

## Case Report

# Presence of Vaginal Secretion on Accused's Underwear in Absence of Semen and Blood on Victims Swabs/Clothings as a Proof of Sexual Assault: A Report

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## Abstract

The sexual assault cases are on a rise now a days. Here we present a case of an eleven years old child who was a victim of sexual abuse. On preliminary examination, blood and semen could not be detected on the swabs/clothing's of victim. However, some white stains were visible on the underwear of the accused, which tested negative for semen. DNA profiling was conducted for the samples. Multiplex STR typing was used to study the 15 STR Loci in addition to a gender identification marker, amelogenin by capillary electrophoresis on 3500 xl Genetic Analyzer. It was observed that the profiles generated from the underwear of accused matched with the victim's DNA profile. Thus with DNA fingerprinting technique the case was concluded, linking the victim and the assailant even in the absence of semen and blood on the samples of victim.

**Keywords:** Semen; DNA; Loci; Electrophoresis; STR

## Introduction

Human genome comprise of  $3 \times 10^9$  base pairs. Out of these only 30,000 base pairs are coding & the rest non coding DNA sequences. STR loci are the polymorphic regions found in the genome that are used as genetic markers for human identification-forensic case work, data basing and paternity testing. DNA fingerprinting was first described by Jeffrey et al. [1]. They found that certain regions of DNA contain repeated DNA sequences which they referred to as variable number of tandem repeats. The microsatellites are short sequences of nucleotides; 3-7 base pairs repeated over and over again a variable number of times in tandem. Polymorphic STR loci are the most informative PCR based genetic markers for attempting to individualise biological material due to variation between individual found in these regions. The combination of DNA based tests and regularly used cytological tests have increased male evidence detection rates in forensic case work [2].

## Case Presentation

Fifty three percent of children in India face some form of child sexual abuse [3]. With childhood sexual abuse, victims are after too young to know how to express or to know what is happening. When not treated properly it leads to depression & anxiety. Here we report a case, where a 10yrs. old child was undertaking music classes from a tutor aged 37yrs. since last 2 years. One evening the mother of the victim saw the daughter lying in bed without clothes and this tutor leaving the room silently. The child narrated to her mother that the tutor strips her clothes daily and assaults her. FIR was lodged on the very next day of the incident under Protection of children against sexual offence Act, 2012 [4] & 376 IPC POCSO. POCSO Act was passed by both the houses of Parliament on May 22, 2012. The act defines a child as any person below the age of 18. It deems a sexual assault to be 'aggravated' under certain circumstances, such as when the abused child is mentally unstable or when the abuse is committed

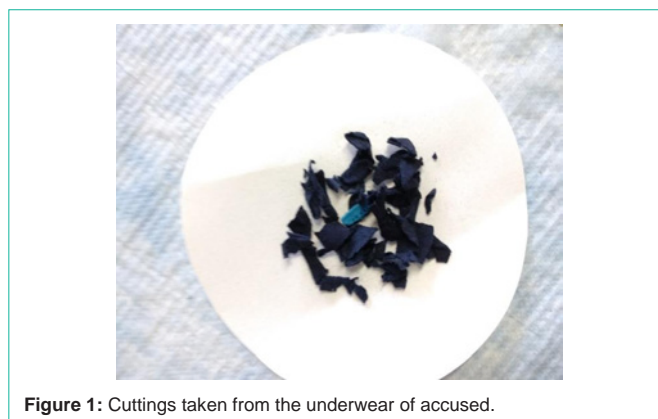


Figure 1: Cuttings taken from the underwear of accused.

by a person in a position of trust or a authority like a family member, family friend, teacher or doctor. Punishments include imprisonment from not less than at least 3 years extending up to 7 years to life imprisonment and a fine. According to IPC section 376, "whoever, except in the case provided for in sub section commits rape, shall be punished with rigorous imprisonment of either description for a term which shall not be less than seven years but which may extend to imprisonment for life, and shall also be liable to fine."

The tutor was arrested. The medical examination of victim and the suspect was conducted on the same day. There was no delay in medical examination of the victim & accused, which proved to be very helpful in the present case. Doctors collected the clothings of the victim along with the swabs and her blood sample using SAFE (Sexual Assault Forensic Examination kit). Doctors opined an old hymen tear and no bleeding. Potency test of the suspect was conducted and the blood sample in gauze along with the underwear of suspect were preserved. The case was deposited/submitted in the biology division of FSL (Forensic Science Laboratory, Delhi) for examination. The

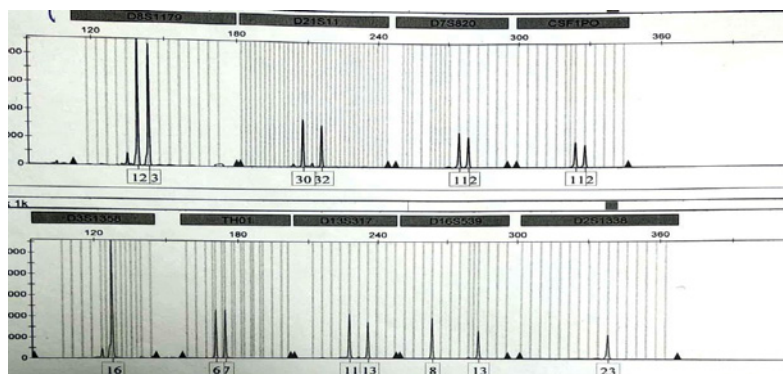


Figure 2: Electropherogram showing panel 1 & 2 of the Blood sample of victim.

