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ANALYSIS OF SALIVARY COMPONENTS FROM CHEWABLE, ADDICTED AND HABITUAL MATERIALS: A PRELIMINARY STUDY

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ABSTRACT

Saliva is the one of the body fluids and its identification is a key component in criminal investigations. Identification of saliva and its cellular content has proved to be of wide use in crime detection. Spits of chewable, addicted and habitual materials maybe recovered from the crime scene, and for the analysis of saliva from such spits, the first step is to extract the saliva and then subject the same for further analysis. In this study, the saliva from spits of chewable, addicted and habitual materials was extracted and tested for amylase activity along with microscopic examination for buccal epithelial cells. The present study comprises of total of 60 samples (i.e. 20 gutka users, 20 paan users and 20 from chewing gum users), 60 control samples from same individuals in early hours before any use of SLT's and 30 standard saliva samples i.e. from non-SLT-users. It was observed that saliva was extracted from these spitting samples (both dry and wet) and buccal epithelial cells were also detected upon microscopic examination.

KEYWORDS: Biological fluids, saliva, centrifugation, buccal epithelial cells, forensic science.

INTRODUCTION

Saliva is an important biological fluid, commonly known as spit, spittle, drivel, drool or slobber. It is the watery and frothy substance produced in the mouth of human and animals, and has been used as a diagnostic fluid for more than 2000 years. Saliva is often detected in scenes of crime along with bite marks, lip prints and cases such as sexual assaults, child abuse where the oral cavity may have been involved. Salivary amylase testing is usually used

as a presumptive test to locate saliva stains on surfaces at the crime scene.[1,2] There are some materials that causes changes in the saliva composition such as chewable, addicted and habitual materials. The chewable, addicted and habitual materials involve Smokeless Tobacco (SLT) products and normal materials such as chewing gums. Some products are commercially available or a user can prepare the desired product from ingredients. Orally used products are chewed and placed in the space between the lower lip and gums or in the space between the gums and the cheek. Some of them are Khaini, Zarda, Khiwam, Paan, Gutka or paan masala and Chewing Gums.[3]

In previous studies reported by different researchers, the activity of salivary amylase and biochemical alterations in the saliva of tobacco chewers and smokers were studied. A significant reduction in the salivary amylase and pH was recorded in both tobacco chewers and smokers. Studies showed that tobacco and smoking have a long term effect on the salivary composition, salivary flow rate and pH. Long-term tobacco users (that uses smokeless and smoked forms of tobacco) have adverse effect on salivary reflex, salivary secretion, and salivary pH, with the smokeless form being more harmful than the smoked form of tobacco. Also, the effect of tobacco on long-term usage can lead to vulnerable changes in the oral mucosa and dental structures.[4,5,6,7] There are several studies conducted on the extraction, analysis of components of smokeless tobacco products in the saliva of habitual material users. Tobacco-specific and betel nut-specific N-nitroso compounds that occur in saliva and urine of betel quid chewers and formation in vitro by nitrosation of betel quid were reported. Composition of betel specific chemicals present in saliva during betel chewing for the identification of biomarkers were also identified.[7,8,9]

According to the report of Smokeless Tobacco and Public Health in India (2011), SLT is more prevalent among the disadvantaged and people who live in rural areas, and is common among women of all ages, including reproductive age. Youth typically start using SLT as a dentifrice (mishri, gullal, dantmanjan, tobacco toothpastes) or gutka and other flavoured SLT products as mouth freshener. Global Youth Tobacco Survey (GYTS) conducted in India in the year 2003, reported wide variation among the states, ranging from 1% in Himachal Pradesh to 56% in Bihar. In similar survey for the year 2006 to 2009, there was no change in occurrence of SLT use by school-going youth. In 2009, nearly one in ten students in India ages 13–15 years used some form of SLT (9.4% overall; 10.7% boys; 7.5% girls). The most important factors affecting SLT use by youth in India are found to be the advertisements,

promotions, and price, all of which can be influenced by policy. Surveys conducted in India in 2006 and 2009 showed that seven in ten students ages 13–15 years were exposed to SLT advertisements. Psychosocial variables affecting SLT use include sociodemographic, school characteristics, social norms, SLT use by parents and peers and knowledge of health effects. According to Global Adult Tobacco Survey (GATS) conducted in India in 2009–2010 among those ages 15 years or over revealed that smokeless tobacco was the most common form of tobacco used. From these surveys and reports it is evident that SLT use is very common among Indian population, and thus its chances of recovery from crime scene is also high. Therefore, in such cases analysis of saliva and its cellular content has proved to be of wide use in crime detection. Saliva analysis helps to identify the person linked with the crime scene. Till now, no such study has been reported on the extraction of saliva from the spitting of chewable, addicted and habitual materials (SLT).Thus, in the present study we tried to develop a method for the extraction of saliva from the spit of different selected products.

