

■ REVIEW ARTICLE

Saliva: A Trump Card in Forensic Odontology

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ABSTRACT

Traditionally, bite-mark analysis has been a vital element in forensic odontology. Salivary analysis is a novel addition to this field. Recovery, detection and analysis of saliva could be crucial in a crime investigation. Hence saliva can act as a significant source of forensic evidence such as drugs, toxins, heavy metals, hormones, sex determinants, DNA and other nucleic acids, blood group antigens and oral microbiota data which can be crucial in identifying the suspect or victim. Additionally, saliva has also depicted some significant benefits over the other biological fluids such as blood. To emphasize on this aspect, the non-invasive nature for collection of saliva and the ease with which it can be collected makes it an edge over blood as a biological sample. Saliva is also comparatively safer to collect especially from some forms of acquired immunodeficiency syndrome and hepatitis. This review summarizes the use of saliva and its implications in forensic as an evidence.

KEYWORDS | forensic odontology, saliva, identification, oralfuid, forensics

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INTRODUCTION

FORENSIC ODONTOLOGY OR FORENSIC DENTISTRY, is a branch of science which applies knowledge in dentistry for the process of law reinforcement by examining dead or living body, including analysis of saliva in crime investigation.¹

Over year saliva is gaining interest amongst the scientific community to use as a diagnostic tool as an alternative to blood or urine.² Saliva is a complex oral fluid which could play a critical role in forensic evidence.³ It comprises of a mixture of secretions released from salivary glands, gingival crevicular fluid and contains exfoliated oral epithelial cells and microorganisms.² The gaining popularity due to its ease and noninvasive method of collection, safety in handling compared to blood which has higher potential risks transmitting diseases

such as hepatitis and acquired immune deficiency syndrome from needle pricks while collecting, makes saliva a desirable fluid for forensic analysis as major source of DNA.⁴

Recovery of traces of body fluids is one of the most important types of forensic evidence. They contain valuable DNA samples which may help in identifying an accused or a victim. Saliva may get deposited unwittingly on skin during acts of biting, sucking or licking. Hence, it can act as a significant source of forensic evidence which can be crucial in law enforcement, including in crime investigation, identifying suspects & victims and also in detecting drug abuse.⁵ This review summarizes the use of saliva and its implications in various aspects of forensics as an evidence.

Detection of and Collection of Saliva from

crime scene

Bite mark analysis has long been a part of forensic odontology as bite marks found in many homicides and assaults. However, elastic and distortable nature of skin compared to a good impression medium, makes it difficult to obtain relevant information. Hence, attention has been shifted to collect saliva deposited on these bite marks to get more relevant information such as DNA, biomarkers and other components that would help in identifying the suspect.⁶

As saliva is a dry colorless stain, it may not be detected by a normal visual inspection. Hence, two special methods are used to detect saliva from the crime scene. Using alternate light sources (ALS), with wavelengths from 415 to 490nm (using orange/red goggles) quartz archtube or argon ion laser, saliva appears as soft edged white spots comparatively less intense than other stains.⁷ Using ultraviolet lights, saliva stains appears bluish white. However, ultraviolet is less preferred as it can degrade the DNA in the sample.⁸

In cases where the above mentioned special methods fail to detect saliva, it can be detected using chemical and immunological methods. Detection of chemicals such as nitrates and thiocyanates, amino acids like tryptophan, reducing carbohydrates, and enzymes such as alkaline phosphatase and alpha amylase, which are found in saliva, is the principle behind it. However, as these chemicals can be found in other body fluids and their presence is not always warranted in saliva, the sensitivity and specificity of these methods varies.⁹

Sheets coated Phadebas®, a reagent that consists of blue dye-linked starch which becomes soluble in the presence of α -amylase, is widely used nowadays in forensic odontology to detect invisible salivary stains. This test is called Phadebas® Forensic Press Test, named after the reagent and the way it is performed.¹⁰

