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Original Article

Effect of Post-coital intervals on DNA STR profiling of semen donor in the post-coital vaginal swabs

Manzoor Hussain[a,b]

[a]*Department of Microbiology and Molecular Genetics, University of the Punjab, Lahore*
[b]*Centre for Applied Molecular Biology, University of the Punjab, Lahore*

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Abstract
Vaginal swabs are considered as most probative evidence material in sexual assault cases to get the DNA profile of the perpetrator. With the increase in post-coital interval, the possibility to get the autosomal DNA STR profile of the perpetrator diminishes rapidly from an alive victim. This becomes more serious problem especially when victims of rape cases provide vaginal swabs after several days of the incidence. The study was designed to find the time interval at which autosomal STR system fails to generate the DNA profile the semen donor in the post-coital vaginal swabs. Sixty two post coital vaginal swabs (x3) were collected from six volunteers at different TSI (time since intercourse). Out of these, 42 post coital vaginal swabs (up to TSI 5 days) were used to generate the autosomal DNA profile of the semen donor and twenty of the post coital vaginal swabs obtained at the TSI of 6, 7, 8, 9 and 10 days were not tested for autosomal STR profile and were saved for subsequent analysis of Y-STRs. It was found that autosomal DNA profile of semen donor up to TSI 48 hours is feasible; however, chances of getting complete male DNA, partial male DNA profile and no male profile at TSI 72 hours are 17%, 67% and 16% respectively. Chances of getting complete male DNA profile becomes zero at TSI 96 hours, however, partial male DNA profile is accessible (40%) at TSI 96 hours. It is concluded that autosomal DNA STR system becomes fail to generate the DNA profile the semen donor in the post-coital vaginal swabs at TSI >96 hours.
Key words: sexual assault cases, post-coital vaginal swabs, TSI, autosomal DNA STR

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INTRODUCTION

Forensic science is helping the criminal justice system by determining the precise circumstances of a crime through analysis of physical evidence recovered from the crime scene. Biological evidence collected from the crime scene provides extraordinarily treasured information with reference to the crime and the assailant(s). DNA technology including biological evidence embraces huge recognition in crime investigations (Kashyap *et al.* 2004). Autosomal DNA STR typing has become the powerful and reliable tool for human identification available today (Vandewoestyne and Deforce, 2010; Grisedale and Van Daal, 2012; Tan *et al.,* 2013; Purps *et al.,* 2015). The most common evidence material in the sexual assault cases is vaginal swabs and the most important aim of forensic analysis is to get the DNA profile of the perpetrator in the vaginal swabs (Benschop *et al.,* 2010). Although autosomal DNA STR analysis has modernized forensic analysis and has enhanced the capacity to get probative information from such type of crimes, however, there are still various situations when the evidence sample contains the cells but it is extremely difficult to get a complete autosomal STR DNA profile from the sample.

One such situation is when victims of sexually assault cases provide vaginal swabs after several days of the incident. In such circumstances, the probability of getting autosomal DNA STR profile of the semen donor reduces substantially as the post coital interval increases. Menstruation, drainage, vaginal lavage and intra-cervicovaginal sperm degradtive changes that take place with the time pass are the major reasons for sperm loss after intercourse. Due to degradation, the few residual sperms are likely to be in a structurally delicate and probably in the degraded condition. The autosomal DNA STR profile of the perpetrator from vaginal swabs can be obtained within 36

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*Corresponding author: manzoor.camb@pu.edu.pk

hours after intercourse, however it becomes impossible to do so as the post coital interval reaches to more than 48 hours (Ballantyne, 2013). However, as per literature of reproductive biology, several spermatozoa remain present in the human cervix up to 7-10 days of coitus and the general perception and recommendation about time limitation to collect the vaginal swabs in sexual assault cases is 72 hours. Although Y-STR analysis has the ability to generate the DNA profile of the semen donor at more extended period as compared to the autosomal STR analysis, however, forensic DNA laboratory always prefer to get autosomal DNA profile of the semen donor first due to fact that autosomal STRs have very high discrimination power as compared to the Y-STRs. Therefore, the present study aims to describe the time since intercourse (TSI) at which autosomal STRs fail to generate the "DNA profile" of the semen donor in the post coital vaginal swabs collected from different volunteer couples of Pakistan. The study is pioneer in the country and may provide the grounds to revisit the policy of time limit of evidence collection in sexual assault cases in the country.

