Original Article

Indian Journal of Forensic Medicine and Pathology
Volume 11 Number 3, July - September 2018
DOI: http://dx.doi.org/10.21088/ijfmp.0974.3383.11318.6

Dried Salivary Stain Detection using Ultraviolet- Light Spectrophotometer, Fluorescent and Raman Spectroscopy

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Abstract

Objectives: To detect the salivary stains, in particular, any improvement in the ability to target an area of DNA analysis. To evaluate the efficacy in detection of salivary stains between UV light, fluorescent and Raman spectroscopy. *Study Design:* Prospective randomised study. *Place & Duration of Study:* Department of Oral & Maxillofacial Pathology, Sri Rajiv Gandhi College of Dental Sciences & Hospital; Forensic science laboratory, Bangalore Test Centre & Indian institute of Science, Bangalore, Karnataka; between August 2013 to December 2014. *Patients & Methods:* Dried salivary stain samples from 20 volunteers were collected and exposed to ultraviolet-light spectrophotometer, Fluorescent spectroscopy and Raman spectroscopy. Water was used as a control sample and Tryptophan, to assess the presence of saliva from the collected samples. *Results:* A total number of 20 volunteers dried salivary stain samples were taken. The absorption spectra of the saliva samples revealed the excited wavelength of 240 to 248 nm coinciding with the excited wavelength of tryptophan in case of ultraviolet spectroscopy; the excited wavelength was 280 to 288 nm coinciding with the excited wavelength of tryptophan with less concentration of saliva in case of fluorescent spectroscopy; and in case of Raman spectroscopy, saliva was not detected instead the diluted content of KCL solution was only noted. *Conclusions:* Ultraviolet light spectrophotometer and fluorescent spectroscopy are a rapid and non-invasive technique for the detection of dried salivary stain, in which the sensitivity and accuracy is best with the fluorescent spectroscopy.

Keywords: Spectroscopy; Saliva; Forensic Science; Salivary Amylase; Light Source.

Introduction

Saliva, one of the biological fluids secreted contains several types of amylase isoenzymes that has been identified exhibiting genetic variations and hence can be used for individualization. Saliva being a oral fluid, is a diagnostic medium that can be easily collected and with minimal invasion [1]. It is usually found deposited in bite marks found in many homicides, assault and other criminal cases and is of medico-legal importance providing circumstantial evidence at the crime scene.[2] It is recovered for DNA extraction and typing to evaluate its usefulness for practical case investigation and discuss the contribution of saliva DNA typing in forensic dentistry [2,3]. Dried salivary stains are invisible, making its recognition and collection difficult. However, the presence of saliva can be confirmed by an amylase assay [2]. Tryptophan, the aromatic amino acid is one of the amino acids present in salivary amylase, a constituent of enzyme in saliva which can be used as a confirmatory subject for the presence of saliva [4]. It appears likely that the content of the aromatic amino acid tryptophan in amylase is largely responsible for emission [5]. Thus tryptophan can act as a prevalent probe in detecting dried salivary stains on human skin [1].

Forensic light source being one of the simplest presumptive test have been used in the detection of biological evidences due to their natural

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Received on 28.08.2018, **Accepted on** 17.09.2018

characteristic light absorption and fluorescent effect. When Forensic light source are emitted on saliva; fluorescence is observed due to the absorption of light by saliva, a biological evidence at particular wavelength re-emitting at a longer wavelength [6]. when saliva is subjected to ultra violet rays, it emits bluish white light which cannot be differentiated with other biological fluids, so the ultraviolet- light spectrophotometer is used that detects saliva when exposed to a certain wavelength of light [6]. Various other techniques were done in past to detect saliva like; starch iodine test, Phadebas reagent test, amylase azure, RSID-saliva test, but the results were not accurate [7]. The most emerging nondestructive confirmatory tests are fluorescent and Raman spectroscopy, in which the tests are still being experimented [8]. Raman scattering is a powerful qualitative and quantitative analytical method based on a process where incident monochromatic photons interact with a sample to produce scattered photons with an energy distribution characteristic of molecular structure. Raman spectroscopy is less sensitive in comparison with fluorescence but has higher selectivity and specificity to biochemical species [8]. The peak fluorescence intensity in saliva samples was found to vary among the volunteer population due to the different protein content of saliva.

This study was aimed to know the high sensitivity and selectivity of dried salivary stain detection which is a source of DNA in forensic science using ultraviolet- light spectrophotometer, Fluorescent and Raman spectroscopy.