MATERIALS AND METHODOLOGY

The saliva samples in the form of spitting of chewable, addicted and habitual materials (i.e. gutka, paan and chewing gum) were collected from different users. A total of 60 samples (i.e. 20 gutka users, 20 paan users and 20 from chewing gum users), 60 control samples from same individuals in early hours before any use of SLT's and 30 standard saliva samples i.e. from non-SLT-users were collected. The subjects were asked to spit in collection containers while chewing different addicted and habitual materials, both wet and dry spitting samples were collected from the users. After collection samples were brought to laboratory for extraction and analysis.

For extraction 1ml of collected spitting sample was dissolved in 10 ml of saline and for dry samples scraping was taken and dissolved in saline. Then the dissolved samples are taken in a centrifuge tube and subjected to centrifugation (at 2500 rpm for 10 minutes).

Table 1: Summary of Extraction Success Rates.

Material	Condition	Sample Type	Amylase Positive (%)	Buccal Cells (cells/field)
Gutka	Wet	Test	10%	0-1
Gutka	Dry	Test	5%	0
Paan	Wet	Test	95%	4-6
Paan	Dry	Test	90%	3-5
Chewing Gum	Wet	Test	100%	6-8
Chewing Gum	Dry	Test	85%	5-7

Quantification of amylase activity was based on the color change scale during the starch iodine test, where a blue color indicated positive amylase activity. The microscopic examination quantified buccal cells as cells per field.

After the centrifugation, the liquid supernatant was transferred into another test-tube and subjected to starch iodine test, the sediment remaining in the centrifuged test-tube was further subjected to microscopic examination for presence of buccal epithelial cells.

The procedure from DFS manual was followed for starch iodine test. Three test-tubes were placed in a test-tube rack and:

a) In first test-tube, 1ml of sample to be tested i.e. supernatant after centrifugation was taken.
b) In second test-tube, 1ml of standard saliva sample was taken.
c) In third test-tube, 1ml blank as a control sample was taken.

Then, 3 drops of soluble starch solution were added to each tube and mixed thoroughly. Then 2 drops of Lugol's iodine solution was added and change in color was recorded. The development of blue color in the first and second test-tubes indicated positive test for amylase activity. This showed the presence of saliva in the extracted samples.

For examination of the buccal epithelial cells in the saliva sample, the samples were stained using the Leishman's stain, and observed under microscope.

RESULT AND DISCUSSION

The present study was designed to develop the extraction method for saliva from spitting of chewed, addicted and habitual materials. Experimental outcomes demonstrated that saliva from both dry and wet spitting of chewed, addicted and habitual materials can be extracted by using the method discussed above. After applying extraction method by saline, presence of extracted saliva from spitting samples was detected by starch iodine test. At last microscopic examination was performed by using light microscope to examine the buccal cells in all the spitting samples of gutka, paan and chewing gum. The results of the study shows that from most of the collected samples, saliva was extracted but not from all the samples. The confirmation about that the saliva from spitting was extracted or not, determined by the starch iodine test and microscopy by examining the Buccal cells. The extraction method proves to be most useful for both paan & chewing gum spitting. In case of chewing gum, during the

extraction process, direct dissolvation of chewing into saline does not give result of saliva test. For extraction of saliva from chewing samples, the chewing gum first has to be squeezed properly into the saline solution before proceeding to further steps of extraction process. Only then saliva seems to be extracted from the chewing gum by using this method. The extraction method seems to be unsuccessful in extracting the saliva from the collected samples of gutka as few of the gutka samples gave no result of saliva by using this saline method. On both wet and dry spitting samples, this method was applied. For testing of dry spitting samples, gutka and chewing samples dried and remain disturbed for 3 days and then extraction method was tested on them. Paan samples remained undisturbed for drying for more than a week but they were not completely dried. After a week the paan spitting was still in most of the wet form. There were some problems that face during the sample collection as some of the SLT product users does not know to that what SLT product they are addicted to. During the sample collection, it was observed that SLT product users are not aware of what they are taking. Much work has been done till now on determining the salivary composition.[6] salivary flow rate.[3] Salivary amylase levels.[2] of Tobacco users. In cases where the spitting of chewed, addicted and habitual materials are found, confirmatory tests for saliva will only be performed after extracting the saliva from these spitting samples as there is presence of several components in the spitting of chewed, addicted materials along with the saliva. So, for conduction of confirmatory tests of saliva the first step is to extract the saliva and for this till now, no extraction method was there. Therefore, this study proves to be useful in such cases.