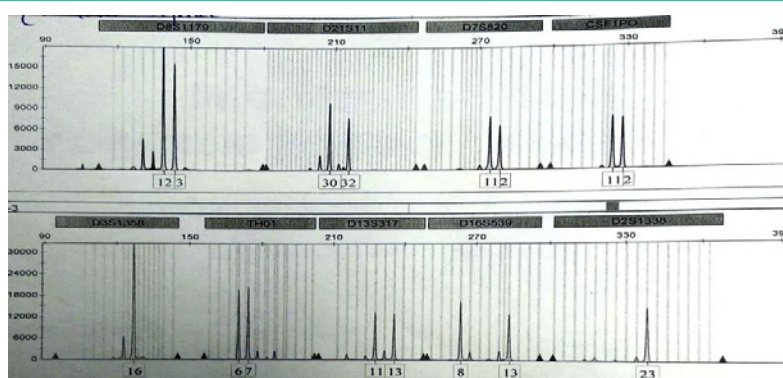


Figure 3: Electropherogram showing panel 1 & 2 of the underwear of the accused.

exhibits were stored at 40C as per standard protocol to prevent degradation of the samples. Due to huge pendency of cases in the laboratory, the case was taken up for examination after about 41 days of the incident.

## Materials and Methods

Preliminary tests for the presence of blood and semen were conducted. Identification of blood was done using tetramethylbenzidine (a derivative of benzedine) followed by adding 3% H<sub>2</sub>O<sub>2</sub> as an assay for the presence of blood. The test depends on the presence of an enzyme peroxidase in haemoglobin. Peroxidase acts as catalytic agent to accelerate the liberation of oxygen from hydrogen peroxide (an oxygen rich compound) in the reagent and thus causing oxidation of benzidine to give rise to blue color [5]. The exhibits were subjected to the preliminary tests for the presence of semen. The Acid Phosphate activity test / sodium alpha naphthyl phosphate method [6] (as prostate gland being the abundant source of AP) and microscopic examination of spermatozoa (Sperm Cytology) were carried out. Sperm heads could not be seen on the same.

Both blood and semen were found to be negative on the exhibits belonging to the victim. Although biological tests were found to be negative still the swabs and garments of victim were subjected to differential DNA Extraction. No male DNA profiles could be generated from these exhibits. On the undergarments of the suspect, whitish stains were found which tested negative for semen, using Acid Phosphate method and microscopic detection of sperm heads

(Figure 1).

Further, the stains were tested for the identification of salivary amylases using starch Iodine test. Amylases are enzymes that cleave polysaccharides such as Starch. The identification of saliva is largely based on the presence of amylase in a sample and Iodine is used to test for the presence of the amylase. Tests were found to be negative.

## DNA examination

Blood sample of victim was taken on FTA card, card was punched into the tube, washed with FTA buffer twice and with 1X TE thrice. Automate Express DNA extraction system using the Prepfil Express Forensic DNA extraction Kit was used to extract DNA from the suspect's underwear.

## DNA quantification

Multiplexing was carried out using this kit and 15 STR loci along with sex determining amelogenin were amplified in a single reaction [7].

Electrophoresis was carried out on ABI 3500xl Automated Genetic Analyser using 36cm capillary on POP4 polymer (Applied Biosystems, Foster City, USA.) 0.5µl of amplified DNA was mixed with 9.5µl HiDi Formamide and 0.3µl Gene Scan 500Liz size standard.

Mixture was denatured at 95°C for 3 minutes followed by lowering the temperature to 4°C for 3 minutes. The denatured samples were loaded on to the sample tray and transferred to auto sampler. Allelic ladder was run as a part of batch of samples.

**Table 1:** Allelic Data table for the samples.

Loci	(Blood sample) of victim		(Underwear) of the accused	
D8S1179	12	13	12	13
D21S11	30	32	30	32
D7S820	11	12	11	12
CSF1PO	11	12	11	12
D3S1358	16	16	16	16
TH01	6	7	6	7
D13S317	11	13	11	13
D16S539	8	13	8	13
D2S1338	23	23	23	23
D19S433	13	13	13	13
vWA	17	17	17	17
TPOX	8	13	8	13
D18S51	14	18	14	18
D5S818	11	12	11	12
FGA	23	24	23	24
AMELOGENIN	X	X	X	X

### Data analysis

Gene mapper Idx software v 1.2 was used for the analysis of samples. The samples were sized according to internal size standard. The software uses the internal size standard to assign a fragment size and a sizing quality value to each peak (Figure 2 & 3).

### Results and Discussions

Sexual assault cases involve the analysis of vaginal samples and garments. The objective behind this analysis is the detection of sperms and the generation of assailant's DNA profile. Microscopic analysis, presumptive testing for body fluid identity, DNA isolation and profiling are performed on those samples. DNA isolation is generally done by differential extraction in which female DNA (mostly from vaginal epithelia) is separated from the male fraction. When less or no sperm cells are present or the ratio of female to male is not favourable partial or incomplete male DNA profiles are obtained [8].

With the advancement of DNA technology and understanding of DNA transfer, the collection of the suspect's underwear along with penile swabs if less than 48 hours had passed between the assault and the apprehension of the suspect is done. These samples are also tested now days for the presence of the victim's DNA on the suspect, thus making a link between the two individuals [9].

### Conclusions

In rape forensic investigations, semen identification is required to corroborate the alleged crime. Such type of cases are frequently examined in forensic laboratories. The presence of female DNA whether through saliva or vaginal fluid of the samples of attacker is expected in cases involving transfer. In the present case where sperm or blood cells are absent, female DNA identified represents the viable source of DNA analysis. It proved that accused person was involved in rape case.

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