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Original Research Article

"Unmasking Salivary Stains – A Tool in Forensic Research Using an *In- vitro* Comparative Method"

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Abstract: ***Background:*** Saliva is an important body fluid having importance in forensic tracing, is identified by its high amylase activity compared to other biological materials using salivary staining method. Whether this activity changes with oral habits, time and temperature, material on which the stain is absorbed or collected and in other biologic materials has to be known. ***Methodology:*** Study samples comprised of fresh saliva samples of 20 subjects each, with and without habits and one sample each of blood and vaginal secretions, collected on both filter paper and cotton cloth. Amylase activity of the pre-weighed blood and vaginal secretions, salivary samples at differing temperature and time and salivary samples diluted with blood & vaginal secretions at differing concentrations were recorded. ***Results:*** Saliva samples showed high specific activity of amylase in cloth and in habituated subjects, but this activity decreased with time and temperature. Whereas blood and vaginal samples showed very less specific activity compared to saliva samples and it decreased with increasing dilutions with saliva. ***Conclusion:*** When studied in a larger scale, the study observations may help in narrowing down the findings at a crime scene which could serve as a guide line for further research in forensics.

Keywords: Unmasking Salivary Stains, *In- vitro* Comparative Method, Saliva.

INTRODUCTION

Saliva is an easily obtainable biological body fluid collected by means of various noninvasive methods. It constituents usually aids as a tool for screening, presumption, and confirmation of evidence during ante-mortem as well as postmortem analysis [1]. From many decades, different methods have been described for identifying saliva stains, constituting detection of human α-amylase (1,4 α-d-glucan glucanohydrolase) particularly [2]. As these alpha amylase (α-amylase) enzymes are the main constituent proteins produced and stored in salivary glands and pancreas, as well it is also present in other body fluids like breast milk, pancreatic juices, seminal fluid and vaginal secretions [3-6]. Presence of salivary amylase in all these biological fluids tends to have an important role in the forensic investigations where a screening method based on amylase activity testing was found to be useful [7, 8].

Tobacco seems to be the single most common preventable causes of death globally. In the 20th century, more than 100 million people died worldwide due to the use of tobacco and tobacco related products like tobacco snuff, bides, cigarettes, chewing tobacco (19%), hookah, cigars, etc [9]. Knowing the risks associated with tobacco use and its possible effects on the chief organs in the human body, it is found indispensable to measure the deleterious carcinogenic effects of tobacco on the organs like pancreas, seems to be utmost importance, as pancreas were responsible for the production of many enzymes used in the digestive process including serum alpha amylase, a major body fluid constituent [10].

Owing to the facts that saliva as an important substance in aiding in most of the body functions, it is also important to know that serological testing and analysis of salivary cellular components are nowadays increasing useful in forensics sciences in crime detection, abuse, and genetic studies [11, 12]. So it was

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found absolutely necessary to analyze and measure the role of saliva and its chef constituent specific salivary alpha amylase activity by using an advanced research tool that is salivary staining. With this background, the present study aims to investigate and compare the specific activity of amylase the three biological fluids like saliva, blood and vaginal stains and also the mixture of stains which were adsorbed onto filter paper and cloth at different time intervals among the tobacco users and non-users.

MATERIAL AND METHODS

A total of 20 participants aged 18-35 years based on their habit of tobacco consumption were selected for the study after without considering their gender or socio-economic status. Categorized into tobacco users and non-tobacco users, those study participants who volunteered and were present on the day of study visiting the outpatient department of oral pathology of the institution. Subjects with long-term systemic diseases were excluded from the study.

Written informed consent from all the participants was obtained and the study was approved by the ethics committee of the institution. All the measurements were carried out by a single examiner who had been calibrated.

Outcomes assessed:

Amylase activity of the pre-weighed blood and vaginal secretions, salivary samples at differing temperature and time and salivary samples diluted with blood & vaginal secretions at differing concentrations were recorded.

A total of three samples were collected from the subjects containing secretions from saliva, blood and vagina. Saliva samples were obtained one hour post meal in sterile plastic containers from all the 20 subjects, followed by blood and vaginal secretions, which will be tested for amylase activity on both filter paper and cotton cloth measuring 2X2 cm. The collected samples were immediately sterilised using centrifugation at 5500 rpm for 5 min at 37°C to remove cell debris and were stored frozen until the time of assay.