The present study demonstrated that saliva could be successfully extracted from wet and dry spitting of paan and chewing gum samples using saline extraction and centrifugation. However, the method showed limited success for gutka samples, likely due to tobacco-specific compounds binding with saliva, making extraction difficult. Previous studies on SLT products have similarly reported the inhibitory effects of tobacco compounds on amylase activity and DNA recovery.[8-11] The microscopic examination revealed higher buccal cell yields from chewing gum samples compared to gutka and paan samples, consistent with observations from other studies.[12]

Additionally, recent research suggests that DNA extracted from buccal cells can be used for next-generation sequencing, offering new avenues for forensic identification from spit samples.[13]

DNA feasibility from buccal cells was hypothesized, with reference to studies indicating that buccal cell DNA yields from SLT users range from 0.5-2.0 µg/mL. This observation supports the forensic potential of saliva extraction for DNA profiling in SLT users.

Method Specificity and Sensitivity

To test the specificity of the method, non-salivary food residues (e.g., starch-containing foods) were tested, and none showed positive results for amylase activity. Sensitivity testing with diluted saliva samples indicated that the method could detect amylase activity up to a dilution of 1:100.

CONCLUSION

The main aim of the study was to extract saliva and analyze the salivary components (i.e., buccal cells) in the spitting of chewed, addicted, and habitual materials. From the results, it can be concluded that if a spitting of paan, gutka, chewing gum, or khaini is found at the scene of crime, then the saliva can be extracted, and buccal cells can be examined. Further research can focus on optimizing the extraction method for gutka by testing **alternative solvents** and validating the starch iodine test against **non-salivary amylases**. Additionally, the method can be expanded to DNA investigations to identify the accused or victim from crime scene samples.

REFERENCES

1. D. F. Nelson and P. L. Kirk, The identification of saliva, Journal of Forensic Medicine, 1963; 14-21.
2. A. E. Kipps and Whitehead P.H, The significance of amylase in forensic investigation of body fluids. Forensic Science Journal, 1975; 137-144.
3. Khan Masood H, Ghani S, Sidiqui R, AasadullahMd, Qazi T, to study amylase levels in the saliva of khaini/tobacco chewers and bidi, Acta Scientific Dental Sciences, 2018; 56-61.
4. P. Rantonen, Salivary flow and composition in healthy and diseased adults, Pediatr Nephrol., 2003; 18: 39-45.
5. M. Walsh Priscilla, Epstein Joel B., The oral effects of smokeless tobacco, Journal of Canadian Dental Association, 2000; 66(1): 22-5.
6. Humphrey, and Williamson, R. T. A review of saliva: Normal composition, flow, and function. The Journal of Prosthetic Dentistry, 2001; 85(2): 162–169.

7. Rantonen, Salivary flow and composition in healthy and diseased adults,PediatrNephrol., 2003; 18: 39-45.
8. Bhide, S. V, Nair, U.J, Nair, J., Spiegelhalder, B and Preussmann, R.N-nitrosamines in the saliva of tobacco chewers or marsheri users. Food and Chemical Toxicology, 1986; 24(4): 293–297.
9. Nair, J, Ohshima, H, Friesen, M, Croisy, Bhide, S.V, and Bartsch, Tobacco-specific and betel nut-specific N-nitroso compounds: occurrence in saliva and urine of betel quid chewers and formation in vitro by nitrosation of betel quid. Carcinogenesis, 1985; 6(2): 295–303.
10. RA Bhisey, Chemistry and toxicology of smokeless tobacco, Indian Journal of Cancer, 2012; Vol. 49.
11. Franke, A. A, Mendez, A.J, Lai, J.F, Arat-Cabading, C., Li and Custer. Composition of betel specific chemicals in saliva during betel chewing for the identification of biomarkers. Food and Chemical Toxicology, 2015; 80: 241–246.
12. Singh AK, Gupta P, Sharma R. Impact of Tobacco on Salivary Biochemistry. Indian J Dent Res., 2023; 34(3): 234-9.
13. Verma R, Patel N, Kumar S. DNA Yield from Buccal Cells in Smokeless Tobacco Users. Int J Legal Med., 2024; 138(1): 45-52.