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The Use of DNA Profiling in Identifying Murderers and Sexual Abusers: A Case Report

استخدام السمات الوراثية في تحديد هوية القتلة والمعتدى عليهم في جرائم الاغتصاب الجنسي: تقرير

حالة



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Abstract

A complaint was registered at a Police Station in the Faisalabad District of Pakistan. A boy aged 7-8 years went to a nearby shop and never came back. He was last seen with the accused by eyewitnesses. The naked dead body of the boy was found tied with his clothing in nearby fields. An autopsy revealed three incised wounds on the neck.

Evidence material from the dead body and crime scene were submitted for forensic analysis. The evidence material was screened for human biological fluids, semen, and blood. The items positive for semen or blood were subjected to DNA extraction. Short Tandem Repeat (STR) markers were amplified from extracted DNA after quantification. The amplicons were subjected to capillary electrophoresis to generate DNA profiles. Mixture DNA profiles were interpreted by EuroForMix, the probabilistic genotyping software.

Post-mortem examination indicated the possibility of sexual abuse of the boy prior to his killing. Seminal material was found on anal swabs collected from the dead body. The DNA profile obtained from anal swabs was consistent with a mixture of the DNA profiles of both the suspect and

Keywords: Forensic Sciences, Child sexual abuse, Short Tandem Repeat markers, Capillary Electrophoresis, Probabilistic Genotyping, anal swabs..

المستخلص

تم تسجيل شكوى في مركز للشرطة في منطقة فيصل آباد في باكستان بدعوى ذهاب صبي يبلغ من العمر 7-8 سنوات إلى متجر قريب ولم يعد أبداً، وشوهد آخر مرة مع المتهم بواسطة شهود عيان، وعثر على جثة الصبي عارية مقيدة بملابسه في الحقول المجاورة، وكشف تشريح الجثة عن وجود ثلاث جروح محفورة في الرقبة. كما تم تقديم أدلة وعينات من الجثة ومن مسرح الجريمة.

وفحصت العينات لتحديد السوائل البيولوجية البشرية والسائل المنوي والدم، وخضعت العينات الإيجابية للسائل المنوي والدم لفحوصات الحمض النووي الجنائي، وباستخدام تقنية تحليل التكرارات المترددة القصيرة (STR) للمواقع المستهدفة، واستخدام الرحلان الكهربائي الشعري تم تحديد السمات الوراثية، وفسرت السمات في العينات المختلطة بواسطة برنامج EuroForMix برنامج التنميط الجيني الاحتمالي.

وأشار تشريح الجثة إلى احتمال تعرض الصبي للاعتداء الجنسي قبل قتله، حيث عثر على مادة منوية على مسحات الشرج التي جمعت من الجثة. وكانت السمات الوراثية التي تم الحصول عليها من المسحات الشرجية متسقة مع مزيج من السمات الوراثية لكل من المشتبه به والضحية. كما تم العثور على السمات الوراثية للمشتبه به في مسحات

الكلمات المفتاحية: علوم الأدلة الجنائية، الاعتداء الجنسي على الأطفال، تحليل التكرارات المترددة القصيرة (STR)، الرحلان الكهربائي الشعري، التنميط الجيني الاحتمالي، المسحات الشرجية.

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the victim. The DNA profile of the suspect was also found in nail swabs taken from the victim. The DNA profile generated from blood stains present on the suspect's clothing matched the DNA profile of the victim.

The DNA of the suspect was present on anal and nail swabs collected from the victim. The victim's DNA was present in blood stains present on the suspect's clothing. The suspect was confirmed to be the perpetrator.

الأظافر المأخوذة من الضحية، كما تطابقت السمات الوراثية الناتجة عن بقع الدم الموجودة على ملابس المشتبه به مع السمات الوراثية للضحية. وبالنتيجة كان الحمض النووي للمشتبه به موجودًا على مسحات الشرح والأظافر التي تم جمعها من الضحية. وكان الحمض النووي للضحية موجودًا في بقع الدم الموجودة على ملابس المشتبه به، وتم التأكد من أن المشتبه به هو الجاني.

1. Introduction

Child sexual abuse (CSA) is an issue of considerable global gravity. A meta-analysis of 55 studies from 24 countries has reported a considerable heterogeneity in prevalence of CSA ranging from 3% to 17% [1]. Another study reported the prevalence to be even higher in India [2]. CSA accompanied by homicide constitutes a significant proportion of all homicides. Sexual aggression is a serious social and public health issue, and crimes of this nature require urgent forensic medical examination. Homicide sex offenders inflict serious physical injuries to the victim which prove fatal. The victim of a sexual assault is murdered to eliminate the witness of the offense or to stop the victim resisting a sexual act [3]. These perpetrators include young adults and mentally ill people with strong antisocial personality disorders [4]. Some authors have categorized them as murderers rather than sex offenders [5].