For mixture of stains, 2 samples from each of the saliva, blood and vaginal secretion were collected and adsorbed on filter paper and cloth of 2x2cms total 12 samples. Dilutions are made from extracts of stains by using 0.1mg/ml in distilled water. All these stains were allowed to dry at room temperature for 4-5 hours and stored at -60°C until use. For short time experiments, salivary stains were usually kept at room temperature for approximately 24 hours. Whereas experiments that cover more longer periods, require keeping the samples at extended temperatures, varying from -20°C to -80°C for about 28 days.

The Human Alpha-amylase (AMY1) ELISA Kit (Salimetrics LLC, State College, PA) was used for the assessment of salivary α-amylase. The quantitative alpha-amylase activity was assessed by a colorimeter assay (595 nm) where the readings in the calorimeter suggest the intensity of color produced as proportional to the activity of the enzyme in the saliva samples. Total protein content in the samples was determined using Lowry method [14].

Specific activity of the study samples was determined using the following formulas:

Specific activity = enzyme units / (vol. in µl x (protein conc. in mg per ml / 1000))

Statistical analyses

To compare three or more mean values one-way ANOVA was applied and to compare proportions, Chi-square test was applied. SPSS version 22.0 (IBM, United States) was used to analyze the data. Significance level was fixed at 5% ($\alpha = 0.05$).

RESULTS

Present study aimed at measuring the specific amylase activities of three different samples including fresh saliva samples and their stains kept at room temperature for 5-48 hours with a assumption that amylase was detected from many stains in addition to saliva stains in the material media.

Table 1 describes the comparison of amylase specific activity in all the three biological fluids in which the salivary amylase activity was higher in the salivary fluids i.e.,14.3 – 150 (70.825 ± 60.33 I.U./mg) when compared to vaginal secretion (0.517±0.286 I.U./mg) and blood (0.202±0.125 I.U./mg).

Amylase specific activity of saliva on filter paper and on cotton cloth at different concentrations of vaginal secretions was shown in Table 2 and Table 3. Respectively, where it can be observed that with an increase in concentration there is a decrease in specific activity of amylase in the salivary fluid (Fig 1 & 2). The same phenomenon observed with respect to different concentrations of blood (Table 4 and Table 5) where with an increase in concentrations there is decrease in amylase specific activity of saliva (Fig 3 & 4).

When comparisons were made about samples collected from tobacco users and non–users, it was found that there is no significant association in relation to filter paper and cloth at all the time intervals i.e., at 5h, 24h and 48h intervals (Fig 5, Table 6 and Table 7).

Table 1: Comparison of amylase specific activity in three biological fluids

Samples	Range	Mean±SD*
Saliva	14.3 – 150	70.825±60.33
Vaginal secretion	0.31	0.517±0.286
Blood	0.13-0.39	0.202±0.125

***SD = Standard Deviation**

Table 2: Amylase specific activity of saliva on filter paper in different concentrations of vaginal secretions

Vaginal secretions (VS)	Pure	25	50	75
First sample of VS	0.49	30	22.5	6.5
Second sample of VS	0.34	15	5.2	2.4

Table 3: Amylase specific activity of saliva on cotton cloth in different concentrations of vaginal secretions

Vaginal Secretions (VS)	Pure	25	50	75
First sample of VS	0.93	12	8.5	6.5
Second sample of VS	0.31	15	4.1	2.3

Table 4: Amylase specific activity of saliva on filter paper in different concentrations of blood

Blood	Pure	25	50	75
First sample of blood	0.15	4.5	3.3	1.9
Second sample of blood	0.13	10.2	3.8	2

Table 5: Amylase specific activity of saliva on cotton cloth in different concentrations of blood

Blood	Pure	25	50	75
First sample of blood	0.39	13.1	7.8	3.4
Second sample of blood	0.14	9.3	7.9	6.2

Table 6: Salivary amylase specific activity comparison between habits and no habits on filter paper

On filter paper			
Time	Mean values		P - value
	Habits	No habits	
5h	230.21	224.12	0.96*
24h	154.08	153.32	0.99*
48h	108.04	105.51	0.96*

