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Impact of biological evidences on DNA profiling of sexual assault cases

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ABSTRACT

Introduction: The identification of perpetrator involved in crime becomes difficult due to lack of evidence. Biological evidence plays an integral role in establishing link between survivor, suspect, and the scene of crime. The era of eye witness is almost diminished and DNA analysis is necessary for the identification of perpetrator. The loss or degradation of biological traces due to urinating, defecating, douching of genitals, showering and delay in medico-legal examination may yield negative results.
Objective: The objective of study was to link DNA profiles generated from biological samples to associate suspect with survivor and other parameters affecting the results of DNA profiling.
Materials and Methods: This present study was conducted in the Biology & Serology Division of Regional Forensic Science Laboratory, Northern Range, Dharamshala and DNA Division of State Forensic Science Laboratory, Himachal Pradesh, Junga, India. A total of 142 sexual assault cases received for examination during the year 2018 and 2019 were studied.
Results: Human spermatozoa were detected in 39 (27.46%) cases and genetic profiles were generated. Spermatozoa were detected even after taking bath and washing of clothes in 3 (2.11%) cases. 10 (7.04%) survivors were menstruating at the time of assault. Hymen was absent in 43 (30.28%) cases followed by old healed tags in 37 (26.06%), recent tears in 15 (10.56%), intact in 9 (6.34%) and partially ruptured in 4 (2.82%) cases. Out of 39 cases, DNA profile resulted as an inclusion of assailant in 27 (19.01%) cases and exclusion in 12 (8.45%) cases. The persistence of spermatozoa on vaginal swabs was found up to 4 days. Only 18 (12.68%) survivors underwent medico-legal examination on first day of assault.
Conclusion: The findings clearly showed that early medico-legal examination is of paramount importance to avoid the potential loss of evidence, which directly affects the results of DNA profiling.

1. Introduction

Over the years, it has been seen that the women are victims of various types of crimes like rape, murder, dowry deaths and domestic violence. Sexual assaults have been the most common type of crime against women. According to the Indian Penal Code (IPC), the rape has been defined as a cognizable, non-bailable and non-compoundable offence in the Sections 375 and 376. Sexual assault is any kind of sexual contact or conduct without the consent of victim.[1] The term consent plays an important role in these crimes. Sexual assaults include sexual activities like any form of genital, anal or oral contact directly or through clothes, finger and object insertion, masturbation, exhibitionism, masochism, molestation, pornography and indecent assault.[2] In reality, sexual assaults are the result of lust or revenge in order to fulfil the sexual desires.[3] In some cases, it has also been seen that the women had misused

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the laws and made false allegations in order to defame the males.[4] The survivors of sexual assaults are often severely traumatized by the incident and face difficulty in functioning in their daily life. The shock and shame of the incident may prevent the survivor from revealing the incident to their close ones for medical help and police assistance.[5]

There has been an increase in the number of sexual assault cases worldwide in the past few years. Worldwide, around 20% of females have experienced some kind of sexual abuse in their childhood.[6,7] According to the international data, 35% of women experience some kind of sexual abuse in their lifetime.[8] Less than 10% women seek help from the police and raise their voice against sexual abuse. In the world, India occupies 39^{th} position in sexual assault cases. According to 2018-19 annual report of National Crime Record Bureau of India, crime against women has increased 7.3%. Himachal Pradesh had reported 344 cases of sexual assaults in the year 2018 and 359 cases in the year 2019.[9]

The sexual assault cases are prevalent in every society, but the conviction rate is very less as compared to total number of cases registered and many cases go unreported due to social stigma attached to rape. When Protection of Children from Sexual Offences (POCSO) Act came into existence in 2012, the reporting of child sexual abuse cases increased tremendously in India and the statistics showed that the child sexual abuse outnumbered the adult sexual assaults. The media reports sexual assault cases in a sensational way, which affect the social status of the survivors and they feel socially degraded.[3] The threats and fear of attack by the assailant also restricts the survivor from reporting the incidence. In some cases, survivor thinks that even after reporting the incidence to the investigation agencies and criminal justice system, there will be no conviction of the assailant. Alcoholism, drug addiction and open defecation in rural areas are also some of the reasons which lead to increase in the incidences of sexual assault. The higher female to male ratio and higher conviction rate may lower the incidences of sexual assault.