The forensic application of modern sciences is tremendously helpful in the proceedings of a criminal trial. In most cases, biological evidence is the only evidence that can prove sexual contact and identify the perpetrator, and it is the most important evidence for legal proof in courts of law. Contrary to other offenses, where major effort is invested in investigating the crime scene, in sexual assault cases the victim is the crime scene [24].

DNA based forensic evidence is stable, and DNA can be isolated from the smallest remains in minute quantities years after a crime has been committed.

Furthermore, forensic DNA analysis is very specific and can identify the donor of the evidence with a high degree of certainty [6].

Presented here is a case study of DNA profiling used to identify the culprit of the rape and brutal murder of a 7-8-year-old boy. Although the victim was seen with the suspect on the day of the incident, there were no eyewitnesses to this heinous crime. Biological evidence and a DNA report proved very conclusive in finalizing the investigation of this case.

2. The Case

An FIR was registered on October 2, 2019 at Tarkhani Police Station (Faisalabad District) under Section 7 of the Anti-Terrorism Act, 1997 Pakistan Penal Code. A 7-8-year-old boy went to a nearby grocery store and did not come back.

A police investigation revealed that the boy was last seen on a motorbike with the suspect. After approximately 8-9 hours, the naked dead body of the boy was discovered in nearby sugarcane fields with his arms and legs tied with his own clothing. A post-mortem examination of the dead body was conducted after approximately 12 hours. A medico-legal officer estimated a probable lapse of 6-12 hours between death and medico-legal examination. Upon autopsy, the following injuries were documented by the doctor:

1. A muscle-deep incised wound in the front of the neck measuring 10 cm x 3 cm on the lower side



- of the chin about 6 cm below the centre of the chin.
2. An incised wound measuring 15 cm x 2 cm cutting overlying muscles, trachea, and larynx. This cut is present about 8 cm below the level of the chin and approximately 2 cm below injury No. 1, extending from the angle of the right mandible to the level of the left ear lobe.
 3. An incised wound measuring 7 cm x 3 cm on the left side of the front of the neck cutting the jugular veins.
 4. A deep wound measuring 4 cm x 2 cm on the front of the abdomen about 2 cm away from the umbilicus on the left side, resulting in protrusion of the gut.
 5. The anal orifice was dilated, the anal sphincter was lax, the anal canal was congested, and a tear was observed at the 6 O'clock position of the anal opening.

The police arrested the accused shortly after the recovery of the dead body. The weapon of offense, a sickle, was recovered from the nearby fields after the suspect revealed its location to the police.

3. Materials and Methods

The evidence items from the dead body of the victim and crime scene pertaining to the investigation of sexual assault and murder were collected. The medico-legal officer conducting the post-mortem collected anal swabs, and forensic experts from the Crime Scene Unit collected oral swabs (as standard reference samples), nail swabs, and clothing from victim. The clothing worn by the suspect at the time of the crime was recovered by the police. Evidence items were submitted to the Punjab Forensic Science Agency (PFSA) for forensic DNA analysis. The evidence items were received at the PFSA through a proper chain of custody. The evidence items were first screened for the presence

of human biological fluids. Anal swabs and clothing from the victim were screened for seminal material. The clothing of the suspect was screened for the presence of human blood. Clothing was inspected visually, with the help of an alternative light source, for the presence of suspected semen and blood stains [7]. The evidence items showing positive presumptive testing were tested through a specific confirmatory test. The anal swabs positive for seminal material were subjected to differential DNA extraction, to separate epithelial and sperm fractions. This was done in order to avoid preferential DNA amplification of the victim's DNA, due to its abundance [8]. DNA extraction by the organic method was used for blood stains, buccal swabs from the suspect and victim, and nail swabs from the victim [9]. The DNA was purified using the amicon filtration technique [10]. The DNA extracts were quantified through real time PCR using a commercial kit, Quantifiler™ Duo (Thermo Fisher Scientific, USA). The quantified extracts were amplified using 1 ng of DNA. Fifteen Short Tandem Repeat (STR) loci (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, FGA) and a non STR locus, Amelogenin, were amplified using the AmpFISTR® Identifier® Plus kit [11]. The amplicons were genotyped by capillary electrophoresis, using the ABI Prism 3500 Genetic Analyzer (Applied Biosystems, USA) (12). The allele size was determined by Gene Mapper ID-X Software V. 1.4. Using the allelic ladder, the alleles in known and questioned samples were determined to generate DNA profiles.

EuroForMix probabilistic genotyping software was used for mixture DNA analysis and interpretation.

The quality check of each stage of DNA analysis (extraction, quantification, amplification, and genotyping) was ensured by the use of reagent blanks,



negative controls, and known positive controls.

