CHAPTER 1

INTRODUCTION

1.1 Background

Sex determination is of great importance as additional information in criminal investigations as well as in identification of missing persons, no suspect cases, and ancient DNA studies (Andreasson and Allen, 2003). Deoxyribonucleic acid (DNA) is extracted from toothbrushes and sex determination is achieved by amplification of the amelogenin gene through AMEL gene-based primers in PCR. Amelogenin-based gender tests are part of various PCR multiplex reaction kits from different manufacturers which are widely used for DNA typing of both reference samples and casework samples in the forensic field (Steinlechner *et al.,* 2002). Forensic biology necessarily involves the application of DNA along with various other branches of sciences which deal with proper handling, examination, evaluation, and presentation of toothbrush evidences, that aids to investigate a crime and deliver justice.

There are many biological samples which can be isolated DNA. However, crime scenes are different from each other and the evidences that can be found at the crime scene is different too. In such cases, toothbrush can be used as an alternative to get the DNA. Sex determination is also a part of forensic biological evidence and an essential priority when traditional identification of the deceased becomes impossible. This research aims to determine Sex by analysis of the amelogenin gene using Polymerase Chain Reaction (PCR) method on Deoxyribose

nucleic acid (DNA) isolated from toothbrushes. This testimony is done by using the toothpaste which include sodium fluoride.

Deoxyribonucleic acid (DNA) purity and concentration from toothbrush for forensic intent is encountered by a number of factors including the used toothpaste constituents like Sodium Monofluorophosphate (SMFP) and Sodium Fluoride (NaF) compounds (Volpe *et al.,* 1995). For fluoride compound in toothpaste, other researchers has suggested that there is an effect caused by the compound in profiling DNA for forensic purposes (Alfadaly *et al.,* 2016; Song *et al.,* 2015). Such effect is also manifested in DNA extraction from toothbrushes with fluoride compound (Adams *et al.,* 2017; Nzilibili *et al.,* 2018). Therefore, this effect might contribute to problematic and challenging identification on individualized investigation.

In human identification, the victim's toothbrush is an invaluable personal item as the deposited cellular material contains DNA from which a reference profile can be produced (Bandhaya and Panvisavas, 2008). DNA recovered from a used toothbrush is a valuable known reference source of DNA that is useful for identification purposes (Riemer, Fairley, and Oc, 2012). Toothbrushes are often selected source of reference DNA sample in victim identification cases. Although DNA profiling from toothbrushes is routinely performed, there have been reports of failure to generate results in some cases for example, some cases occurred in Thailand (Alfadaly *et al.,* 2016).

If the toothbrush has been shared, it is possible that a mixed DNA profile will be required. One cause could be attributed to the moisture in bathrooms together with the tropical temperature and humidity, which promotes growth of

bacteria and thus accelerates DNA degradation. Furthermore, although the common practice of using all of the bristles (stiff hairs of toothbrush) improves the chance of collecting enough intact genetic material, it may also increase the concentration of PCR inhibitors found in toothpaste residue, which compromises the quality of the resulting DNA profile (Alfadaly *et al.,* 2016). In most cases, there are enough preserved biological material in used toothbrushes to give DNA in quantities and quality good enough for all kinds of DNA work related to human identification (Jobim *et al.,* 2004).

Amelogenin is a major protein constituent of the developing enamel matrix. Amelogenin constitutes about 90% of the total enamel matrix proteins and play a major role in the mineralization and morphological changes in enamel (Chowdhury *et al.,* 2018). The human amelogenin gene has been located on X-chromosome at Xp22.1–p22.3 and on Y chromosome at Yp11.2 with 90% of the transcripts expressed from the X-chromosome and the rest from the Y-chromosome. The X and Y copies of the amelogenin gene do not undergo homologous recombination (the trading of DNA that occurs between equivalent segments on paired chromosomes). Because of this, the amelogenin gene is the preferred genetic marker for sex determination in forensics (Bansal *et al.,* 2012).

In forensic casework, analysis of the X and Y homologues of the amelogenin gene is the basis of the use of amelogenin to determine the sex of a DNA donor in forensic analysis (Bulter and Li, 2014). The biological sex of human remains is a very important part of identifying victims. Moreover, human gender identification, based on the amelogenin gene, has important applications in forensic

casework, prenatal diagnosis, DNA data basing, and blood sample storage. The amelogenin gene, located on the X and Y chromosomes in humans (1), produces a protein important in the development of the tooth enamel matrix (2). Using specific amelogenin PCR primers, different bp fragments are amplifiable from the X and Y chromosomes, respectively (3) (Reddy *et al.,* 2011). Hence, it has been a central system to differentiate males from females especially in forensic casework and prenatal diagnosis.

Current methods of human identification in forensic science rely heavily upon the analysis of human DNA. It has long been known that cellular DNA is present in saliva and that this DNA is suitable for forensic purposes. The preferred source of DNA in human genetics research is blood, or cell lines derived from blood, as these sources yield large quantities of highly quality DNA. However, DNA extraction from saliva can yield high quality DNA with little to no degradation/fragmentation that is suitable for a variety of DNA assays without the expense of a phlebotomist and can even be acquired through the mail (Goode *et al.*, 2014). Saliva samples contain multiple enzymes and antibacterial components, as well as large quantities of nucleated buccal (epithelial) cells, leukocytes and bacterial DNA (Bruinsma *et al.,* 2018). Amplification of amelogenin from salivary DNA samples have a high potential to be used for forensic individualization process *(*Khare *et al.,* 2012).

