The Effect of Seawater on the Quantity of Dental Pulped DNA in Forensic Odontology Identification

Nazaratun Thaiyibah[1], Amalia Rozaiza Ightikhoma[1] and Ahmad Yudianto[1,2]
[1]*Forensic Science Program, Postgraduate School, Universitas Airlangga Campus B, 4-6 Airlangga Rd, 60286 Surabaya,Indonesia*
[2]*Human Genetic Laboratory, Institute of Tropical Disease, Universitas Airlangga Surabaya, Indonesia*

Keywords: Dental Pulp DNA, Forensic odontology, Seawater

Abstract: The identification of forensic odontology on victims of sea natural disasters is highly efficient. Its contrast with the identification of soft tissue that are susceptible to damage is a consequence of seawater exposure. Seawater affects the decomposition of corpses and creates potential difference due to salinity (content of salt) in the water. The identification of odontology can consist of DNA analysis of the dental pulp, because the dental pulp is shielded by hard tissue such as dentine and enamel which are makes the pulp capable to protect the DNA, but the enamel is semipermeable (water permeable), which can affect the extraction of DNA degree and its purity. This study aims to determine the effectiveness of seawater to the quantity of teeth pulp DNA. The research was conducted experimentally by observing and explaining situations that occurred (cause and effect) in dental pulp DNA quantity exposure to seawater from 1 day and 7 days using spectrophotometer method with wavelengths of 260 nm and 280 nm. The results of this study indicated that the waters of the Lombok Strait with 28.74 ‰ of salinity degraded the DNA content of the dental pulp, but the DNA purity remained stable.

1 INTRODUCTION

Identification of forensic odontology is part of branch of dentistry that uses dental knowledge for social or criminal problems for victim identification. Field of dentistry involves collection and interpretation of dental evidence and other evidence that related with criminal. Identification of forensic odontology is carried out in mass disasters that are naturally occurring, as well as those caused by human negligence such as fire, explosion, body decay, or accidents at sea (Krishan, 2015).

For the victims of mass disasters at sea, nidification of *forensic odontology* is very efficient, while identification of soft tissue easily leads to broken tissues because of the seawater exposure that makes decomposition occur more quickly. The effectiveness of seawater can be seen from the total of salt or salinity of the water. Research done by Putri (2016) stated that there is an impact of seawater to DNA quantity from victims in terms of psoas muscle. This impacts the identification action because visual action and fingerprint cannot be used anymore (Irnamanda, 2016). If accidents at sea occur where body parts are destroyed, the only specimen needed for sample is tooth (Datta, 2012).

Tooth is one of human body structure which is most sturdy and most resistant to bad conditions such as decomposition, microbes' action, incineration, and also environment attack. Therefore, tooth is used as an identification tool for forensic odontology investigation. There are pieces information that we can get from human tooth identification, such as age, sex, race, facial shape, blood type, and it is also valuable source of DNA (Rai, 2012). Most forensic odontology investigation cases might fail because of insufficient appropriate antemortem records. If no sufficient amount of antemortem records is not available, appropriate identification become complex and the investigators could only obtain the biological profile of molecular system, which is the DNA that expose the true identity of someone (Datta, 2012).

Deoxyribonucleic Acid (DNA) genome obtained from tooth sample can contain about 6 µg to 50 µg of DNA. Datta (2012) said that Polymer chain reaction (PCR) allows individual differenciation from others with high reliability concentration and only 1ng (one per one billion grams) from DNA target. Deoxyribo

Thaiyibah, N., Ightikhoma, A. and Yudianto, A.
The Effect of Seawater on the Quantity of Dental Pulped DNA in Forensic Odontology Identification.
DOI: 10.5220/0007544604400445
In *Proceedings of the 2nd International Conference Postgraduate School (ICPS 2018)*, pages 440-445
ISBN: 978-989-758-348-3

Nucleic Acid (DNA) abudance quantity can be extracted from tooth. This is the important advantage from DNA analysis, which is that the tooth DNA is contained on the tooth pulp layer.