Rapid stain Identification test (RSID) is a parallel stream immune-chromatographic test similar to pregnancy testing kit is used for identifying saliva detecting the alpha amylase

in it. Even though not widely used, ELISA test can also be used to detect alpha amylase.¹¹ Near Infrared (NIR) Raman Spectroscopy and Fluorescence Spectroscopy are also widely used techniques.¹²

After identifying the presence of saliva, classically it is collected from the skin using a wet cotton swab or a wet filter paper. This method is referred to as Single Swab Technique. Sweet et al in 1999 developed Double Swab Technique for better yield of saliva. It was achieved through rehydration of the dry salivary stain with a sterile swab wetted in nuclease free water. This swab is rolled over the stain for about ten seconds applying moderate pressure. Then a second swab is used to collect the saliva.¹³

The suspect's saliva is collected directly from the oral cavity suspect as well in order to analyze and compare with the saliva obtained from the crime scene. Non-stimulated saliva can be collected simple rinse with mouth wash. Paraffin or citric acid crystals are used to stimulate production of saliva. For more concentrated saliva, it can also be collected directly from the opening of Stenson's duct using commercially available kits like Oragene.¹⁴

Laboratory Analysis of Saliva

General laboratory tests of saliva are used to identify if the stain is saliva or something else. Those tests are parts of detection of saliva and mentioned in the above section. Apart from those tests, forensic odontology has devised various tests of saliva that would help in identifying the suspect or the victim by finding out various characteristics of a person including age appropriate and gender appropriate hormones, presence of drugs, toxins, gender appropriate chromosomal characteristics, and genetic materials such as RNA and DNA. Some of the important ones are mentioned as follows.

Salivary Drug Screen

Compared to the traditional urine drug screen, used to detect presence of drugs in human body,

which has the risk of getting wrong sample as patients collect the sample themselves in their privacy, salivary drug screen has an advantage of getting the right sample. It is analyzed by radio immuno assay (RIA) technique to detect various drugs such as opioids, barbiturates, benzodiazepines, cocaine, amphetamines and cannabis. Nicotine can be detected in saliva of smokers using ELISA technique to detect alkaloid nicotine also known as cotinine which is considered as a biomarker for nicotine exposure.^{5,15} Recently immuno chemical strip test called Drug Wipe Test has been introduced for quick detection of commonly abused substances in saliva.¹⁶

This information can assist the investigative team to characterize the suspect and rule out people in their list who are less likely to abuse substances. This information can also help to know if the victim has been drugged prior to the assault or homicide.

Saliva Toxicology Screen

Presence of various poisons can be detected in saliva using appropriate techniques. Organophosphate, organochlorine and other toxic substances can be detected in saliva. For example, Atrazine, Diazinon can be detected using ELISA technique, Ethion using Liquid-Liquid Extraction (LLE) or Gas Chromatography techniques. While PVC can be identified using Light Chromatography and Mass Spectrometry, Acetone intoxication can be detected using Solid Phase Micro Extraction (SPME), Gas Chromatography and Mass Spectrometry. Paraquat is better identified using diode-array electrophoresis.^{17,18}

Detecting toxicological status can verify if the victim has been poisoned before the act of crime has occurred, which would help the investigation team to assume the modus operandi.

Salivary Drug Screen

Heavy metals such as Mercury, Nickel and Zinc which can appear in saliva as a result of leaching dental materials can also be detected in saliva using various techniques such as atomic

absorption spectroscopy or mass spectroscopy. Identifying the heavy metal in saliva can help in identifying the suspect or victim by matching with the records of dental procedures involving restoration and prosthesis using various dental materials.^{17,18}

Salivary Hormone Detection

Various hormones can be detected in saliva using Radioimmuno assay (RIA). Detecting testosterone, estrogen and progesterone can identify the sex of a person whereas hormones such as beta-human chorionic gonadotropin can identify the pregnancy status of the person. Low cortisol levels and high testosterone levels were correlated to criminal behavior in a few studies. Hence, detecting such hormones can assist a forensic odontologist in identifying the suspect or the victim¹⁵.