Before the advent of DNA technology, the punishment to perpetrator was given on the basis of oral testimony of the survivor and eye witnesses, but the advances in DNA technology have changed the scenario. DNA technology has been recognised as a great tool in investigation of crime cases as DNA can be easily extracted from hair, teeth, skin, bone, tissue and body fluids such as blood, saliva, urine and semen.[10] DNA technology plays an important role in investigation of sexual assault cases and considered to be the most important evidence in individualization of a suspect. With the help of DNA technology, multiple assailants involved in a crime can be identified by generating genetic profile from the mixed stains found on body and clothes of survivor. Early medico-legal examination of the survivor is required to collect and preserve the biological evidence in order to reach at concrete scientific conclusion. According to the Section 53-A of Code of Criminal Procedure (CrPC) of the Amendment Act of 2005, the medical examination of accused of rape has also become a necessary requirement.[11] Delay in medico-legal examination, douching of genitals and changing of clothes prior to examination lead to loss or degradation of biological evidence. The medical examination of survivor should be done without taking bath or changing clothes so that secretion of biological fluids and other trace evidences present on the body and clothes of survivor and accused may not be destroyed or degraded.[12,13]

The purpose of the study is to establish a link between biological evidences recovered and interrelationship of factors affecting DNA profiling. The study demonstrates a significant relationship of factors such as time gap between the date of incidence and medico-legal examination, status of survivor, anogenital and extra genital injuries and presence of spermatozoa in sexual assault cases received for examination in forensics laboratory.

2. Materials and Methods

2.1. Study group and design

This present study was carried out in the Biology & Serology Division of Regional Forensic Science Laboratory (RFSL), Northern Range, Dharamshala and DNA Division of State Forensic Science Laboratory, Junga, Himachal Pradesh, India. A total of 142 sexual assault cases (75 POCSO and 67 adults) registered under section 376, POCSO Act and other sections of Indian Penal Code (IPC) received from three districts of northern range of Himachal Pradesh during the year 2018-2019 were studied. 72 cases were received for forensic examination in 2018, out of which 39 were registered under POCSO Act whereas in 2019, 70 cases were received, out of which 36 were registered under POCSO Act.

2.2. Collection and analysis of data

The data was collected from the police forwarding letter, first information report (FIR), medico-legal report and results from the reports of Forensic Science Laboratory. Dried forensic exhibits and evidences were stored in exhibit storage chambers and liquid evidences such as blood, semen, saliva were stored at 4°C in refrigerator of forensic science laboratory. The variables such as time gap between the date of assault and medico-legal examination, menstruation status, barrier method of contraception used, status of survivor regarding bathing, changing and washing of clothing, undergarments, status of hymenal tears, type of assault, presence of seminal stains, anogenital and extra genital injuries, presence of spermatozoa in various samples such as pubic swab, vaginal swab and smear (lower, higher, external and internal), perineal swab, fornix swab (anterior and posterior), cervical swab and smear, periurethral swab,

urethral swab, anal swab (inner and outer), vulval swab, rectal swab, introitus swab, buccal mucosa swab, hymenal swab, cervix slide, pericervical slide, posterior fornix smear slide, buccal mucosa slide, perineal swab slide, vaginal smear slide (internal and external), perineal smear slide, anal smear slide, cervical canal slide, pubic hair, seminal stain scrapping, fingernail clippings/scrapings, clothes of survivor and results of DNA profiling were entered in a self-designed proforma on Excel spreadsheet and statistically analysed.

3. Results

All the female survivors underwent medico-legal examination and the biological evidences were collected and preserved during the examination. 18 (12.68%) cases were examined in the first day, 19 (13.38%) on the second day, 18 (12.68%) within a week, 17 (11.97%) within 8 days to one month, 4 (2.82%) within 2 months, 2 (1.41%) within 3 months, 2 (1.41%) within four to six months and 3 (2.11%) within seven months. 10 (7.04%) survivors were menstruating at the time of assault.

In 29 (20.42%) cases, survivors were not menstruating at the time of medico-legal examination whereas 21 (14.79%) were menstruating and 12 (8.45%) had not attained menarche. 2 (1.41%) survivors were found to be pregnant and assailants were found to be biological father of the offspring, 2 (1.41%) had attained menopause and 1 (0.7%) had an abortion. In 7 (4.93%) cases, the assailant used condom during sexual assault, in 18 (12.68%) cases, the assailants did not use any barrier method of contraception and 9 (6.34%) survivors did not know whether the assailant used any barrier method or not. 29 (20.42%) survivors had taken bath and washed clothes followed by 23 (16.20%) who took bath only and 22 (15.49%) who neither took bath nor changed clothes. 11 (7.75%) survivors had taken bath, changed clothes but did not wash clothes, 3 (2.11%) had taken bath but did not wash their clothes, 1 (0.7%) had not taken bath, changed clothes but did not wash clothes and 1 (0.7%) had not taken bath but changed clothes before going for medico-legal examination.

Hymen was absent in 43 (30.28%) cases followed by old healed tags in 37 (26.06%), recent tears in 15 (10.56%), intact in 9 (6.34%) and partially ruptured in 4 (2.82%) cases. Hymen was not visualized due to menstruation in 5 (3.52%) cases. Vaginal penetration was the pattern of crime in 115 (80.99%) cases followed by 3 (2.11%) cases of molestation and 2 (1.41%) cases of pornography and vaginal penetration. Vulval fingering, vaginal touching, object and finger insertion, touching and kissing, oral and anal penetration, masochism, masturbation, pornography, intoxication and molestation were also reported in some cases. Dry seminal secretions were found on both legs in 1 (0.7%) case. Abrasion and bruises were observed in 7 (4.93%) and 6 (4.23%) cases respectively followed by laceration and bleeding in 2 (1.41%) cases each. Erythema (skin redness) on body, cut genitalia and contusion were reported in 3 (2.11%) cases. No injury was found in 114 (80.28%) cases.