4. Results

A presumptive test indicated the presence of seminal material on anal swabs. A trace number of spermatozoa were observed on smears after KPICI staining.

Human blood stains were identified on the trousers and shirt of the accused suspect. Human blood was also identified on the blade of the sickle, the weapon of murder.

The concentration of human DNA in sperm and epithelial fractions from anal swabs was 0.34 ng and 104 ng, respectively. The DNA concentration

found in blood stains recovered from the suspect's clothing was 9.6 ng; whereas, 0.22 ng of DNA was recovered from nail swabs collected from the victim.

The DNA STR profiles of the victim and suspect are shown in Figure-1. The DNA profile obtained from the sperm fraction from anal swabs was consistent with the mixture of the DNA profiles of the suspect and the victim. The contribution of the victim and the suspect to this DNA mixture was 0.56 and 0.44, respectively.

The DNA profile obtained from nail swabs from the victim was a mixture of DNA from two people. This DNA profile was consistent with the mixture of the DNA profiles of both the suspect and the vic-

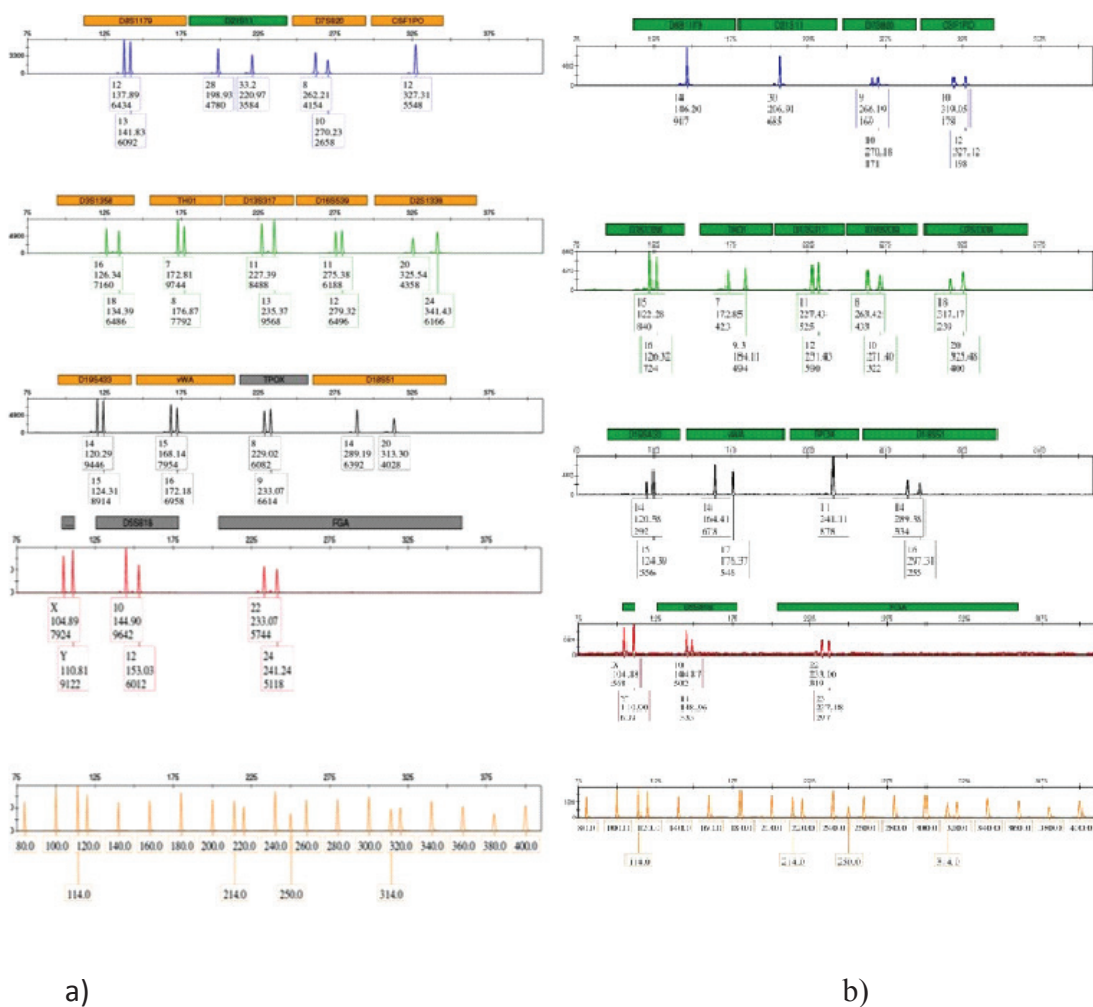


Figure 1 - Electropherogram of the DNA profile of the victim (a) and Electropherogram of DNA profile of the suspect (b).



