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Review Article

Saliva as a Forensic Tool: A Comprehensive Review

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***ABSTRACT*:**
Saliva has gained much interest among researchers in the past few years, especially in the field of forensic sciences. Scientists concur that the diagnosis and prevention of diseases using human saliva is about to be explored as more and more laboratories and medical practitioners get ready for this new technology. Unlike blood testing, saliva analysis looks at the cellular leveland therefore saliva is truly a representative of what is clinically relevant. Hence; in this review, we aim to highlight some of the important aspects and applications of saliva in the field of forensic.
Key words: Forensic, Saliva

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Introduction

Saliva is produced and secreted from salivary glands. The basic secretary units of salivary glands are clusters of cells called acini. These cells secrete a fluid that contains water, electrolytes, mucus, and enzymes, all of which flow out of the acinus into collecting ducts. Within the ducts, the composition of the secretion is altered. Much of the sodium is actively reabsorbed, potassium is secreted, and large quantities of bicarbonate ion are secreted. Small collecting ducts within salivary glands lead into larger ducts, eventually forming a single large duct that empties into the oral cavity.[1, 2]

Oral samples that are useful for the diagnosis of systemic diseases include saliva, gingival crevicular fluid (GCF), oral swabs, dental plaque, and volatiles. Indeed, published data indicates the successful use of all of these types of oral samples to detect or predict susceptibility to systemic diseases.[3, 4]

Forensic medicine has been largely by-passed by the tide of health systems research and evidence based medicine. Murder victims form a central part of forensic medical examiners' case load, and women murdered by intimate partners are an important subgroup, representing the most severe form and consequence of intimate partner violence.[3]

The term "Forensic odontology" is derived from Latin, meaning forum or where legal matters are discussed. Oneof the main advantages of forensic odontology inforensics is the multiple sources from where DNA can be extracted. This includes blood samples, saliva, bone, teeth, and tissues, mouth mucosa cells which may be presenton cigarettes, envelopes, lip prints and other biologicalmaterials. Forensic studies have demonstrated that use of saliva which is deposited in prints, bite marks,cigarette butts, marks and prints left on stamps and other objects may help in individual identification.[4-6]

Saliva is usually deposited in bite marks found in many homicides, assault and other criminal cases. Since many problems are encountered in bite mark analysis, primarily because of elastic and distortable nature of skin and lack of good impression medium, the saliva deposited during biting has received an important alternative focus in bite mark analysis. It is difficult to collect saliva stains from skin, clothing, paper or other inanimate objects since it remains invisible and substrate on which saliva is deposited, mainly skin, cannot be submitted directly to extraction procedures.[7, 8]

Methods of detection of dried saliva stains

- **Chemicals: -** various chemicals and enzymes have been tried to detect dried saliva stains. Saliva detection from stamps and envelopes has been done by the chemical which acts on reducing sugars and gives a red insoluble precipitate. Most commonly used enzymes are alkaline phosphatase, starch and amylase. Salts like nitrate and thiocyanate have also been used for the detection. All these methods have limitations and variable sensitivity depending upon the age of the saliva stain and quantity of deposit
- Lasers and ultraviolet light, quartz arch tube and argon ion laser
- Fluorescent spectroscopy:- the aromatic amino acid, tryptophan, in α-salivary amylase gives a characteristic emission spectrum on fluorescent spectroscopy, thus possessing a good sensitivity in detecting dried saliva stains on the skin.[9]

Saliva recovery from skin

Traces of salivary evidence can be recovered for identity testing. The classical technique using a single wet cotton swab or section of wet filter paper laid passively on the skin has been effectively used to collect saliva from the skin. A technique using a wet cotton swab (similar to the classical method) followed by a dry cotton swab, known as the double swab technique, was studied by Sweet et al., and found to provide a better yield of saliva recovered from the skin surface. Deoxyribonucleic acid (DNA) from saliva and skin-deposited saliva samples can be extracted by the phenol-chloroform method.[10]

Deoxyribonucleic acid profiling/fingerprinting

Except for identical siblings, DNA profiling is unique for individualization. Heat, moisture, sunlight, surface contaminants and other factors can accelerate DNA degradation. Contamination of evidence with DNA from animals or bacteria does not pose a serious problem as the probes used in DNA profiling are specific to humans or at least primates. Bacteria does have an effect on the stability of human DNA, especially soil bacteria which are rich in nucleases. The polymorphisms within the DNA molecule are the basis of all inherited polymorphisms and they do not change over the lifetime of an individual. DNA samples are amplified by polymerase chain reaction for DNA typing using short tandem repeats (STRs).[11]