Nzilibili et al., (2018) in their study, used toothpaste containing 0.1% of SMFP. After using it for seven days tooth brushing, the result came out to be negative when determination of purity and concentration was measured. So, in this

experiment, DNA purity, concentration and sex determination will be measured by using a toothpaste which contains 0.221% of NaF in the first, third and seventh day tooth brushing. People usually have their own toothbrushes and seldom use others; one can usually expect to obtain DNA of the person in question from his/her toothbrush without any contamination by other people's DNA. Therefore, we can determine DNA purity and concentration from toothbrush and we can also analyze sex determination for forensic evidence in crime scenes.

1.2 Statement of the Problem

Today evidences have too many released failures that result in too many rollback failures. If we ignore this problem; resources will need to increase to handle the cascading problems, and we may miss critical deadlines which could result in lost evidence and further damage to our quality reputation. The main problem of this study is to determine the effectiveness of using sodium fluoride toothpaste in forensic evidence sex determination. Understanding of DNA biological evidence samples' diversity in various environmental conditions made, some questions are left unanswered; for instance, what is the effect of DNA purity and concentration from toothbrush by using sodium fluoride containing toothpaste on forensic DNA evidence? What is the suggestive crime scene lifespan estimation when using amelogenin gene from toothbrush to identify sex? All these questions seek to explore maximized possible contribution of DNA from toothbrush at crime scene. The toothbrush evidence is to help in application of investigation of a wide range of crimes, DNA test, paternity test, sex test, missing persons and victims of mass disasters and evidence for court, etc.

Biological evidence with forensic interest may be found in several cases. Although, in some cases, DNA biological evidence sample cannot be found and is limited under natural environmental treatment in crime scene. In such cases, toothbrush can be used as an alternative to get the DNA purity, concentration and sex determination. However, this alternative way of using toothbrush to obtain DNA is not commonly used in forensic field. Therefore, DNA from toothbrush evidence need to be more accurate and sufficient services for crime scene investigation and personal identification. But, a number of factors can affect the amount of DNA that is recovered on such samples, including environmental conditions, handling time, the contacted surface, and individual differences in cell shedding among people. Furthermore, samples containing degraded or low quantities of DNA are common to forensic DNA analyses, and maximizing the amount of DNA recovered has been an important area in forensic science. Therefore, sample collection and storage are important issues as well when working with personal effects for victim identification or as evidentiary material.

The finding of this study will redound to the benefit of forensic science that plays an important role in crime scene investigation. The finding of forensic evidence with toothbrush needed to be more effective to the court. The outcomes to be considered are consist of the followings: using toothbrush to determine DNA purity, concentration and sex identification; development of a forensic evidence towards legal system/court; knowing sodium fluoride toothpaste effect on forensic DNA; increase in forensic biological evidence for crime scene; and enhancing the DNA forensic evidence from toothbrush.

1.2.1 Research Questions

1) How does the effect of toothpaste which contains sodium fluoride influence DNA purity and concentration at exposure time on day 1, 3 and 7?
2) How is the result and accuracy percentage of identifying sex from amelogenin gene isolated from toothbrush by using sodium fluoride containing toothpaste at exposure time on day 1, 3 and 7?

1.3 Research Objectives

1.3.1 General Objective

To examine the analysis of amelogenin gene to identify sex from toothbrush as a forensic evidence using sodium fluoride containing toothpaste.

1.3.2 Specific Objectives

The following specific objective guide the study:

1) To analyze how sodium fluoride influence towards DNA purity and concentration at exposure time on day 1, 3 and 7
2) To estimate how tooth brushing affects both men and women at exposure time on day 1, 3 and 7
3) To estimate the percentage of the accuracy of identifying sex from amelogenin gene isolated from toothbrush at exposure time on day 1, 3 and 7.

1.4 Research Significance

1.4.1 Theoretical Significance

The result of the study can enrich the evidence for crime scene investigation. This study is expected to contribute significantly in understanding the trending in purity and concentration of DNA and sex identification from toothbrush as caused by influence of sodium fluoride exposure from day 1, 3 and 7 brushing. The result of the study can encourage the researchers those who want to determine sex for forensic evidence, especially for court or personal identification.

1.4.2 Practical Significance

According to National Institute of Justice in the United States, Forensic science plays a vital role in the criminal justice system by providing scientifically based information through the analysis of physical evidence. During an investigation, evidence is collected at a crime scene or from a person, analyzed in a crime laboratory. Nzilibili et al., (2018) in their study, concentration analyzed portrays the detrimental effect that damage and destroy DNA molecules into increased segmented molecules. Purity readings suggest the lowered amount of intact DNA molecules that would be enough to make into PCR. Also, PCR and electrophoresis method portrayed inhibition and failed visualization of sex gene band contrast (Nzilibili *et al.*, 2018). This study can apply various techniques in understanding DNA purity and concentration of forensic evidence, and will investigate sodium fluoride containing toothpaste effect on forensic DNA. Furthermore, the analysis of amelogenin gene to identify sex from toothbrush will need to be more effective for forensic evidence in crime scene lifespan estimation.