MATERIALS AND METHODS

Samples

In order to determine the post-coital interval at which autosomal STR system becomes fail to generate the DNA profile of the assailtant(s), Sixty two post-coital vaginal swabs (x3) were obtained from six volunteers (PS-1 to PS-6) with proper consent who collected the swabs at different intervals/TSI (time since intercourse) including 24 hrs, 48 hrs, 72 hrs, 78 hrs, 84 hrs, 90 hrs, 96 hrs, 102 hrs, 108 hrs, 114 hrs, 120 hrs, 6-day, 7-day, 8-day, 9-day and 10-day after the sexual intercourse (Table I). To ensure the proper recovery of post-coital vaginal swabs, each couple was equipped with a comprehensive set of guidelines and information including complete detail of sampling intervals, set of sterile swabs and do`s and don'ts (Mayntz *et al.,* 2008). Oral swabs (x2) from each partner of the couples were also collected for reference DNA profile. Throughout the study secrecy/ privacy of the volunteers was ensured completely.

DNA extraction and quantification

DNA isolation from the oral swabs was carried out by using organic extraction procedure while DNA extraction from the post coital vaginal swabs was performed through differential extraction protocol (Hanson and Ballantyne, 2004). The aqueous phase extract after the Phenol:chloroform treatment were purified through Microcon® YM-100 (Millipore Corporation, USA) in accordance with the manufacturer`s instructions. DNA quantification was performed through Quantifiler™ Human DNA Quantification Kit on 7500 SDS Real Time PCR machine (Applied Bio-systems Foster City, CA, USA) as per manufacturer`s instructions.

DNA profiling

All DNA samples of post-coital vaginal swabs and of reference samples were amplified through AmpFℓSTR® Identifiler™ kit (Applied Bio-systems Foster City, CA, USA) on 96-Wells Gold-plated "GeneAmp PCR System 9700" (Applied Bio-systems) in accordance with the user's manual instructions. The amplified products were subjected for capillary electrophoresis at 3130xl Genetic Analyzer machine (Applied Bio-systems). GeneMapper® ID Software v3.2 was used for data analysis.

RESULTS AND DISCUSSION

Complete male DNA profile of the semen donor was detected in all post-coital vaginal swabs taken at 24 Hrs of intercourse. However, male DNA profile obtained from post-coital vaginal swabs of 48 Hrs was found partial in PS-1, PS-2, PS-5 and PS-6. At TSI 72 hrs, PS-1 did not generate the male profile; however, male DNA profile detected in all other samples remained partial except PS-3 which generated full male DNA profile (Table I).

At TSI 78, 84 and 90 hours, PS-1 and 4 did not generate the male DNA profile; samples of PS-2 and PS-3 were not available at these TSI. Partial male DNA profile was detected in PS-5 and PS-6 at TSI 78 hrs. complete and partial male DNA profile was detected in PS-5 and PS-6 respectively at TSI 90 hrs; however, no male DNA profile was detected at TSI 84 hrs. probably due to some sampling or processing error (Table II). Surprisingly, a partial male DNA profile was detected in two couples (PS-2-3) at TSI 96 hrs (Fig. 2-3). As no male DNA profile was detected at TSI 102, 108, 120 hours in any post-coital vaginal swab so remaining post-coital vaginal swabs > 120 hrs (6-10 days) were not processed for autosomal DNA STR analysis and were saved for subsequent analysis with Y-

STRs (Table I). Number of loci amplified at each TSI is given in Figure 1 and the probability to get full, partial or no male profile at different TSI is shown in Table II. These results are in accordance with the previous conducted studies (Mayntz *et al.,* 2008; Ballantyne, 2013; Speck and Ballantyne, 2014) with a slight variation that although the probability to get full male profile of the semen donor is maximum up to 48 hrs after intercourse however, extended post coital intervals up to 96 hrs may generate the full/ partial profile of the semen donor.