The polymorphic repair of STR mainly in small fragments also makes it possible to evaluate DNA from samples with a significant grade of degradation. In addition to genomic DNA, cells derived from saliva contain mitochondrial DNA (mtDNA), the sequence of building blocks of which can be determined to assist in identification. The main advantage of mtDNA is that there is a high copy number in each cell caused by the high number of mitochondria present in most cells. Chromosomal DNA is inherited from both the mother and father whereas mtDNA is strictly maternally inherited. Therefore, mtDNA testing may be successful when nuclear DNA testing fails or when genomic DNA cannot be analyzed, possibly because it is too degraded.[12]

ANALYSIS OF DRUGS OF ABUSE IN SALIVA

The most frequently used biological specimen for the determination of drugs of abuse is urine since it is a noninvasively obtained sample and is acceptable for routine collection. Yet even the acceptability of a urine sample is being disputed in view of the potential invasion of privacy, especially if a directly observed collection is advisable to prevent adulteration or substitution of the sample. Improved analytical techniques have made it possible to analyze a large number of drugs in a small amount of oral fluid. The major advantage of oral fluid over urine is the easy, rapid and nonintrusive sampling procedure.[13]

Drugs that can be identified in saliva are amphetamines, barbiturates, benzodiazepines, phencyclidine, cocaine and opioids. Saliva can be used to detect recent marijuana use by means of radioimmunoassay. A major psychoactive component of marijuana can be detected in saliva for at least 4 h after marijuana is smoked.

Most drugs appear to enter saliva by simple passive diffusion which is characterized by the transfer of drug molecules down a concentration gradient with no expenditure of energy. Salivary drug concentrations generally reflect the free fraction of the drug in the blood. Peel et al. found measurable quantities of drugs in saliva extracted with methanol and analyzed by enzyme multiple immunoassay technique and gas chromatography/mass spectrophotometry. This methodology, both specific and sensitive appears to be a useful adjunct to serological testing in bite marks for identification purposes. A number of drugs such as phenobarbital, amphetamine and morphine have been detected in saliva and saliva stains by radioimmunoassay (RIA) by a number of investigators.[14]

SALIVARY ANIMAL BITE MARK ANALYSIS

Fletcher et al. described an enzyme-linked immunoassay technique using monoclonal antibody based on the presence of salivary immunoglobulin A for species identification in stains upto 16 months old. Crossover electrophoresis and double gel diffusion techniques were used for comparison in cases with poor monoclonal antibody results. This technique would appear to have value in bitemark examinations from nonhumans where the biting animal is not known. Evolutionary relationships among species are most directly determined by comparisons at the DNA sequence level.[15]

SEX DETERMINATION FROM SALIVA IN BITEMARKS

The possibility of obtaining exfoliated buccal epithelial cells in saliva on bite marks has increased the possibility of sex determination of the perpetrator. The duration of this line of inquiry is apparently possible for several weeks, post deposition, depending on the materials containing the impressions and environmental factors. Two parameters have been proposed, both based on

successful efforts to identify the sex using blood stains: (1) The presence and detection of sex chromatin (Barr bodies in females and F bodies in males) and (2) sex hormone level determinations based on detectable quantities and ratios of testosterone and 17B-estradiol by RIA. The former parameter has been demonstrated successfully in saliva stains.[16]

Laboratory tests

Laboratory tests for saliva remained presumptive until the late 1980s, when a group of researchers in Japan succeeded in developing a monoclonal antibody that is specific for the alpha-amylase variant that is present in human saliva in particular. Therefore, instead of testing for enzymatic activity, now we can detect the alpha-amylase molecule itself, and specifically, the alpha-amylase from human saliva. This ushered the development of test kits that are now being used in forensic laboratories around the world to screen for human saliva (known to many as Lateral Flow Immunochromatographic Strip Test or Rapid Stain Identification (RSID) Saliva kits). The SBI lab used a combination of the presumptive Phadebas test and the RSID test to "confirm" the presence of human saliva. The SBI lab protocols have been updated recently and reflect important changes in interpretation language. Under current protocols, the SBI lab acknowledges that the RSID test for saliva is a presumptive test.[17]

The RSID-Saliva kits have been tested on samples from various types of surfaces such as paper, cigarette butts, plastic and glass bottles, and metal cans. The specificity of RSID-Saliva kits has been scrutinized by researchers. Although RSID-Saliva kits were found to be sensitive and specific to human saliva, positive reactions were also noted in samples containing alpha-amylases from mammals such as gorillas and rats. Positive reactions were also noted in other bodily fluids such as semen, blood, vaginal discharge and sweat. The RSID-Saliva test gives positive results from breast milk, likely because the presence of alpha-amylase aids the nursing infant in food digestion. High reactivity of this test is also observed in samples containing human feces. Not surprisingly, humans swallow copious amounts of saliva, which travels through the entire digestive system and, as a result, alpha-amylase from saliva (as well as alpha-amylase from the pancreas) is thought to reach the colon where it can mix with fecal material. Reactivity was also noticed in urine samples, a result that remains inconsistent between studies.[18]

