A SYSTEMATIC REVIEW

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Saliva as a diagnostic tool in forensic odontology

Jessica, Elza I. Auerkari*

Abstract

Objective: This paper aims to review the studies of the saliva analysis for forensic odontology purposes, starting from identifying the saliva stain and extracting the saliva sample.

Methods: A literature review was performed using textbook (2016) and online search in scientific databases: PubMed®, ScienceDirect®, JDMFS online, EBSCOhost® (from 2002 to 2017). The studies generated were screened and selected by a single examiner based on the following criteria: full text articles with abstracts reporting on saliva and forensic odontology or personal identification and all articles are written in English.

Results: From the articles gained by the author, the author found that saliva stain can be detected through many ways and can be extracted using single swab or double swab technique. Saliva analysis can be used to detect drug and hormone abuse, to detect intoxication, to identify suspects and victims of a crime or disaster, and also to identify bitemark's perpetrator.

Conclusion: Saliva is a very useful diagnostic tool in forensic odontology. Its detection and analysis is very important in verifying the existence of a crime, in identifying suspects and victims of a crime, and in personal identification in mass disaster. Despite all the usefulness of saliva analysis, the existence of saliva in crime scene is often difficult to be confirmed, therefore it is better to combine more than one saliva stain identification tool to confirm.

Keywords: Forensic odontology, Law reinforcement, Personal identification, Saliva analysis, Saliva stain

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Department of Oral Biology, Sub-division Forensic Odontology, Faculty of Dentistry University of Indonesia, Jakarta, Indonesia

*Corresponding to:
Auerkari, Department of Oral Biology, Sub-division Forensic Odontology, Faculty of Dentistry University of Indonesia, Jakarta, Indonesia
eiauerkari@yahoo.com

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Introduction

Forensic odontology or forensic dentistry is a branch of dentistry science which applies dentistry knowledge in law reinforcement process by examining living or dead body, including saliva analysis in crime investigation.[1]

Saliva is mouth fluid. Its production and secretion is affected by stimulation, diet, fasting, age, sex, and medications.[2,3] Saliva analysis can determine one's body condition due to substances movement from blood to saliva through several mechanisms, such as passive diffusion, active transport, and ultrafiltration (gap junction).[4,5] Previous studies found that saliva analysis can determine Cystic Fibrosis, Sjogren's Syndrome, Squamous Cell Carcinoma, periodontal disease, stress, acute myocardial infarct, cholesterol, pancreatic cancer, and the disability to absorb Calcium.[2,4,5] Besides that, saliva analysis can also be used to detect viral infection (HIV, Human Herpes, Cytomegalo, Epstein-Barr, Hepatitis C, Hepatitis A, Hepatitis B), as well to detect H.pylori infection (the bacteria causing peptic ulcer).[2,4-6]

Previous study stated that most of the drugs found in blood and urine, can also be found in saliva, thus enabling saliva analysis to detect drug abuse.[7] Recently, drug abuse is increasing so fast. Usually, to detect whether or not someone consuming drug, the authority will perform urine collection and analysis, but the urine collection is often considered as an invasion of one's privacy.

Medical check-up or drugs examination using saliva has many advantages, such as low sample quantity, higher sensitivity, non-invasive, easy collection, not inducing stress, increases subject cooperation, can be collected anywhere, no need of special equipment or training, can examines children or adults, correlation with level in blood, more accurate in detecting many oral and systemic diseases, less expensive to screen large population, minimum cross-infection risk between subject and examiner as well as risk between subjects.[1,4-8]

Saliva can be found in crime scene.[7,9] Saliva can be deposited on things in crime scene or on bitemark on victim's or suspect's body. Data obtained from saliva analysis is very important in law reinforcement, including in proving the existence of crime, in crime investigation, in identifying suspects & victims and also in detecting drug abuse.[4-6,8,9]

This paper will review how to identify the presence of saliva and review several use of saliva in forensic odontology. The authors hope that this paper gives contribution in forensic odontology development in Indonesia.

Methods

We performed a literature review using textbook (2016) and online search in scientific databases: PubMed®, ScienceDirect®, JDMFS online, EBSCOhost® (from 2002 to 2017). The studies

generated were screened and selected by the first author only (JJ) based on the following criteria: full text articles with abstracts reporting on saliva and forensic odontology or saliva and personal identification and all articles are written in English.