*** Not statistically significant at p ≤ 0.05 level.**

Table 7: Salivary amylase specific activity comparison between habits and no habits on cotton cloth

Cloth			
Time	Mean values		P - value
	Habits	No habits	
5h	216.99	172.5	0.59*
24h	158.55	123.99	0.58*
48h	113	91.7	0.63*

***Not statistically significant at p ≤ 0.05 level.**

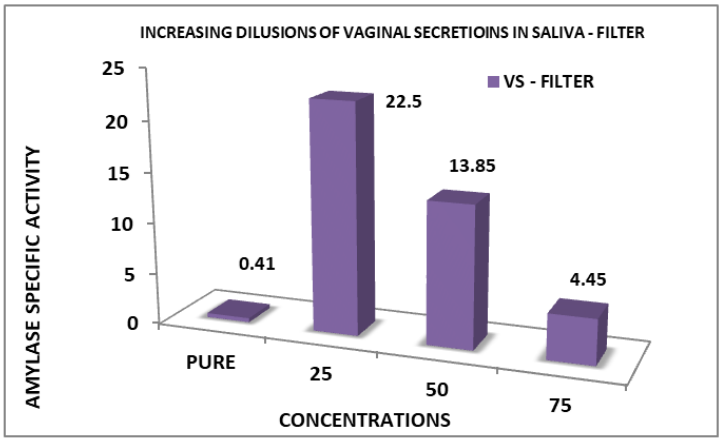

Figure 1: Increasing dilutions of vaginal secretions in saliva on filter paper

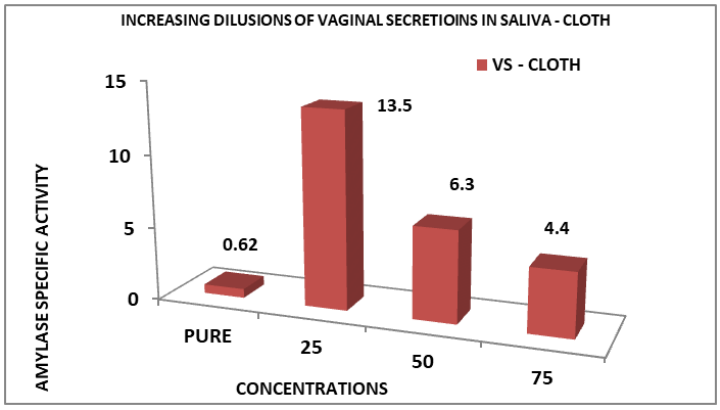

Figure 2: Increasing dilutions of vaginal secretions in saliva on cotton cloth

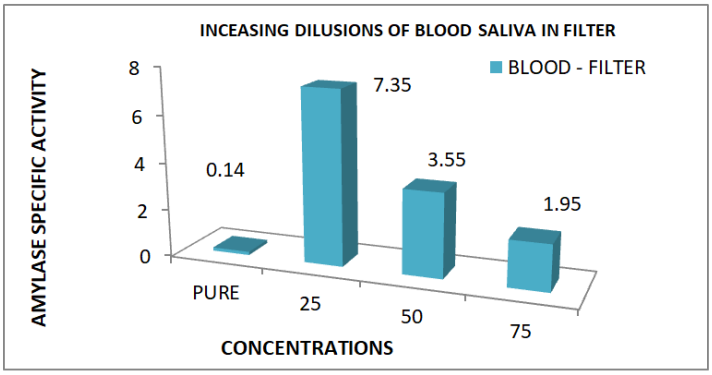

Figure 3: Increasing dilutions of blood in saliva on filter paper

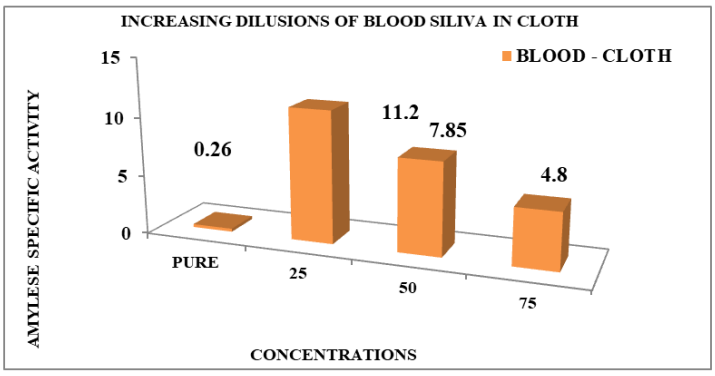

Figure 4: Increasing dilutions of blood in saliva on cotton cloth

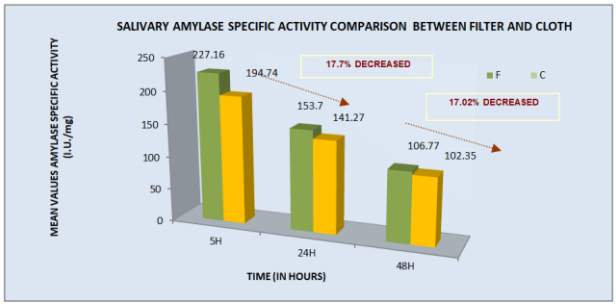

Figure 5: Salivary amylase specific activity comparison between filter paper and cotton cloth

DISCUSSION

Salivary attains are one of the important forensic trace evidence. So these salivary stains should be differentiated from other biological fluid stains. Identification of salivary stains forms the basis for further investigations like DNA analysis. From the present study it is observed that after drying of the study samples stored at -20°C for 5hours at room temperature, showed a decrease in concentrations of salivary amylase activity to 17% around 5-24 hours and remained the same 17% from 24-48 hours which is similar to a study conducted by H T Sutsumi *et al*., (1991) [15] where it was observed that the saliva stain amylase activity gradually decreased up to 24hrs & remained practically constant until at least 28 days when stored at -20°. The same study has found that on increasing the contaminants like blood and vaginal secretions in saliva there is a decrease in the salivary amylase specific activity, which was also similar to the present study findings.

A study conducted by T Nagaya and M Okuno (1993) [16], with an aim to investigate the biological effects of smoking tobacco and drinking alcohol on the digestive function of human found that these habits does not show any significant influence on the salivary protein or amylase activity. The same finding was observed in the present study where there is no significant influence of habits on salivary