The presence of spermatozoawas detected on salwar, underwear, T-shirt, dress/frock, shirt, lower/pajami, pants/trouser, handkerchief, inner and outside of condoms, plastic sack, bag, bed sheet and pillow cover in 39 (27.46%) cases. The specimen such as vaginal swabs, cervical swabs, perineal swab, vaginal smear slides, cervical and pericervical slides, and seminal stains scrapings collected during the medico-legal examination yielded positive results for semen. After the confirmation of spermatozoa in forensic samples, the genetic profiles were generated through DNA profiling. The DNA profiles obtained from the biological samples considered as exclusion and an inclusion of assailant (Figure 1). The DNA profile is determined as an inclusion, when the DNA profile of a survivor or suspect is consistent with the DNA profile recovered from the forensic samples whereas the DNA profile is determined as exclusion, when the DNA profile of a survivor or suspect is inconsistent with the DNA profile recovered from the forensic samples.

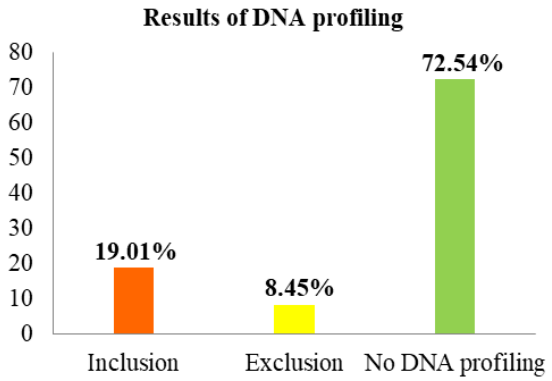

Fig. 1: Bar graph showing the results of DNA profiling in sexual assaults

Table 1: Associated factors showing interrelationship with DNA profiling in sexual assaults

S. No.	Parameters		Frequency and percentage	Detection of spermatozoa and DNA profiling of samples
1.	**Status of survivor**	Neither taken bath nor washed clothes	12 (8.45%)	• T-shirt, underwear and handkerchief • Vaginal swab and vaginal slide • Vaginal and perineal swab, vaginal smear slide and dry seminal stains from both legs • Vaginal and cervical swab, vaginal and cervical slide • Underwear and handkerchief • Salwar and plastic sack • Outside and inside of condom • Vaginal, perineal and cervical swab • Vaginal swab, vaginal slide and pants/trouser • Shirt and salwar • Underwear • Bed sheet
		Taken bath, changed clothes but did not wash clothes	8 (5.63%)	• Dress/frock and lower/pajami • Underwear and salwar • Salwar • Salwar • Lower/pajami • Salwar • Bed sheet • Lower/pajami
		Taken bath but did not wash clothes	4 (2.82%)	• Bed sheet and pillow cover • Underwear • Bed sheet • Salwar
		Taken bath and washed clothes	3 (2.11%)	• Handkerchief • Salwar • Bag
		Taken bath	2 (1.41%)	• Bed sheet • Lower/pajama
		Changed clothes but did not take bath	1 (0.7%)	• Salwar
2.	**Barrier method of contraceptionused during act of intercourse**	No-contraceptive barrier used	10 (7.04%)	• Bed sheet • Shirt and salwar • Underwear and handkerchief • Dress/ frock and lower/pajami • Lower/ pajami

Continued on next page

Table 1 continued

				• Salwar • Vaginal swab and vaginal slide • Salwar • Handkerchief • T-shirt, underwear and handkerchief
		Condom	3 (2.11%)	• Inside condom • Bed sheet • Outside and inside of condom
		Survivor does not know	2 (1.41%)	• Underwear • Bed sheet and pillow cover
3.	**Menstruation status of survivor at the time of medico-legal examination**	Not- menstruating	10 (7.04%)	• Bed sheet • Underwear • Pants/trouser and underwear • Lower/pajami • Salwar • Underwear • Vaginal swab and vaginal slide • Bed sheet • Handkerchief • T-shirt, handkerchief and underwear
		Menstruating	8 (5.63%)	• Shirt and salwar • Outside and inside of condom • Underwear and handkerchief • Bed sheet • Vaginal, cervical swab, vaginal and cervical slide • Salwar • Lower/pajami • Bed sheet
		Not attained menarche	1 (0.7%)	• Salwar and plastic sack
4.	**Time gap between the date of assault and medico-legal examination**	Within a day	6 (4.22%)	• Bed sheet • Underwear • Vaginal swab, vaginal and peri-cervical slide and salwar • Shirt and salwar • Vaginal swab and vaginal slide • Bed sheet
		Second day	11 (7.75%)	• Salwar • Inside condom • Vaginal swab, vaginal slide and pants/trouser