Results

From the articles gained by the author, the author found that there are many ways to detect saliva stain and in forensic odontology, saliva is used to help investigator to detect drug and hormone abuse, to detect intoxication, to identify suspects and victims, and also to identify what species made the bitemark.[1,4,7,8,10]

Drug and Alcohol Abuse Detection

Urine collection to detect drug and alcohol consumption has weaknesses, such as possibility of getting wrong sample and considered as invasion of one's privacy. Saliva analysis by using Radioimmunoassay (RIA) technique can be used to detect opium (opium will be detected 4 hours after consumption), phenobarbital, amphetamine, morphine.[4,7] Alcohol abuse can be detected by determining ethanol level in saliva 20 minutes after consumption by using Gas Chromatography or ELISA technique.[4,8]

Saliva stain that is thought to contain drug or alcohol can be analyzed using Enzyme multiple immunoassay and Gas Chromatography or Mass Spectrophotometry followed with RIA technique.[7]

Cigarette Consumption Detection

Cigarette consumption on active smoker or nicotine intoxication on passive smoker can be detected by determining cotinine level in saliva using RIA or ELISA technique. Cotinine is tobacco's main metabolite (alkaloid nicotine), thus cotinine is considered as nicotine exposure biomarker.[8]

Organic Substances Intoxication Detection

Pesticides, such as Atrazine and Diazinon can be detected in saliva using ELISA technique, while pesticide such as Ethion can be detected in saliva by using Liquid-Liquid Extraction (LLE) or Gas Chromatography technique.[8]

Paraquat can be detected in saliva by using electrophoresis technique with diode-array detection. Phthalates, common material used in production of PVC (polyvinyl chloride), can be detected in saliva by using Light Chromatography and Mass Spectroscopy technique. Acetone intoxication can be detected from saliva by using Solid Phase Micro Extraction (SPME), Gas Chromatography and Mass Spectroscopy technique.[8]

Heavy Metals Intoxication Detection

Some metals that can be detected in saliva by using Atomic Absorption Spectroscopy or Mass Spectroscopy technique are Cadmium (Cd) and Lead (Pb). We can also detect leaching of dental materials, such as Mercury, Nickel, Zinc, etc by analyzing saliva.[8]

Hormone Detection

Radioimmunoassay (RIA) can be used to determine the presence and the quantity of testosterone, estradiol and progesterone, cortisol and cortisone in saliva.[4,7] Solid phase immunoassay can be used to detect Aldosterone hormone in saliva.[4]

Personal Identification

Saliva can be deposited on things in crime scene or on bitemark on victim's or suspect's body. Saliva analysis can be very useful to identify suspect. Therefore, it is very important to detect and analyze saliva stain.

Detection of Saliva Stain

There are many ways to detect the presence of saliva stain, i.e. using chemicals (Alkaline Phosphatase enzyme, iodine, starch; nitrate salt & Thiocyanate), using laser and ultraviolet light (quart arch tube and argon ion laser), fluorescent spectroscopy technique, RNA marker, and by detecting the presence of oral Streptococci.[7,9,11]

Starch; Phadebas® is widely used to detect saliva stain. Phadebas® reagent consists of blue dye-linked starch which becomes soluble in the presence of α-amylase. There are two forms of Phadebas®, i.e. sheet coated with Phadebas® reagent and tablet of Phadebas® reagent. The test using sheet coated with Phadebas® reagent (i.e. called Phadebas® Forensic Press Test) is used to locate invisible saliva stains by detecting the high α-amylase enzyme activity in saliva. It is done by pressing the sheet directly on the samples. If the stain location can be visibly identified or estimated, a tablet of Phadebas® reagent is used with several methods.[9]

Fluorescent Spectroscopy; optimal light to detect saliva stain is the light having 450 nm wave length, where Tryptophan in salivary α-Amylase will emit typical spectrum, providing good sensitivity in detecting the presence of saliva stain on skin.[7,9] But, the fluorescence emitted from saliva is relatively weak compared with that of other body fluids, such as semen and is not always detectable depending on the materials on which the saliva is present.[9]

RNA-based Saliva Marker; Watanabe et al found that simultaneous detection of mRNA Statherin (STATH) and Histatin 3 (HTN3) by the real-time PCR method is a powerful supplementary tool

to detect the presence of saliva even in a sample suspected of containing other body fluids with amylase activity. However, their study demonstrated that the RNA saliva markers were less stable than amylase (especially in wet conditions) and also less stable than DNA in dry conditions although DNA was also unstable in wet conditions.[9]