Tooth pulp mitochondria DNA is obtained from tooth pulp, which is a connective tissue that is rich in blood vessels and nerves contained within the core layer of the tooth (Girish, 2010). Tooth pulp gets protection from dentin coating and enamel. This protection causes tooth pulp DNA to be 100% usable for gender analysis using polymerase chain reaction (PCR) analysis after the tooth is heated at temperature 100° C for 15 minutes (Febri, 2013). However, the research done by Irnamanda (2016) said that there is seawater influence on accuracy of ABO blood type determination from tooth pulp. Therefore, the research of seawater effectiveness on quantity of DNA pulp tooth from concentrations and purity after DNA isolation using DNAzol method should be conducted.

2 MATERIALS AND METHODS

2.1 Materials

This study was carried out in the Laboratory of Analytical Chemistry of Science Faculty and Technology. And the DNA was analyzed in Institute Tropical Disease, Universitas Airlangga Campus C, Surabaya.

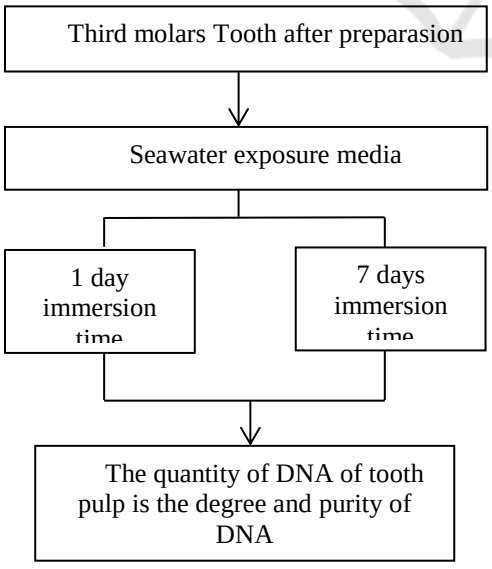

Figure 1: Flow chart of working plan.

This research was done by experiments, which were directed to observe and explain a situation that occurred (cause of effect) within certain time that could not be controlled by researchers. The purpose of this research was to measure the DNA tooth pulp quantity exposure to seawater for 1 day and 7 days based on concentration and purity of DNA for forensic odontology.

This research used third molars tooth sample post-retraction in healthy condition and without caries as variables studied and DNA quantity as dependent variables, and also the media of seawater and exposure time as independent variables. The analysis of total salt or salinity used the argentomtric method. The seawater sample that were analyzed were filtered first for remove impurities and then diluted. Afterwards, argentometric titration had to be done.

2.2 Methods

2.2.1 Preparation of Third Molars Tooth Samples

Third molar teeth exposed to seawater for 1 day and 7 days were drilled to obtain a tooth pulp portion which was then dissolved into 300 µl aquadest in a centrifuge tube. Then, they were vortexed immediately, and the supernatant was removed and then added with DNAzol 1000 µl (Invitrogen, ThermoFisher Scientific, Waltham, MA, USA). The solution was then vorteed again and incubated for 1 hour.

2.2.2 Extraction of Tooth Pulp DNA

The prepared sample was added with chloroform 200 µl (Merck KGaA, 64271 Darmstadt, Germany), vortexed, and incubated overnight. After an overnight incubation, the sample was vortexed for 2 minutes. Then, the liquid of the sample was transferred to the new centrifugation tube. The solution was centrifuged at 8,000 rpm for 10 minutes. The supernatant was taken carefully and fed into a 1.5 ml eppendrof tube, which was then added with 70% isopropanol (EMSURE*, Merck KGaA, 64271 Darmstadt, Germany) until the tube was full and the contents were homogenized. The process continued with incubation for 30 minutes at room temperature. The solution was centrifuged at 12,000 rpm for 10 minutes, then the supernatant was discarded. The pellets were washed with 70% ethanol (EMSURE*, Merck KGaA 64271 Darmstadt, Germany) by 1 ml, and vortexed and incubated for one hour at room temperature. The pellet was dried by means of

opening the tube for 5-15 seconds after the 70% ethanol was removed. This protocol followed Putri et al (2016). It was followed by the addition of nuclease fice water to the pellet of 50 µl as a DNA solvent, which was vortexed and stored at -20 ° C to make ready volume of DNA pellet for DNA quantification with Ultraviolet-visible Spectrophotometer (UV-1601, Shimadzu, Japan).