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Table 1 continued

			• Salwar, lower/pajami and bed sheet • Salwar and bed sheet • Vaginal, perineal and cervical swab • Outside and inside of condom • Salwar and plastic sack • Underwear and handkerchief • Lower/pajami • Handkerchief
		Within a week	5 (3.52%)	• Pants/trouser and underwear • Bed sheet • Vaginal and cervical swab, vaginal and cervical slide • Vaginal and perineal swab, vaginal smear slide and dry seminal stain scrapping from both legs
		Within 8 days to 1 month	8 (5.63%)	• Salwar • Dress/frock and lower/pajami • Bag • Underwear and salwar • Lower/pajami • Salwar • Bed sheet and pillow cover • Bed sheet
		Within 2 months	3 (2.11%)	• Bed sheet • Salwar • Salwar • Handkerchief
		Within 7 months	1 (0.7%)	• Lower/pajama

An inclusion of assailant as a source of biological sample was reported in 27 (19.01%) cases whereas exclusion was reported in 12 (8.45%) cases. Out of 39 cases, 12 (8.45%) survivors had neither taken bath nor washed clothes, 8 (5.63%) had taken bath, changed clothes but did not wash clothes, 4 (2.82%) had taken bath but did not wash clothes, 3 (2.11%) had taken bath and washed clothes, 2 (1.41%) had taken bath and 1 (0.7%) had changed clothes but not taken bath before medico-legal examination. Condom was used by the assailant in 3 (2.11%) cases. No barrier method of contraception was used by the assailant in 10 (7.04%) cases and 2 (1.41%) survivors were not knowing whether the assailant used any barrier method or not. 8 (5.63%) survivors were menstruating during medico-legal examination, 10 (7.04%) were not menstruating and 1 (0.7%) had not attained menarche. 6 (4.22%) survivors underwent medico-legal examination within a day, 11 (7.75%) within 2 days, 5 (3.52%) within a week, 8 (5.63%) within 8 days to one month, 3 (2.11%) within 2 months and 1 (0.7%) within 7 months Table 1. Date and time of assault was not mentioned in First Information Report of 5 (3.52%) cases.

Anogenital and extra genital injuries were observed in 7 (4.93%) cases. No injury was observed in 31 (21.83%) cases. Hymen was ruptured with old healed tags in 24 (16.90%) cases followed by absent in 6 (4.23%) cases, intact in 2 (1.41%), ruptured with recent tags in 1 (0.7%) and partially ruptured in 1 (0.7%) case. The pattern of assault was vaginal penetration in 33 (23.24%) cases, Vaginal and oral penetration, and vaginal fingering in 1 (0.7%) case, kissing and touching in 1 (0.7%) case and objects and finger insertion in 1 (0.7%) case. Dry seminal stains were found on both legs in 1 (0.7%) case (Table 2).

DNA profiling was not conducted in 103 (72.54%) cases as spermatozoa were not detected in samples tested negative. The time duration of presence of human spermatozoa detected in various biological samples and clothes of survivor was 2 days 2 hours 15 minutes in vaginal and perineal swab, 4 days 4 hours 25 minutes in cervical swab, 4 days 4 hours 25 minutes in vaginal and cervical slide, 7 hours 23 minutes in pericervical slide, 2 days 2 hours 15 minutes in vaginal smear slide, 6 months 11 days 18 hours 30 minutes in lower/pajami, 6 days 3 hours 30 minutes in pants/trouser, 1 month 21 days 35 minutes in salwar, 15 days 6 hours 43 minutes in dress/frock, 7 days 22 hours 5 minutes in underwear, 1 month 13 days 17 hours 50 minutes in handkerchief, 13 days 2 hours 30 minutes in bed sheet, 13 days 2 hours 55 minutes in bag, 1 day 7 hours 10 minutes in plastic sack and 1 day 4 hours 30 minutes in condom.

4. Discussion

4.1. Detection of spermatozoa

According to Locard's principle of exchange 'every contact leaves traces'. Likewise every act of sexual assault leaves behind some important trace evidences such as semen, blood, vaginal fluid, saliva, sweat, hair, fingernails or epithelial cells on the body and clothes of survivor, assailant and at scene of crime.[14,15] These biological evidences are of great importance in establishing the occurrence of crime. The presence of spermatozoa on survivor's body, genital swabs and slides is considered as an absolute positive evidence of sexual assault. Other biological trace evidences can be found in case of any kind of struggle for self defense. Acid phosphatase test and microscopic examination were done in forensic evaluations to confirm the detection and presence of human semen and spermatozoa.[16] In the present study, spermatozoa were detected and identified in 39 (27.46%) cases. The findings of our study were close to the study of Pal et al[17] and AlMadaniet al.[18] who detected spermatozoa in 56(30.93%) and 25 (28.74%) cases.