Table I: Autosomal DNA STR Profiling at different TSI of the post coital vaginal swabs

Volun-teer ID	Hours										Days				
	24	48	72	78	84	90	96	102	108	120	6	7	8	9	10
PS-01	CP	PP	N	N	N	N	N	N	-	-	-	-	-	N.T	N.T
PS-02	CP	PP	PP	-	-	-	PP	-	-	N	N.T	N.T	N.T	N.T	N.T
PS-03	CP	CP	CP	-	-	-	PP	-	-	N	N.T	N.T	N.T	N.T	N.T
PS-04	CP	CP	PP	N	N	N	N	N	-	-	-	-	-	-	-
PS-05	CP	PP	PP	PP	N	CP	-	-	-	-	N.T	N.T	N.T	N.T	N.T
PS-06	CP	PP	PP	PP	N	PP	N	N	N	N	N.T	N.T	N.T	-	-

CP=Complete male DNA profile; PP=partial male DNA profile; N = no male profile; - = sample not available; N.T= not tested

Table II: Chances to get male autosomal DNA profile at different TSI

S. No.	TSI (hours)	Chances of full male profile (%)	Chances of partial male profile (%)	Chances of No male profile (%)
1	24	100	0	0
2	48	33	67	0
3	72	17	67	16
4	96	0	40	60
5	102	0	0	100
6	108	0	0	100
7	120	0	0	100