2.2.3 Measurement of Concentration and Purity of Tooth Pulp DNA

Measurements were made using UV-VIS instrumentation (UV-160, PC, Shimadzu, Japan), where the eppendorf tube DW of 695 µl was added with 5 µl isolated DNA, then vortexed. The measurements of UV spectrophotometer absorbance were conducted at wavelengths of 260 nm and 280 nm. This protocol followed Simon et al (2018), in which DNA concentration was given by absorbance reading at 260 nm and purity at 280 nm in UV-1061.

3 RESULTS AND DISCUSSION

Seawater media that was used came from the Straits of Lombok, Indonesia, with 28.74% of total salt or salinity. Salinity measurement was done by argentometric method, which are the quantitative analysis method of formation of sediment from salt. The salinity of sea water media was still within the normal range, because based on Praseno (2000) research, the normal salinity range for tropical seawater waters is 28 ‰ - 32 ‰.

The quantity of dental pulp DNA based on the concentration of dental pulp DNA is as follows:

Table 1: Measurements of dental pulp DNA concentration.

Seawater exposure	Absorbance λ : 260 nm	DNA Concentration (ng/µl)	Average ± SD DNA concentration (ng/µl)
1 day	0.577 0.535 0.562	1009.75 936.25 983.50	961.50 ± 41.53
After (7 days)	0.454 0.490 0.423	794.50 857.50 740.25	797.42 ± 58.68

The results of measurements of dental DNA pulp exposure to seawater for 1 day and 7 days with a wavelength of 260 nm using UV instrumentation yielded results in the form of absorbances. The values of absorption or absorbance obtained in the conversion in the form of DNA content were determined by the equation:

DNA Concentration = λ260xFPx50ng/µl

The average ± SD dental DNA pulp concentration 1-day exposure of sea water was 961.50 ± 41.53, while for 7 days it was 797.42 ± 58.68. The decrease of DNA dental pulp concentrations in this study indicated that there was a structure of DNA dental pulp damage that resulted in reduced DNA concentrations.

The quantities of dental pulp DNA based on the purity of dental pulp DNA were as follows:

Table 2: Measurements result of tooth pulp purity

Seawater exposure	Absorbance		Purity of DNA	Average ± SD Purity of DNA
	λ 260 nm	λ 280 nm		
1 day	0.577 0.535 0.562	0.549 0.521 0.547	1.05 1.03 1.03	1.04 ± 0.01
7 days	0.454 0.490 0.423	0.446 0.457 0.409	1.02 1.07 1.03	1.04 ± 0.03

The purity of dental pulp DNA exposure to seawater for 1 day and 7 days with wavelengths of 260 nm and 280 nm using UV instrumentation obtained results in the form of absorbance. The value of absorption or absorbance obtained in the conversion were in the form of DNA purity obtained by the equation:

Purity of DNA = λ260 : λ280

The average value of ± SD DNA purity of dental was stable at 1.04. This indicates that the selection of DNA extraction was accurate and the next stage could be conducted.

Third molars tooth pulp DNA isolation post extraction was done by DNAzol method. The result of tooth pulp DNA isolation was followed by measurement of concentration and purity of DNA using spectrophometer UV-vis at wavelengths of 260 nm and 280 nm (Putri, 2016). The measurement function is to identify the tooth pulp DNA, since measurement of concentration and purity of DNA affected the success of next stage in DNA identifications. Decrement of DNA concentration up to 1 ng/µl potentially against decrement of detection

ability of STR up to 95% (Putri, 2016). If the DNA in bad degradation condition, it would cause the primer to not attach to DNA target which would be duplicated.