Sex Determination from Saliva

Apart from relative concentrations of various sex hormones, presence of barr body in exfoliated oral mucosal cells in saliva can help identifying the sex of the person. Barr body is inactive in X chromosome in cells with more than one X chromosome,⁵ which are usually cells from people off male sex (XX), but it can also be seen in males with Klinefelter syndrome (XXY).

F-bodies are fluorescent stained Y chromosomes seen in sperm head. Detecting the presence of F-bodies in saliva can identify the presence of spermatozoa in saliva.⁵ This information may be crucial in suspected cases of sexual abuse.

Salivary DNA Isolation and Fingerprinting

DNA of the suspect or victim can be obtained from the exfoliated oral mucosal cells found in saliva. DNA from saliva and skin-deposited saliva samples can be extracted by using phenol-chloroform method.¹⁶ Those DNA samples are amplified by PCR. Thus amplified DNA is typed using a set of 15 STRs. This is used to establish a link between biological evidence and a suspect or victim in a criminal investigation. This is called DNA fingerprinting.⁶

Usually cells contain genomic and mitochondrial DNA. Matching genomic DNA

with available sample or a first degree relative can aid in establishing the identity of a person. Chromosomes containing genomic DNA undergoes meiotic division and recombine with paternal chromosomes during fertilization. Hence, variations occur even amongst parents and children, or between siblings except for monozygotic twins. So this method may not be very effective when a sample for comparison or a close relative is not available. Mitochondrial DNA (mtDNA) is inherited through cytoplasm of the gametocyte from mother. This does not undergo meiotic division or recombination during fertilization. Hence copy of same mtDNA can be found even in distant maternal relatives across generations. Hence, matching mtDNA can identify even distant maternal relatives of the person. This can be particularly useful when close relatives are not available.¹⁹

Extra cellular Nucleic Acid Detection in Saliva

Apart from DNA, other nucleic acids such as mRNA and microRNAs (miRNAs) are secreted in extra cellular body fluids such as breast milk, semen, saliva, urine. RNAs has homology with DNA, which makes them useful when DNA is not available. RNA obtained from saliva is used to identify the victim or suspect by comparing it with reference sample by comparing various characteristics of RNA such as Copy number variations (CNV) or Single nucleotide polymorphisms (SNPs).²⁰

Blood Group Antigens

Approximately 80% of people secrete ABO blood group antigens in their saliva. If the suspect has bitten the victim, the suspect's ABO antigens can also be extracted from his saliva. Identifying blood group of the suspect or victim can assist in establishing one's identity in forensic investigations.²⁰

Salivary Microbiota

Bacteria of oral microflora could be recovered and analyzed from saliva. Streptococcus

mutans, Actinomyces, and Veillonella are commonly found in saliva. They can be identified by methods such as bio-typing, serotyping, and bacteriocin typing. Out of the common organisms found, the uniqueness of Streptococcus mutans for every person makes it the most important species used in identification in forensic odontology. The unique Streptococcus mutans for each person is identified using genotyping it with Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) analysis.²¹

CONCLUSION

Since the human skin is not a good impression medium because of its elastic and distortable nature, analysis of bite marks, as traditionally practiced in forensic odontology poses a limitation to the advancement of the field. This led to opening of another frontier in using saliva as a forensic tool. Saliva is a biological fluid that can be easily obtained using noninvasive techniques. It constitutes various products such as drugs, toxins, heavy metals, hormones, sex determinants, genetic materials such as DNA and RNA, blood group antigens, and micro biota, which would help in identifying victim or suspect in a criminal investigation. It is a new research area still in infancy and highly underutilized. It needs to be explored further for its better utilization and raise awareness to facilitate appropriate legal policy changes. **IJFMP**

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