forensic DNA samples from toothpicks that can be found in the crime scene.

Keywords: DNA, Purity, Concentration, Toothpicks, Forensic Evidence

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INTRODUCTION

The oral cavity area has its uniqueness; it can contain individualistic characteristics to identify someone (1). In the field of investigation of forensic odontology, not only angulation, occlusion, configuration, and dental fillings make teeth unique to a person as they can be used as a means to identify an individual based on the teeth precision and clarity, but also for deoxyribonucleic acid (DNA) analysis that has been widespread in the scope of forensic dentistry. DNA can be extracted from epithelial cells contained in saliva and/or teeth (2, 3).

A forensic standard for investigating and resolving various crimes such as murder, mutilation, and other major crimes is DNA evidence (4). The orofacial region is a good source for sampling for DNA analysis because the oral tissues are more resistant to environmental degradation and damage from temperature, electricity, or mechanics. DNA analysis can be carried out for very accurate identification if used correctly (5).

In Indonesia, the Ministry of Health of The Republic of Indonesia recommends using toothbrushes and dental aids such as toothpicks to help clean the teeth (6). Objects that are at the crime scene and its surroundings are crucial for the examination of forensic identification. Specimens that are widely used in examinations for identification that contained DNA are blood spot, saliva, other body fluids, and bones, including objects used by the perpetrator or victim for the last time (7), among them are toothpicks. The advantage of a toothpick is its practicality, especially to pick leftover food that is still attached after eating (8). Almost all restaurants and homes in Indonesia are provided with toothpicks (9).

DNA found at crime scenes is very limited both in quantity and quality. The perpetrators of the crime did not leave much evidence, causing them to be difficult to prosecute in court. Therefore Polymerase Chain Reaction (PCR) amplification technology in DNA is very suitable for analysis of forensic DNA samples because it is sensitive, fast, and not limited by DNA quality (10). Based on their problem, this study aims to analyze DNA purity and concentration in toothpicks that have been used.

MATERIALS AND METHODS

The experiment of this study was conducted at the Human Genetic Laboratory of Universitas Airlangga, Surabaya, Indonesia, during March 2020 involving samples obtained from eight female volunteers who still have complete permanent teeth. Volunteers were instructed to eat fibrous food and drink water before cleaning the teeth gaps in the fourth tooth region of the posterior teeth with a toothpick. Toothpicks that have been used are extracted for the DNA.

The DNA extraction process was started by soaking the toothpicks separately overnight in eight sterile centrifuge tubes. Tubes were each filled with 5 cm^3 of distilled water to allow dissolution and settling of DNA samples from toothpicks. After 24 hours, the samples were centrifuged at 12,000 rpm for 10 minutes. Supernatant fluids were then removed. The DNA sample solution was added with DNAzol, and then vortexed and incubated again for 15 minutes before being centrifuged at 8,000 rpm for 10 minutes. The above separated supernatant was then mixed with isopropanol, followed with another centrifugation at 12,000 rpm for 10 minutes. The left pellet was washed by 70% ethanol and then centrifuged again at 12,000 rpm. This protocol was proposed by Nzilibili et al (11). The pellet was then added by 50 µL sterile aquades before undergoing a spindown of 30 seconds for the spectrophotometer. The final DNA extraction and isolation were performed using the Invitrogen™ DNAzol™ reagent.

DNA purity was given by the optical density (OD) and calculated based on OD260/280 ratio. DNA concentration was measured at 700 µL dilution factor (10 µL from DNA and 690 µL distilled water). DNA concentration was obtained from the formula as follows: OD260 Ч dilution factor Ч 50 µg/ml double-stranded DNA (dsDNA). The ethical clearance was issued by the Health Research Ethical Clearance Commission Faculty of Dental Medicine, Universitas Airlangga, under ethical clearance number: 155/HRECC.FODM/III/2020.

RESULTS

The results of this study are illustrated in Table I below. Data are presented according to optical densities of UV light absorption spectrophotometer measurements at OD260 and OD280. Based on Table I, the lowest DNA purity was 1.069 µg/µl and the highest was 1.265 µg/µl. The average level of DNA purity in toothpicks was 1.164 µg/µl. It can also be seen that the highest DNA concentration was 381.5 µg/µl and the lowest was 150.5 µg/µl, with the average being 245.87 µg/µl.