The measurement result of tooth pulp DNA concentration and purity from third molar tooth samples were shown in Tables 1 and 2. The tooth pulp DNA concentration and purity seawater exposure for 1 day was 961.50 ng/µl and 1.04, while the tooth pulp DNA concentration and purity for a seawater exposure of 7 days were 797.42 ng/µl and 1.04; The graphic below (Figure 2) shows the decrement of concentration after exposure seawater media. There was decrement of tooth pulp DNA concentration in this study, showing that the broken structure of tooth pulp DNA caused the decrement of DNA concentration.

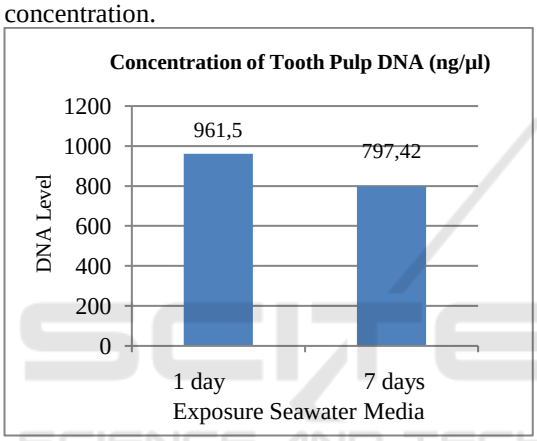

Figure 2: Graphic of decrement of tooth pulp DNA concentration

In groups of teeth, seawater exposure showed conformity concentration of tooth pulp DNA concentration and purity of 961.50 ng/µl dan 1.04. Irnamanda (2016) found that tooth pulp had high conformity concentration with post-extraction of tooth sample within 6 months (180 days) without treatment. Since morphology is composed by the hardest enamel substance and dentine that protects tooth pulp. Therefore, the pulp can protect DNA and experienced slow postmortem changes. However, after the exposure of seawater media treatment was done, there were degradations of DNA concentration. This is caused by enamel composition consisting mostly of hydroxyapatite salt, soluble materials (mucopolysaccharide), and insoluble substance (keratin), which could easily absorb water, causing the enamel to become semipermeable (penetrating by water). The diffusion path gap between crystal arrangement caused the enamel to be said as microporous solid material. The gap between apatite crystals containing the organic and water matrix occurred due to the structure of the hexagonal hydroxyapatite crystals that caused a perfect bond to be difficult to make.

Enamel is composed by inorganic substances and organics substance, but they do not bind together because of the hexagonal crystal structure, thus the lead gap between apatite crystal arrangements (Manjunatha, 2013).

Seawater is liquid, which means that it can penetrate the enamel and dentin due to a gap between the apatite crystal structure, since seawater, which is used for soaking tooth, cause liquid outside cells to diffuse into cells due to potential differences of sea water and red blood cells (Irnamanda, 2016).

Most salt-containing seawater has a higher potential than red blood cells. Therefore, sea water enters erythrocytes and causes the erythrocyte membrane to rupture and erythrocyte cytoplasm to exit. The higher the osmotic pressure, the easier the red blood cells in the tooth are pulled out because of the semipermeable cell membrane of water. The teeth exposed to sea water can affect the structure of DNA contained in the dental pulp (Irnamanda, 2016). Damages to the structure of DNA caused by exposure to seawater media result in the destruction of hydrogen DNA bonds that are irreversible. One of them is on the damage of base pairs purine-pyrimidine in DNA, which is the main component in the structure of DNA (Putri, 2016).