4.2. Role of medico-legal examination and DNA results

When semen is detected on genital swabs, slides and clothes of survivor, then individualization of semen was done by obtaining a genetic profile through DNA technology. The developed profile was compared with the profiles of survivor and suspect. On the basis of genetic profiles, the suspect was included or excluded as a possible assailant of crime. In our study, an inclusion of assailant in sexual assault was reported in 29 (20.42%) cases and exclusion in 12 (8.45%) cases. In 103 (72.54%) cases, spermatozoa were not detected on clothes, genital swabs and slides of survivor so, the DNA profiling could not be done. Spermatozoa may not be detected in cases of fabrication of facts and false allegations by the survivors against the perpetrator or due to the loss and degradation of samples due to post assault activities like vaginal drainage, urinating, washing, bathing, changing clothes, douching of genitals and use of condom during the act and increasing intervals between the assault and medico-legal examination.These post assault activities by the survivor affect the quality and quantity of DNA. The reasons for delay in medico-legal examination may be non-disclosure of incident due to fear of reprisal of perpetrator, fear of shame, guilt, blemish on the character and reputation. The biological evidences got lost or degraded and there are chances of growth of microorganisms such as bacteria and fungi which breaks down the sperm and yields negative results.[19–21]

In many cases, it was observed that the survivors due to lack of knowledge had taken bath and changed clothes prior to medico-legal examination which resulted in loss or elimination of the important biological evidences. Among 142 cases of sexual assaults, 18 (12.68%) were examined

Table 2: Type of assault and injuries on body of survivor

S. No.	Parameter	Type of injury	Frequency and percentage
1.	Type of assault	Vaginal penetration	33 (23.24%)
		Vaginal and oral penetration, and vaginal fingering	1 (0.7%)
		Kissing and touching	1 (0.7%)
		Object and finger insertion	1 (0.7%)
2.	Status of hymenal tears	Ruptured (old healed tags, recent tears and partially ruptured)	26 (18.31%)
		Absent	6 (4.23%)
		Intact	2 (1.41%)
3.	Anogenital and extragenital injuries	Multiple bruises on body	7 (4.93%)
		Abrasion on knee	
		Reddish erythema on genitalia	
		Bruises over right thigh	
		Rashes and abrasion on vaginal wall and scratch marks on thighs	
		Multiple abrasions on body	
		Two bluish coloured bruises on left breast	
	No injury		31 (21.83%)

on the same day of the assault. Our findings were similar to the study of Arifet al.[22] who reported the same day examination in 18 (12.1%) cases. The results of present study were close to the findings of Pal et al,[17] Sharaf El. Din[23] and Pal et al[24] who reported same day examination in 27 (14.91%), 12 (9.2%) and 3 (8.55%) cases respectively. In our study, 19 (13.38%) survivors underwent for medico-legal examination on second day, 18 (12.68%) within a week and 17 (11.97%) within 8 days to one month and 11 (7.75%) after one month. Our study was consistent with the study of Tamuli et al.[6] who also reported medico-legal examination after one month of the assault in 28 (7.33%) cases. This shows that the maximum number of survivors underwent medico-legal examination on the second day of assault.

4.3. Status of survivor and spermatozoa on clothes

In the present study, 29 (20.42%) survivors had taken bath and washed clothes, 23 (16.20%) had taken bath and 22 (15.49%) had neither taken bath nor washed clothes, 11 (7.75%) had taken bath, changed clothes but did not wash clothes, 3 (2.11%) had taken bath but did not wash clothes, 1 (0.7%) had not taken bath, changed clothes but did not wash clothes and 1 (0.7%) had changed clothes but not taken bath before medico-legal examination and tested negative for the presence of semen/spermatozoa.

Only 39 cases were tested positive for spermatozoa, out of which 12 (8.45%) survivors had neither taken bath nor washed clothes, 8 (5.63%) had taken bath, changed clothes but did not wash clothes and 3 (2.11%) had taken bath and washed clothes, 4 (2.82%) had taken bath but did not wash clothes, 2 (1.41%) had taken bath and 1 (0.7%) had changed clothes but not taken bath before medico-legal examination. The results of our study were in contrast to the results of Arif et al.[22] who reported that 122 (82.4%) survivors had changed clothes, 26 (17.57%) had changed clothes but did not wash and handed over the clothes worn at the time of assault. In the present study, dried secretions were found on clothing, bed sheets and bag of survivor in 34 (23.94%) cases. Our study was consistent with the study of Lavelle and Kellogg[25,26] who reported that dried secretions on clothing remain quite stable, so semen can be detected after a long time. The stains present on the associated evidences such as clothing and bedding will not be affected if they are kept in dry environment.[20] Spermatozoa can be detected on washed clothes but the retention of sperm on clothes depends on the type of fabric, amount of ejaculation, type of detergent used, laundering temperature and time of washing of clothes which affects the presence of spermatozoa.[27–29]