Therefore, Watanabe et al suggest that the RNA method could be introduced to saliva identification procedures and should be used as a supplementary method to strongly support identification of saliva by the amylase-based method.[9]

Detection of Saliva Stain by detecting Oral Streptococci; Nakanishi et al.[11] found that saliva stain can be identified by detecting the presence of S. salivarius and S. mutans using Polymerase Chain Reaction (PCR), and they suggest that S. salivarius is more reliable than S. mutans. It is a promising new saliva marker because these streptococci were not detected in semen, urine, vaginal fluid, or on skin surfaces.[11]

Saliva Sample Extraction

After saliva stain is detected, we can extract the saliva by using single swab or double swab technique. Single swab technique is a technique in which a sterile cotton end, dipped in sterile distilled water for 10 seconds, swabs saliva stain in circular motion and light pressure. After that, the cotton is put in an evidence box for at least 30 minutes to dry it.[1,7]

In double swab technique, the steps in single swab technique are followed by swabbing the wet stain with a new dry sterile cotton in circular motion and light pressure in 10 seconds after the first swab. The second cotton is put in the evidence box too for at least 30 minutes to dry it.[1,7]

After the cottons are dry, they are taken out from the evidence box. The next steps are extracting saliva DNA with Phenol-chloroform method and amplifying the DNA sample with PCR using Short Tandem Repeats (STRs).[1,7]

DNA Analysis

Persons can be identified by performing serologic and cellular analysis. The cell in saliva contains genomic DNA and mitochondrial DNA (mtDNA) which are very useful in identification. There are many mtDNA in each cell because most cells have many mitochondria. mtDNA is inherited maternally, so in the absence of any close relatives, distant maternal relatives can be used as a reference source to support identification. Therefore, mtDNA analysis may be successful when nuclear DNA analysis fails or when genomic DNA cannot be analyzed, such as in the case where the sample is too degraded or in the absence of nuclear DNA (e.g. rootless hair and tooth).[7]

Sex Determination

Cells found in saliva can be used to determine one's sex by detecting the presence of sex chromatin and by determining sex hormone level.[7] Barr bodies are sex chromatin in females, while F bodies are in males.[7] Sex hormone levels are determined based on detectable quantities and ratios of testosterone and 17B-estradiol by RIA.[4,7]

Bitemark Analysis

Rapid Stain Identification (RSID) is used on saliva stain to confirm that the perpetrator is human and not animal. RSID detects the presence of human saliva α-amylase. RSID will give positive result if the perpetrator is human and will give negative result if the perpetrator is animal.[10]

In the case where animal is definitely the perpetrator, species identification is done by using Enzyme-linked immunoassay (ELISA) technique using monoclonal antibody to determine species salivary immunoglobulin A (sIgA). Crossover electrophoresis and double gel diffusion techniques are used for comparison in cases with poor monoclonal antibody results. Evolutionary relationships among species are most directly determined by comparisons at the DNA sequence level.[7]

Discussion

Despite of its usefulness and advantages, saliva analysis is difficult to be performed because saliva is unstable. Salivary proteins are easily degraded due to proteolytic activity of proteolytic enzymes released by cell substances and microbes. Besides that, the complexity of saliva substances also makes saliva preservation difficult.[6,12]

Saliva stains are not always detected, depending on the material where saliva is deposited.[9] Ohta and Ohmura found that citric acid and positively charged ion (e.g. Fe3+ and Mg2+) will inhibit α-amylase enzyme activity, causing saliva undetected.[13] Citric acid is contained in citric fruits and in soft drinks.[13] Fe3 + ion is one of the blood substances which will be contained in blood stains found in many crimes.[13] Mg2+ ion is contained in foods, medicines, and fertilizers.[13]

RSID might give false positive result.[10] RSID gave negative result to all animal saliva tested by Casey and Price, but Pang and Cheung reported

positive result on rat saliva using RSID, therefore RSID result is better validated with other saliva detection technique.[10,14]

Conclusion

Saliva is a very useful diagnostic tool in forensic odontology. Its detection and analysis is very important in verifying the existence of a crime, in identifying suspects and victims of a crime, and in personal identification in mass disaster. Despite all the usefulness and advantages of saliva analysis, the presence of saliva is often difficult to be confirmed, therefore it is better to combine the common detection method (which is based on α-amylase enzyme activity detection) with other detection method (e.g. with S. salivarius detection using PCR or with RNA marker).

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Conflict of Interest

The authors report no conflict of interest.

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