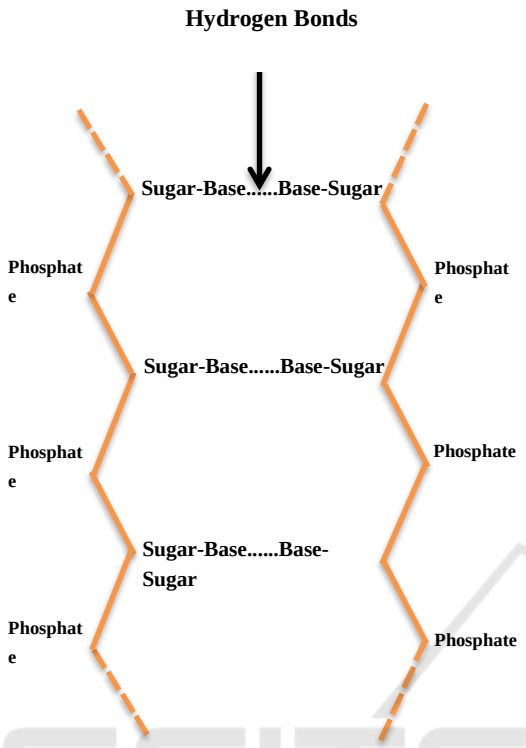

Figure 3: DNA structure of the purine-pyrimidine nitrogen base pair

The osmotic pressure on seawater is related to the salinity of seawater. The greater the salinity in sea water, the higher the osmotic pressure. In this study, the sea water used comes from the Strait of Lombok with total salt or salinity of 28.74‰. Salinity measurement was done by argentometric method, that is quantitative analysis method of formation of sediment from salt. The salinity of sea water media was still within the normal range, because based on Praseno (2000), the normal salinity range for tropical seawater waters is 28‰ - 32‰.

4 CONCLUSION

The quantity of dental pulp DNA processing in forensic DNA profiling, together with quality (purity), are important parameters for human identification. This study determined the effectiveness of seawater to the quantity of teeth pulped DNA by observing and presenting the trend. The factor's (sea water) exposure yielded an effect on DNA quantity for day 1 and 7, in which the amount of DNA decreased. This acts as an evidence that sea water has a potentially interfering and damaging outcome when targeted sample is found to be in contact. Effectiveness is suggested by high concentration of salinity contained in sea water, though it did not establish the minimal concentration of salinity. This study therefore concluded the existence of an impending effect from exposure to seawater. In addition, this study also significantly contributes to the understanding of the sea water medium in prediction of DNA quantity that could be expected at the prescribed duration of PMI. Furthermore, the finding triggers discussion towards experimentation of the minimal benchmark values of sea water salinity significance.

ACKNOWLEDGEMENTS

The authors would like to thank the technicians of the Human Genetic of Tropical Diseases (ITD) Laboratory of Airlangga University and all those who have assisted in the completion of this research.

REFERENCES

Datta, P., 2012. *DNA Profiling in Forensic Dentistry*. J Indian Acad Forensic Med. April-June 2012, Vol. 34, No. 2.

Febri, K.A., 2013. *Heteroplasmy Analysis of Dental Pulp Mitochondrial DNA in Forensic Personal Identification*. Dental Journal Vol. 46, Number 3, September 2013.

Girish, JK., 2010. *Dental DNA Fingerprinting in Identification of Human Remains*. J Journal Forensic Dent Sci. 2010. Jul-Dec 2(2): 63-68.

Irnamanda, D.H., 2016. *Effect of Seawater on ABO Blood Type Determination Accuracy of Dental Pulp*. KONAS PDFI 2016.

Krishan, K., 2015. *Dental Evidence in Forensic Identification-An Overview, Methodology and Present Status.* The Open Dentistry Journal, 2015, 9, 250-256.

Nzilibili, S.M.M et al, 2018. *Concentration and Purity DNA Spectrophotometer: Sodium Monofluoro phosphate Forensic Impended Effect*. Egyptian Journal of Forensic Science 8:34.

Manjunatha BS, 2013. *Dental Anatomy and Oral Physiology Including Occlusion and Forensic Odontology*. Jaypee Brothers Medical Publishers (P) LTD: New Delhi, India.

Putri, N.P and Yudianto A., 2016. *Effect Of Soil and Sea Water to Quality Of Psoas Muscle DNA Corpse With STR Methode*. Journal Bioscience Pascasarjana Vo. 18 (2016) No. 3, December 2016.

Prasero, D.P., 2000. *Retaid di Perairan Indonesia*. LIPI. Jakarta: Hal 82.

Rai, B., 2012. *DNA Technology and Forensic Odontology*.
Spinger Link: Evidence Based Forensic Dentistry.