amylase specific activity. Contrary to that the, a study conducted by Hasan *et al*., (2017) [17], showed that saliva α-amylase increased significantly in smokers when compared with tobacco non-consumers. In the present study we didn't restrict only to the smokers. we considered any form of tobacco consumption and the difference in results can be depend on diurnal variation of salivary amylase activity. According to Urs M. Nater (2007) [18] salivary amylase activity on momentary stress, mood, food, or body activity.

In the present study, it was observed that with an increase in duration of time, the mean specific amylase activity of saliva shown a gradual decline when observed for around 48hrs post staining on filter paper and cotton cloth. This finding is similar to a fact that levels of amylase in saliva vary with time as observed in the studies conducted by C Dawes (1972) [19] and A E Kipps and P H Whitehead (1975) [20]. Stains on filter paper showed high amylase specific activity when compared to cloth but it is of no statistical significance as observed in the current study, similar to the observations from an experimental study conducted by Wornes DJ *et al*., (2018) [21]. Current study has many limitations when it comes to generalizability of the study findings and the number of samples observed or the quality of the testing done and the absence of testing on live objects, but it has few advantages over all these limitations when it come to the effectiveness, less technique sensitive, easy sample procurement and less cost or expenditure for the process.

CONCLUSION

Results of the present study showed that saliva samples have higher specific activity of amylase in cloth and in habituated subjects, but this activity decreased with time and temperature. The blood and vaginal samples showed very less specific activity compared to saliva samples and it is decreased with increasing dilutions with saliva. Filter paper and cloth has shown almost same adsorbing capacity. Amylase specific activity in saliva stain is not affected by tobacco usage habits. However, these interesting observations when studied in a larger scale, could help in narrowing down the findings at the crime scene and also help in establishing basic guide lines for further research in forensic sciences.