4.4. Barrier methods of contraception used

Nowadays, rapists have been increasingly using some barrier methods of contraception during the sexual intercourse to avoid pregnancy and sexually transmitted diseases due to awareness of sex education through internet, electronic and print media. Condom is the most commonly used barrier method. The use of any barrier method of contraception may result in absence of seminal fluid on the body and clothing of survivor.[33–35]Sexual dysfunction, vasectomized, oligospermic or azoospermic and even potent assailants with no ejaculation during the act could lead to absence of seminal fluid as semen without sperm cells may generate no DNA profile or a partial profile which is less informative. In such cases, other biological evidences like fingernail scrapings, epithelial cells, saliva, etc. play a

major role. Fingernail scrapings and clippings can be a good source of foreign DNA, if the survivor has struggled with the perpetrator during the assault as blood, semen, saliva and epithelial cells of skin may be present on fingernails. Saliva is ample source of DNA as genetic profile of suspect can be generated from mucosal epithelial cells of the mouth using STR or Y-STR typing from saliva on genitals and other body parts of survivor where the suspect had kissed, licked or bitten during the assault.[20,30]Our study reported that the assailant used condom during sexual assault in 7 (4.93%) cases. The results of our study contradicts with the study of O'Neol et al.,[31] Davis et al.[32] and Cerdes et al.[33] who mentioned that 112 (13.32%), 10 (29.4%), 190 (12%) assailants used condom during the assault.

4.5. Link between menstruation, semen and spermatozoa

Earlier, it was assumed that spermatozoa and seminal secretions might outflow during menstruation from the vagina of survivor thus decreasing the probability of finding semen in genital swabs and slides. But, the studies showed that menstruation did not affect the probability of finding semen in vagina and did not hamperthe results of DNA profiling.[38]In our study, 10 (7.04%) survivors were menstruating at the time of assault and semen and spermatozoa were detected in 6 (4.22%) cases. The results of our study were in contrast to the results of Cerdaset al.[38]conducted study on the 170 survivors who were menstruating at the time of assault and reported detection of spermatozoa in 101 (59.41%) cases. Cerdas et al.[34] mentioned that semen was detected in 149 (55%) and spermatozoa were detected in 90 (33%) samples.[33] 2 (1.41%) survivors were pregnant and assailants were found to be biological father of the offspring. The results were in line with the study of Arif et al.[22] who reported pregnancy in 2 (1.3%) cases after the assault. The results of our study were close to the results of Pal et al.[24] who mentioned that 1 (2.85%) survivor was pregnant after the sexual assault.

4.6. Type of assault and status of hymeneal tears

The results of our study demonstrated that vaginal penetration was the most common pattern of crime in 115 (80.99%) cases. The results of our study were in line with the study of Kauret al.[39] and Adegoke and Adefolau[35] who reported vaginal penetration in 76 (76%) and 394 (73.78%) cases.Earlier hymen was considered as a sign of virginity and Two Finger Test or Per Vaginal (PV) examination was conducted to determine whether the survivor is habitual of having sex or not, but now this practice is banned as this violates right to privacy and hymen could also be ruptured during some intense activities like cycling, swimming, horse riding and use of menstrual cups and tampons during menstruation. The present study reported that hymen was ruptured in 56 (39.44%) cases and intact in 9 (6.34%) cases. The result of our study was close to the results of Pal et al.,[17] AlMadani et al.[18] and Abdellah and Ali[36] who reported old healed tags in 40 (22.09%), 12 (29.3%) and 6 (13.04%) cases respectively. Our study was in agreement with the study of Tamuli et al.[6] who reported intact hymen in 25 (6.54%) cases. The results of our study were close to the results of Pal et al.,[17] Arif et al.,[22] and Pal et al.[24] who reported intact hymen in 22 (12.15%), 5 (3.3%), 4 (11.42%) cases. The hymenal tags or tearsdo not prove that vaginal penetration has occurred or not buttenderness, bruise, redness, perforation or tear around the vulva gives the indication regarding penetration.

4.7. Anogenital and extragenital injuries

Any kind of sexual contact without consent of the survivor may lead to injuries on the body. The injuries are caused due to struggle and self-defense by the survivor during the assault. During the assault, skin cells or hair may get deposited on the survivor. Even DNA profile can be generated from touch or other skin contact which have sufficient amount of DNA.[37] Injuries on genitals, breasts, thighs and scratches on the back are commonly observed in sexual assault cases. In our study, the most common type of injuries found on the body of survivors were scratch marks over cheeks, thighs, contusion over thigh, abrasion on knee, left leg, vaginal wall, neck, front part of skull, posterior part of introitus and multiple abrasions, laceration/tear on lower part of vagina, multiple bruise on body, right thigh, fourchette, thigh, vulva, left breast, tear on sides of urethral opening on labia majora,rashes on vaginal wall, cut genetalia,reddish erythema on genitalia, bleeding on vaginal introitus opening. In 114 (80.28%) cases, no injury was found on the body of the survivor whereas anogenital and extra genital injuries were observed in 20 (14.08%) cases. Abrasion and bruises were the most common injuries found in 7 (4.93%) and 6 (4.23%) cases respectively. The results of our study were in contrast to the results of Pal et al.,[24] Sherif et al.[38] and Arif et al.[22] who reportedanogenital and extragenital injuries in 11 (31.43%), 77 (33.62%) and 16 (10.8%) cases respectively. The presence of injuries showed that struggle has occurred between assailant and survivor during the forcible act of sexual assault. No injury on the body indicated that the survivor might be known to the assailant and due to acquaintance no resistance was shown by the survivor during the assault. In some cases, injuries got healed and could not be observed on the body due to delay in reporting the case to investigation agencies and medico-legal examination.