Methodology

This study includes dried salivary stain samples taken from 20 volunteers between August 2013 to December 2014 conducted in the Dept of Oral & Maxillofacial Pathology, Sri Rajiv Gandhi College of Dental Sciences & Hospital; Forensic science laboratory; Bangalore Test Centre & Indian institute of Science; Bangalore, Karnataka. This study was approved by the Ethical committee of Sri Rajiv Gandhi College of Dental Sciences & Hospital, Bangalore, Karnataka.

The criteria for the sample selections were volunteers free of any oral diseases. The equipment used were plastic and quartz cuvettes, micropipettes, glass slides, Ultraviolet - Visible light Spectrophotometer, fluorescent spectroscopy, Raman spectroscopy. The volunteers were instructed to clean their forearm with soap and water. The area where saliva has to be deposited was marked which was on the ventral side of the forearm and water as a control sample to be deposited on the contra lateral arm. Both water and saliva were allowed to dry for 45 minutes to 1 hour. The fibre free Cotton was dipped in buffer solution of pH 7.4; the excess solution was squeezed out and samples were collected by rubbing over the marked area. Then the collected samples were transferred to the cuvettes.

They were subjected to ultraviolet emission, fluorescence emission and Raman emission spectrum by adding 0.1M KCL solution making up to 2 ml solution. In case of ultraviolet spectroscopy and fluorescent spectroscopy, emission spectrum was recorded from 200 to 400 nm. In case of Raman spectroscopy, a drop of saliva sample is dropped on the glass slide and emission spectrum was recorded from 500 to 1750 Raman shift/cm^{-1}.

The emission spectrum of tryptophan was recorded by dissolving 0.5mg/ml of tryptophan in 0.1M KCL solution. This solution was excited at a wavelength of 244 nm and 290 nm for UV-light and fluorescence spectroscopy respectively.

Results

The absorption spectra of the saliva samples varied in each type of spectroscopy. The absorption spectra of the saliva samples were recorded on excitation at a wavelength of 240 nm to 300 nm and the excitation peaks were recorded. The

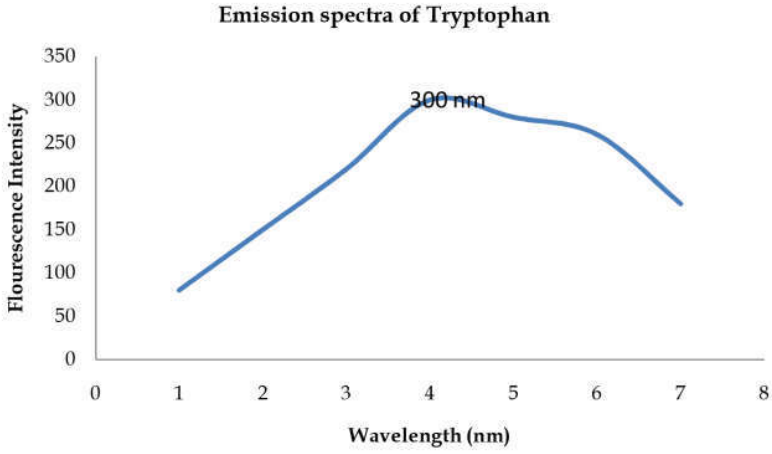

Fig. 1: Emission spectra of Tryptophan (control)