Conflicts of interest: None
Source of funding: None

REFERENCES

1. Chatterjee, J., Tahmasebi, T., Mertens, S., Kar, G. S., Min, T., & De Boeck, J. (2014). Seed layer effect on the magnetic properties of ultrathin Co/Pt multilayers with perpendicular magnetic anisotropy. *IEEE Transactions on Magnetics*, *50*(11), 1-4.
2. Hochmeister, M. N., Schlatter, P., Rudin, O., & Dirnhofer, R. (1997). High levels of alpha-amylase in seminal fluid may represent a simple artifact in the collection process. *Journal of forensic sciences*, *42*(3), 535-536.
3. Auvdel, M. J. (1986). Amylase levels in semen and saliva stains. *Journal of forensic sciences*, *31*(2), 426-431.

4. Virkler, K., & Lednev, I. K. (2009). Analysis of body fluids for forensic purposes: from laboratory testing to non-destructive rapid confirmatory identification at a crime scene. *Forensic science international*, *188*(1-3), 1-17.
5. Da Vela, G., Bertino, M. G., Fusini, F., & Ricci, U. (2006, April). Evaluation of an automated system for amylase detection in forensic samples. In *International Congress Series* (Vol. 1288, pp. 636-638). Elsevier.
6. Bauer, M. (2007). RNA in forensic science. *Forensic Science International: Genetics*, *1*(1), 69-74.
7. Hedman, J., Dalin, E., Rasmusson, B., & Ansell, R. (2011). Evaluation of amylase testing as a tool for saliva screening of crime scene trace swabs. *Forensic Science International: Genetics*, *5*(3), 194-198.
8. Carboni, I., Rapi, S., & Ricci, U. (2014). Stability of human α-salivary amylase in aged forensic samples. *Legal Medicine*, *16*(4), 214-217.
9. WHO Report on the Global Tobacco Epidemic. Tobacco Free Initiative (TFI), WHO, Switzerland, Geneva; 2017.
10. Bishop, M. L. Schoeff, L. E., & Fody, E. P. (2013). Clinical Chemistry Techniques, principle and Correlation. Lippincott Williams & Wilkins, Pennsylvania, USA, 784 p.
11. John, S. J., Rajaji, D., Jaleel, D., Mohan, A., Kadar, N., & Venugopal, V. (2018). Application of Saliva in Forensics. *Oral & Maxillofacial Pathology Journal*, *9*(2).
12. Saxena, S., & Kumar, S. (2015). Saliva in forensic odontology: A comprehensive update. *Journal of oral and maxillofacial pathology: JOMFP*, *19*(2), 263-265.
13. Salivary alpha-amylase enzymatic kit. https://salimetrics.com/assay-kit/salivary-alpha-amylase-enzymatic-assay/
14. Classics Lowry, O., Rosebrough, N., Farr, A., & Randall, R. (1951). Protein measurement with the Folin phenol reagent. *J biol Chem*, *193*(1), 265-275.
15. Tsutsumi, H., Higashide, K., Mizuno, Y., Tamaki, K., & Katsumata, Y. (1991). Identification of saliva stains by determination of the specific activity of amylase. *Forensic science international*, *50*(1), 37-42.
16. Teruo, N., & Masanori, O. (1993). No effects of smoking or drinking habits on salivary amylase. *Toxicology letters*, *66*(3), 257-261.
17. Hasan, H. R., & Jabir, R. J. (2017). Salivary and serum α-amylase activity and their correlations with oxidative stress index among different iraqi smokers groups. *MOJ Addict Med Ther*, *4*(1), 224-230.
18. Nater, U. M., Rohleder, N., Schlotz, W., Ehlert, U., & Kirschbaum, C. (2007). Determinants of the diurnal course of salivary alpha-amylase. *Psychoneuroendocrinology*, *32*(4), 392-401.
19. Dawes, C. (1972). Circadian rhythms in human salivary flow rate and composition. *The Journal of physiology*, *220*(3), 529-545.
20. Kipps, A. E., & Whitehead, P. H. (1975). The significance of amylase in forensic investigations of body fluids. *Forensic Science*, *6*(3), 137-144.
21. Wornes, D. J., Speers, S. J., & Murakami, J. A. (2018). The evaluation and validation of Phadebas® paper as a presumptive screening tool for saliva on forensic exhibits. *Forensic science international*, *288*, 81-88.

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