4.8. Discharge of semen

The presence of seminal stains on clothes and body of survivor do not directly prove the fact that any type of

penetration has occurred or not, as the seminal stains can be present as a result of masturbation, any sexual perversions or discharge/spillage of semen by the assailant on the body parts of survivor. In our study, the dried seminal secretions were detected on both legs in 1 (0.7%) case and resulted in inclusion of assailant though dried stains on the skin have a limited amount of DNA.[39] In the present study, 2 (1.41%) survivors narrated that assailant had kissed, touched, inserted an object and finger in genitals of survivor. In these cases, semen was detected on bed sheet, plastic sack, and salwar and DNA profile generated from these samples matched with the DNA profiles of assailant. This clearly suggests that the survivor's statement providing information regarding the assault must be taken into consideration which can streamline the overall DNA analysis of the samples.

5. Conclusion

Biological evidences are of great importance in sexual assault cases as their presence proves sexual contact and leads to identification of perpetrator through DNA profiling. Various factors such as douching, showering, changing of clothes by the survivor and delay in medico-legal examination affect the results of DNA profiling. Awareness should be created among investigation agencies, medicos, and other stake holders regarding early medical examination of the survivor so that sufficient evidences could be collected and preserved for forensic investigations. Our study demonstrated that dried biological samples on clothes can persist for longer time but trace evidences on the body of survivor and assailant should be collected at the earliest after the sexual assault as they may be eliminated during routine activities.

6. Author Contributorship Statement

1. **Dr. Surender Kumar Pal** conceived of the research study, made the protocol, wrote the study manuscript, drafted the study tool (schedule, statistically analysed the data and interpreted the results) and guided throughout the research study.
2. **Vinita Kumari** helped in the data collection, entry of the data in Microsoft excel spreadsheet along with the coding and decoding of data.
3. **Nisha Devi** helped in proofreading and finalisation of the manuscript.

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None.

8. Conflict of Interest

The authors declare no conflict of interest.

References

1. Ellison SR, Subramanian S, Underwood R. The general approach and management of the sexual assault patient. *Missouri Med*. 2008;105(5):434–40.
2. Putnam FW. Ten-year research update review: child sexual abuse. *J Am Acad Child Adolesc Psychiatry*. 2003;42(3):269–78.
3. Venkitakrishnan U, Kurien SG. Rape Victims in Kerala. Thiruvananthapuram: Centre for Development Studies; 2003. p. 1–64.
4. Kaur S, Lamba M, Gupta R. Y chromosome STR typing: a distinguishing tool for exclusion in a casework of sexual assault. *J Forensic Res*. 2017;8(5):1–4.
5. Awasthi A. A critical examination of recent rape cases and the criminology involved; 2013. Available from: https://www.academia.edu/6608945/A_Critical_Examination_of_Recent_Rape_Cases_and_the_Criminology_Involved.
6. Tamuli RP, Paul B, Mahanta P. A statistical analysis of alleged victims of sexual assault- a retrospective study. *J Punjab Acad Forensic Med Toxicol*. 2013;13(1):7–13.
7. Krug KG, Mercy JA, Dahlberg LL, Zwi AB, Lozano R. World Report on Violence & Health; 2003. Available from: https://www.paho.org/en/documents/world-report-violence-and-health-paho-2003-1.
8. Facts and figures: Ending violence against women; 2020. Available from: https://www.unwomen.org/en/what-we-do/ending-violence-against-women/facts-and-figures.
9. National Crime Record Bureau; 2021. Available from: https://ncrb.gov.in/sites/default/files/CII-2021/CII_2021Volume%201.pdf.
10. Briody M. The effect of DNA evidence on sexual offence cases in court. *Curr Issues Crim Just*. 2018;14(2):159–81.
11. The Code of Criminal Procedure (Amendment) Act; 2005. Available from: https://www.mha.gov.in/sites/default/files/2022-09/TheCCP%28Amendment%29Act%2C2005%5B1%5D.pdf.
12. Hobbs CJ, Wynee JM. Sexual abuse of English boys and girls: The importance of anal examination. *Child Abuse Neglect*. 1989;13(2):195–210.
13. Watkeys JM, Price LD, Upton PM, Maddocks A. The timing of medical examination following an allegation of sexual abuse: is this an emergency? *Arch Dis Child*. 2008;93(10):851–6.
14. Farmen RKB, Haukeli I, Ruoff P, Frøyland ES. Assessing the presence of female DNA on post-coital penile swabs: relevance to the investigation of sexual assault. *J Forensic Leg Med*. 2012;19(7):386–9.
15. Sweet D, Lorente M, Lorente JA, Valenzuela A, Villanueva E. An improved method to recover saliva from human skin: the double swab technique. *J Forensic Sci*. 1997;42(2):320–2.
16. Gingras F, Paquete C, Bazineta M, Granger D, Marcoux-Legault K, Fiorillo M, et al. Biological and DNA evidence in 1000 sexual assault cases. *Forensic Sci Int Genetics Supple Series*. 2009;2(1):138–40.
17. Pal SK, Rana A, Sharma A, Sehgal A. Forensic study of child sexual abuse in northern range of Himachal Pradesh. *Forensic Gen Sci*. 2018;1(3):1–6.
18. Almadani O, Bamousa M, Alsaif D, Kharoshah M, Alsowayigh K. Child physical and sexual abuse in Dammam, Saudi Arabia: A descriptive case-series analysis study. *Eqypt J Forensic Sci*. 2012;2(1):33–7.
19. Suarez SS, Pacey AA. Sperm transport in the female reproductive tract. *Hum Reprod Update*. 2006;12(1):23–37.
20. Burg A, Kahn R, Welsh K. DNA testing of sexual assault evidence: The laboratory perspective. *J Forensic Nurs*. 2011;7(3):145–52.
21. Butler JM. Fundamentals of forensic DNA typing. San Diego: Elsevier Academic Press; 2010.
22. Arif M, Ahmed M, Hanif F. Natural sexual offences; Medicolegal assessment in Punjab. *Professional Med J*. 2014;21(5):980–6.
23. El-Din A, Elkholy SMS, Metwally ES, Farag HA. Pattern of Female Sexual Assault in Qalyubia Governorate, Egypt, During the Period From 2009 to 2013: A Retrospective Study. *Am J Forensic Med Pathol*. 2015;36(4):276–84.
24. Pal SK, Sharma A, Sehgal AK. A study of sexual assaults in northern range of Himachal Pradesh. *Int J Med Toxicol Forensic Med*. 2015;5(2):64–72.