Saliva as a fluid for Omics study

Genomic DNA from saliva is found to be highly informative and discriminatory. Progress in salivary genomics is favoured by the availability of sufficient quantity of DNA in saliva, its stability when stored at high temperatures even for extended periods of time and reliable polymerase chain reaction (PCR) / exome sequencing results. Its application in forensic and clinical investigations is augmented by high-throughput technology platforms like genome-wide microarrays. The oral microbiota (commensal and pathogenic) and remnants of food also contribute to DNA extraction from saliva along with desquamated oral mucosal cells (source of human genome). Though it can be overcome by careful collection techniques, this contamination has led to the identification of variations in the oral microbiome under pathological conditions. Latest advancements in kit based saliva collection procedures enable isolation of contamination free high quality DNA which can be used for several genetic analyses such as clinical genetic testing, pharmacogenomic testing, population studies etc. DNA tests can be categorized into five domains: diagnostic, predictive, pre-symptomatic, carrier and prenatal. Salivary DNA based tests are being used in many diagnostic laboratories for mutations and polymorphisms associated with disease susceptibility such as cancer, periodontal disease, Mendelian diseases etc..Transcriptome based studies have identified majority of investigated salivary RNA to be of human genome origin, in spite of the presence of a vast oral microbiome.[19]

Pharmacovigilance potential of saliva

Considerable investigation has been carried out on qualitative and quantitative analysis of drugs (including narrow therapeutical index drugs and illicit drugs for forensic purpose) and detection of commonly abused substances in saliva. A definable relationship between concentration of a therapeutic drug in blood (serum) and its concentration in saliva has been considered as a fundamental prerequisite for diagnostic application of saliva. Presence of a drug in saliva is influenced by its physicochemical characteristics (molecular size, lipid solubility, degree of ionization, degree of protein binding etc), its interaction with salivary glands, extravascular metabolism, effect of salivary pH, mechanism of its transfer into saliva, salivary flow rate and drug stability in saliva. Non-ionizable drugs or those that are not ionized within salivary pH range are considered suitable for salivary drug monitoring. Only the unbound fraction of the drug in serum is available for diffusion into saliva as serum-binding proteins do not cross the membrane owing to their size. The unbound fraction usually being the pharmacologically active one, may present an advantage of drug monitoring in saliva over serum. Majority of studies have observed a significant positive correlation between salivary and serum drug levels. Stimulation of salivary flow and storage duration did not affect this correlation. However, pharmacokinetics of drugs in oral fluid is more complex than that of blood. Drug detection in this specimen will depend on a range of factors including dose, frequency of use (i.e. acute versus chronic use) and detection limits of analytical assays.[20- 24]

Forensic age prediction for saliva samples using methylation-sensitive high resolution melting: exploratory application for cigarette butts

In forensic science, predicting the age of a victim or a suspect can trigger the quick solution of a crime. Nonetheless, forensic scientists have had few options for

estimating the age of the person of interest in actual practice, such as examining bones morphologically1 or analysing the amino acid racemization of teeth. These techniques are not versatile methods, as they limit sample sources. In addition, biological fluids, which are more commonly found at crime scenes, cannot be analysed with these morphological techniques. For this reason, forensic scientists have begun to apply knowledge of genetics to forensic cases, e.g. signal joint T-cell receptor excision circles (sjTREC)3, telomere length4, and somatic gene arrangements. However, these genetic biomarkers exhibit relatively low accuracy or are severely influenced by the degradation of DNA collected from evidentiary materials found in actual crime scenes.[25]
Epigenetics have recently come to play an important role in forensic age prediction. Cytosine methylation at CpG sites has been well investigated as a novel epigenetic marker of chronological age. Hannum et al. built a predictive model for aging blood with 71 methylation markers selected from the IlluminaInfinium HumanMethylation450 BeadChip, resulting in an error of 4.89 years. Huang et al. also developed a predictive model for bloodstains using 5 CpG sites analysed by pyrosequencer with a mean absolute deviation (MAD) of 7.98 years. Although these methods are novel, none are routinely applied for actual criminal investigations currently, likely because of their high cost and time requirements.[26]

CONCLUSION

The importance of saliva as an investigative body fluid is increasing steadily over the years in forensic laboratories. These laboratories are required to have automated settings for saliva as is routinely done for blood or urine. Safety in its handling, the ease and noninvasive methods of saliva collection has gained popularity in the field of forensic testing for drugs of abuse. Sex determination and individualization of accused in scenes of crime with the help of salivary exfoliated cellular examination and DNA profiling is proving to be of immense help in forensic investigations. More research dedicated towards this particular innocuous body fluid should be aimed at for gaining detailed information in forensic sciences.

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