Table I : UV Spectrophotometer Result

Sample	OD260	OD280	Purity	Concentration
A	0.062	0.058	1.069	217
C	0.098	0.084	1.167	343
F	0.054	0.045	1.2	189
I	0.089	0.074	1.203	311.5
K	0.054	0.049	1.102	189
N	0.043	0.034	1.265	150.5
R	0.053	0.049	1.082	185.5
S	0.109	0.089	1.225	381.5

OD=Optical Density

DISCUSSION

The initial stage of PCR amplification is to analyze purity and concentration of DNA, where the minimum requirement for DNA purity is 1-2 (ideally 1.8 - 2), where the DNA concentration for DNA profiling was 20 µg/ml (12). In this study, the results of DNA purity from existing samples ranged between 1.069 µg/µl and 1.265 µg/µl, in which the requirements for PCR amplification were fulfilled. Meanwhile, the results of the DNA concentration were 150.5 µg/µl and 381.5 µg/µl. Considering that the requirements for the PCR amplification stage were fulfilled, the results could be used in forensic identification.

Toothpicks is one of the objects that can become evidence at a crime scene. Saliva sticks to the toothpick of the alleged perpetrator where there are epithelial cells that contain DNA from saliva so it allows the toothpick to become DNA evidence (13). In addition, several study prove some objects that can be DNA evidence from saliva that can be found at a crime scene besides toothpicks include toothbrushes, siwak, and bite marks on food and human skin (14).

Analysis of DNA purity and concentration is very important in the interpretation of sample profiles relating to forensic DNA. DNA purity analysis showed a lowered amount of intact DNA molecules sufficient for PCR amplification. The analysis of DNA concentration describes the effect of how the damaged and broken down DNA molecules become increased segmented molecules (11). DNA purity value that is lesser than the minimum value indicates protein contamination in the DNA sample. One the other hand, DNA purity value which shows any number above than 2 indicates RNA contamination (15).

CONCLUSION

The numbers of DNA purity and concentration of toothpicks are proved to be qualified for a stage PCR, in which PCR amplification technology is an ideal method in the analysis of forensic DNA samples from the toothpicks that can be found in the crime scenes.

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REFERENCES

1. Krishan K, Kanchan T, Garg AK. Dental Evidence in Forensic Identification – An Overview, Methodology and Present Status. Open Dent J. 2015;
2. Babu ARS, Rose D. Molecular advancements in forensic odontology. West Indian Med J. 2016;
3. Peridis OK. Bitemarks and Evidence in Forensic Odontology. J Forensic Sci Crim Investig. 2017;
4. Bond JW, Hammond C. The value of DNA material recovered from crime scenes. J Forensic Sci. 2008;53(4):797–801.
5. Muruganandhan J, Sivakumar G. Practical aspects of DNA-based forensic studies in dentistry. J Forensic Dent Sci. 2011;
6. Kemenkes RI. Buku Panduan Pelatihan Kader Kesehatan Gigi dan Mulut di Masyarakat. Kementerian Kesehatan RI. 2012. 16 p.
7. Yudianto A, Margaret N. Effect of Room Temperature on The Quality of DNA from Earphone Swab by Observing Mitochondrial DNA [MtDNA] D-Loop Region Of 126 bp (HVS II, nt 34-159) AND 143 bp (HVS I, nt 16268-16410). Folia Medica Indones. 2017;53(2):86.
8. Ng E, Lim LP. An Overview of Different Interdental Cleaning Aids and Their Effectiveness. Dent J. 2019;7(2):56.
9. Emailijati K, Hamsar A, Marthias EM, Aini N. Hubungan Penggunaan Tusuk Gigi terhadap Terjadinya Saku Gusi Kecamatan Patumbak Kabupaten Deli Serdang Tahun 2016. 2016;03(2).
10. Butler JM. Advanced Topics in Forensic DNA Typing: Methodology. National Institute of Standards and Technology, Gaithersburg, Maryland, USA; 2012. 69 p.
11. Nzilibili SMM, Ekodiyanto MKH, Hardjanto P, Yudianto A. Concentration and Purity DNA Spectrophotometer: Sodium Monofluorophosphate forensic impended effect. Egypt J Forensic Sci. 2018;
12. Yudianto A, Kusuma SE. Dna Isolation From Sweat Stain in Clothes As Forensic Identification Material. Folia Medica Indones. 2006;42(4):205–8.
13. Hyde J. DNA fingerprinting OK. Nature. 2011;325(6101):183–183.
14. Rizky BN. DNA forensic evidence from oral cavity. Biochem Cell Arch. 2020;
15. Oxford Gene Technology (OGT). Understanding and measuring variations in DNA sample quality. Oxford Gene Technol Mol Genet Rev. 2011;