25. Lavelle J. Forensic evidence collection, Child maltreatment. In: Giardino AP, Alexander R, editors. A clinical guide and reference. St. Louis, MO, USA: Medical Publishing; 2005. p. 856–60.
26. Kellogg K. The evaluation of sexual abuse in children. *Pediatrics*. 2005;116(2):506–12.
27. Schlagetter TG, Glynn CL. The effect of fabric type and laundering conditions on the detection on semen stains. *Int J Forensic Sci*. 2017;2(2):1–7.
28. Noëls LK, Raymond S. Repeatedly washed semen stains: optimal screening and sampling strategies for DNA analysis. *Forensic Sci Int Genet*. 2019;38:1–20.
29. Karadayi S, Moshfeghi E, Arasoglu T. Evaluating the persistence of laundered semen stains on fabric using a forensic light source system, prostate-specific antigen semiquant test and DNA recovery-profiling. *Med Sci Law*. 2020;60(2):122–13.
30. Magalhães T, Dinis-Oliveira RJ, Silva B, Corte-Real F, Vieira DN. Biological evidence management for DNA analysis in cases of sexual assault. *Sci World J*. 2015;doi:10.1155/2015/365674.
31. O'neal EN, Decker SH, Spohn C, Tellis K. Condom use during sexual assault. *J Forensic Legal Med*. 2013;20(6):605–9.
32. Davis KC, Schraufnagel TJ, George WH, Norris J. The use of alcohol and condoms during sexual assault. *Am J Mens Health*. 2008;2(3):281–90.
33. Cerdas L, Arroyo C, Gómeza A, Holst I, Angulo Y, Vargas M, et al. Epidemiology of rapes in Costa Rica: characterization of victims, perpetrators and circumstances surrounding forced intercourse. *Forensic Sci Int*. 2014;242:204–9.
34. Cerdas L, Herrera F, Arrieta G, Morelli C, Álvarez K, Gómez A. Menstrual cycle phase at the time of rape does not affect recovery of semen or amplification of STR profiles of a suspect in vaginal swabs. *Forensic Sci Int*. 2016;259:36–40.
35. Adefolalu AO. Fear of the perpetrator: a major reason why sexual assault victims delayed presenting at hospital. *Tropical Med Int Health*. 2014;19(3):342–7.
36. Abdellah NZ, Ali HF. Pattern of reported sexual assault cases in Aswan Governorate, a retrospective study (2010-2015). *Eqypt J Forensic Sci Appl Toxicol*. 2018;18(2):1–17.
37. Lowe A, Murray C, Whitaker J, Tully G, Gill P. The propensity of individuals to deposit DNA and secondary transfer of low level DNA from individuals to inert surfaces. *Forensic Sci Int*. 2002;129(1):25–34.
38. Sherif MK, El-Gohary MM, El-Kelany RS, El-Noor MA. Pattern of sexual assault in Gharbia Governorate during the period between 2011-2014: retrospective and cross section study. *Ain Shams J Forensic Med Clin Toxicol*. 2018;30:128–38.
39. Anzai-Kanto E, Hirata MH, Hirata RDC, Nunes FD, Melani RFH, Oliveira RN. DNA extraction from human saliva deposited on skin and its use in forensic identification procedures. *Braz Oral Res*. 2005;19(3):216–22.

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