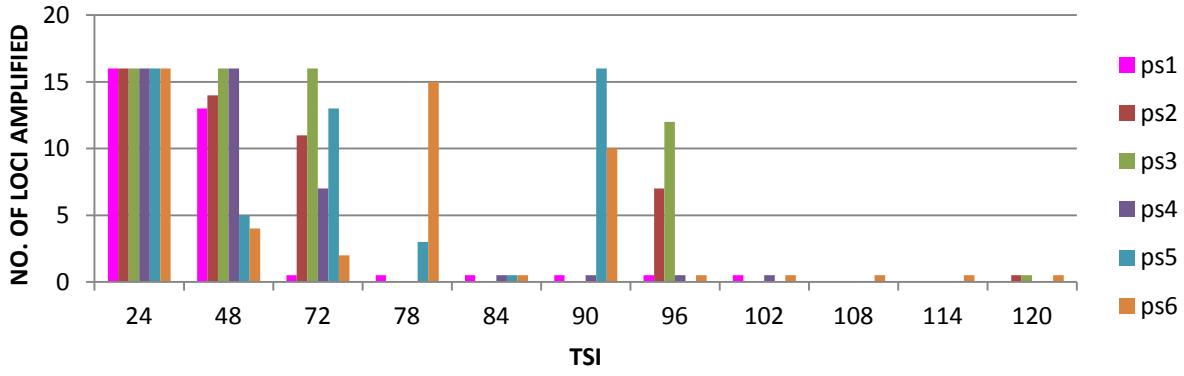

Figure 1: Number of loci amplified at different TSI

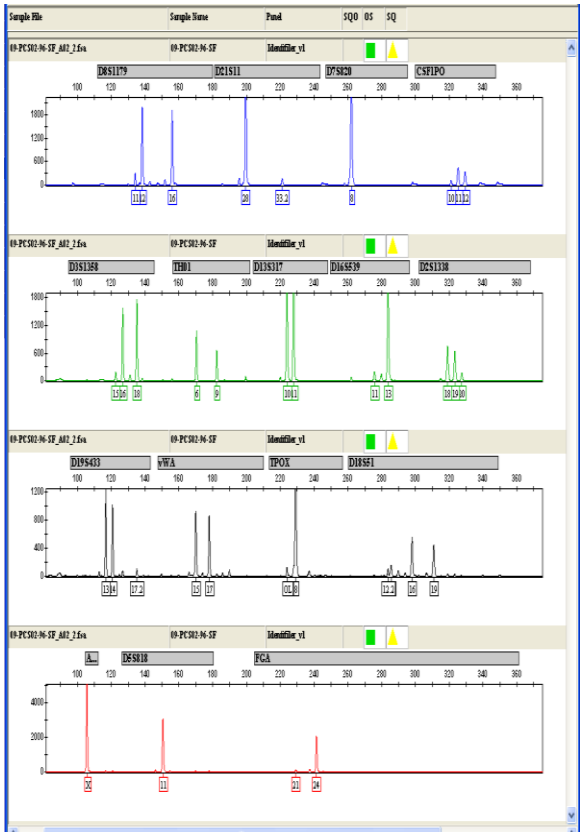

Figure 2: PS-2 at TSI 96 hours

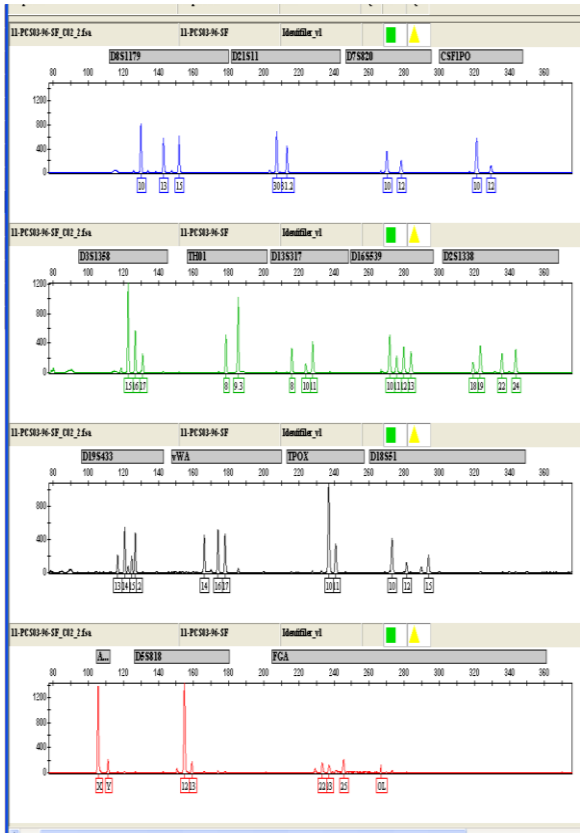

Figure 3: PS-3 at TSI 96 hours

The current study has revealed the complete autosomal STR profile of semen donor at TSI 72(17%) and partial autosomal STR profile of semen donor at TSI 96. The findings were also compared with the casework samples results of sexual assault cases (data not shown) in which DNA profile of the perpetrator was detected at TSI 72 Hrs in 19% cases (7/37), partial or minor male DNA profile was detected in 27% cases while 54% did not generate any male DNA profile. The above findings add momentum to make a suggestion for policy and practice in the country to revisit the time of evidence collection in sexual assault cases, because failure to collect the vaginal swabs from the victims after 72 hours may result in the loss of value-able and decisive evidence in such type of crimes. In the future research, effect of different physiological conditions of body of female and effect of age of female /male partners on the recovery of DNA from post coital vaginal swabs may be studied to make stuff more elaborated. The analytical approach to enhance the recovery of STR profiles is another aspect for future research.

Conclusion

It is concluded that autosomal DNA profile of the semen donor from the post coital vaginal swabs is detectable up to 96 hours after intercourse beyond which it becomes unsuccessful to generate the DNA profile the semen donor. A pilot scale study by increasing the number of donors may generate more reliable conclusions in future.

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