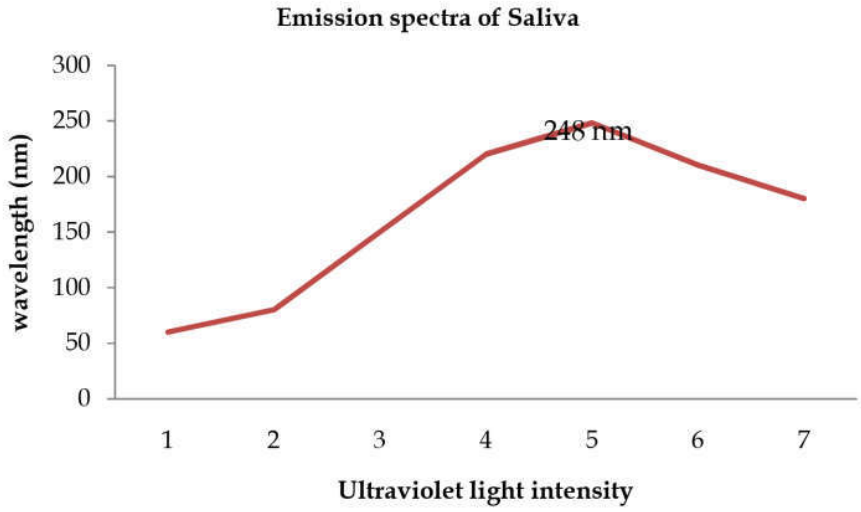

Fig. 2: Emission spectra of Saliva – Ultraviolet Light Spectrophotometer

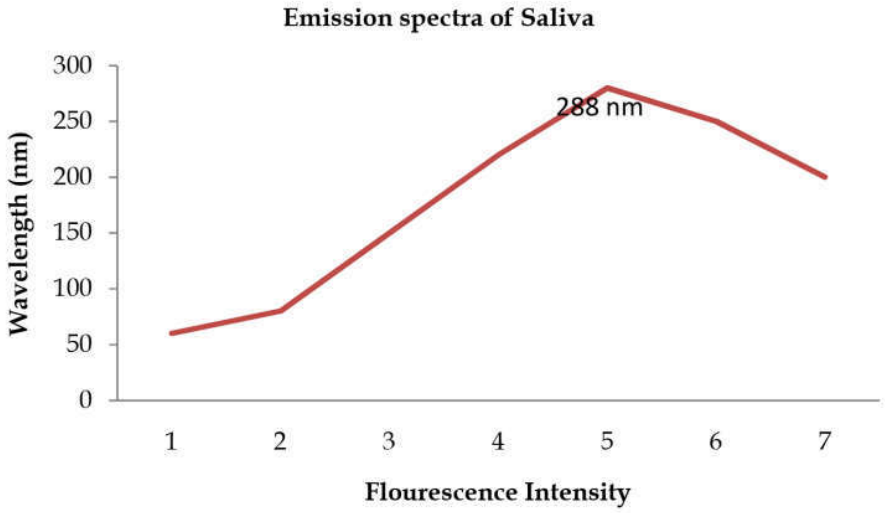

Fig. 3: Emission spectra of Saliva - Fluorescent spectroscopy

control, tryptophan's excited wavelength is 240 nm to 300 nm [Figure 1].

In case of Ultraviolet light spectroscopy, the excited wavelength was 240 nm to 248 nm [Figure 2] that coincided with the excited wavelength of tryptophan. But more the dilution rate of saliva, the rate of excitation peak increased. The excited wavelength was 280 to 288 nm [Figure 3] coinciding with the excited wavelength of tryptophan with less concentration of saliva in case of Fluorescent spectroscopy. In case of Raman spectroscopy, saliva was not detected instead the diluted content of KCL solution was only noted. The data were illustrated in Figure 1, Figure 2, and Figure 3.

Discussion

Both Ultraviolet light spectrophotometer and fluorescent spectroscopy methods were able to detect the diluted saliva samples; but as the dilution rate of saliva increased, the rate of excitation peak increased in Ultraviolet Spectrophotometer indicating its low specificity compared to

fluorescent spectroscopy. Fluorescent spectroscopy showed the peak excitation of 288 nm coinciding with the excited wavelength of tryptophan with less concentration of saliva. Thus Ultraviolet light spectrophotometer and fluorescent spectroscopy are a rapid and non-invasive technique for the detection of dried salivary stain, in which the sensitivity and accuracy is best with the fluorescent spectroscopy. In case of Raman spectroscopy, various studies have proved to identify the traces of saliva using confocal Raman microscope and also have the potential to detect the saliva in a mixed multiple body fluids. Raman spectroscopy is used to characterize the entire composition of the fluid instead of probing a specific chemical group or compound. But in the present study a particular chemical compound Tryptophan was used, thus was not able to detect the sample. Each body fluid has a complex biochemical composition which is heterogeneous in nature and no single characteristic spectrum can satisfactorily represent the experiment. Even though Raman spectroscopy has higher selectivity and specificity, it is less sensitive to detect through the methodology used in this study.

Conclusion

Results from our study showed that dried salivary stain can be detected through both Ultraviolet light spectrophotometer and Fluorescent spectroscopy. But the fluorescent spectroscopy showed more sensitivity and specificity than the Ultraviolet light spectrophotometer comparatively. In case of Raman spectroscopy method, it failed to detect the salivary constituent tryptophan. Even though Raman spectroscopy has higher selectivity and specificity, it is less sensitive to detect through the methodology used in this study. So different methods can be used to detect saliva and further studies have to be experimented with more sample size.

However our study was effective in detecting the saliva through a non-invasive method accurately with Fluorescent spectroscopy that acts as a diagnostic aid in detection of DNA in the field of forensic science.

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