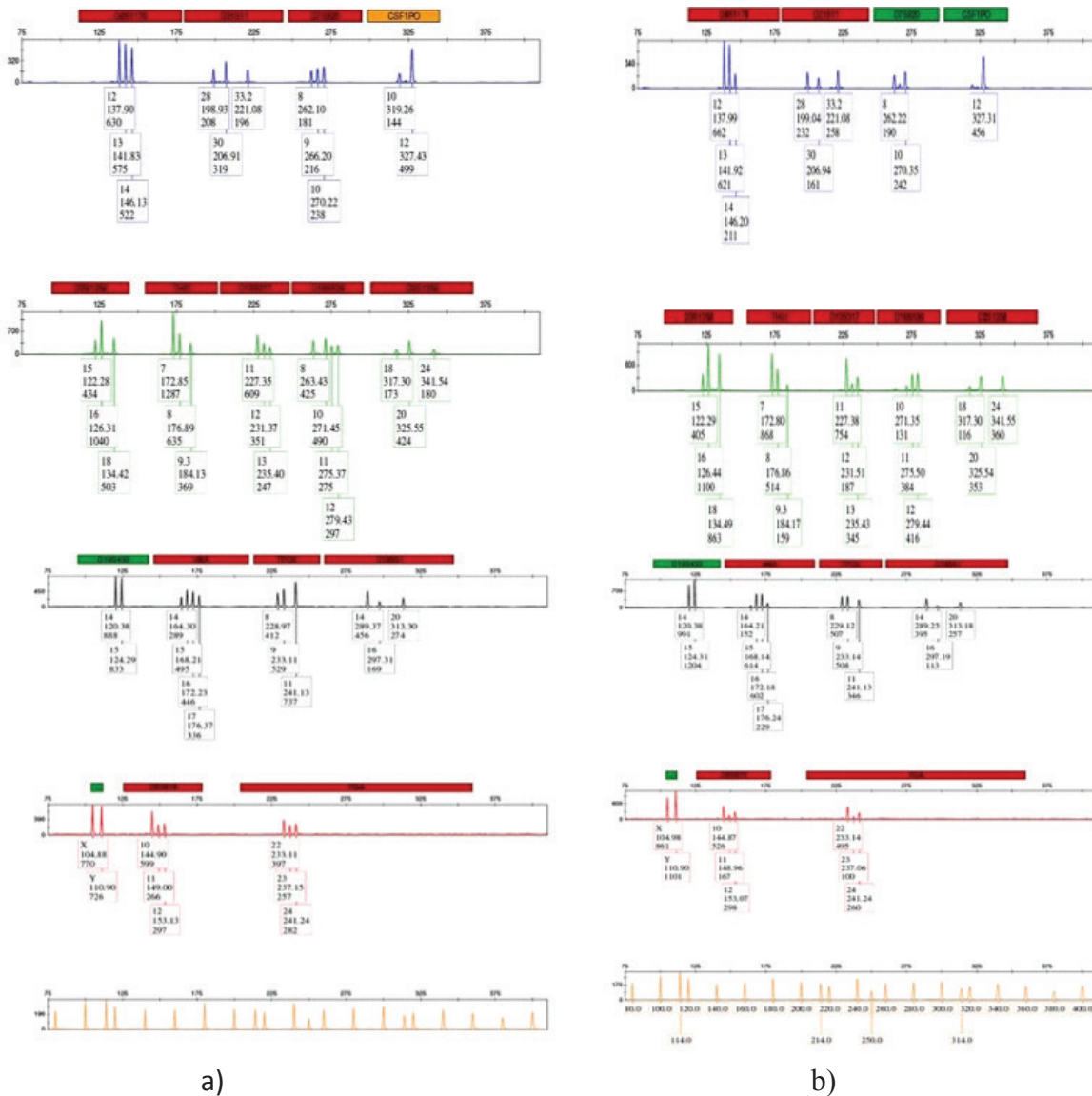


Figure 2 - Electropherogram of the DNA profile obtained from sperm fraction of anal swabs (a) and Electropherogram of the DNA profile obtained from the nail swabs (b).

tim. The mixture proportions of the victim's and suspect's DNA were 0.74 and 0.26, respectively. The DNA profiles obtained from anal swabs and nail swabs are shown in Figure-2.

The DNA profile obtained from the epithelial fraction of anal swabs was of a single source and matched the DNA profile of the victim. The DNA profile obtained from blood stains present on the suspect's clothing was also of a single male origin and

matched the DNA profile of the victim.

Positive and negative controls were also successfully genotyped as expected. No profile was observed in negative control, and a known single source profile was obtained from positive control (Figure-3).

The complete DNA profiles obtained from the suspect, victim, sperm fraction of anal swabs and nail swabs positive and negative controls are given in Table-1.



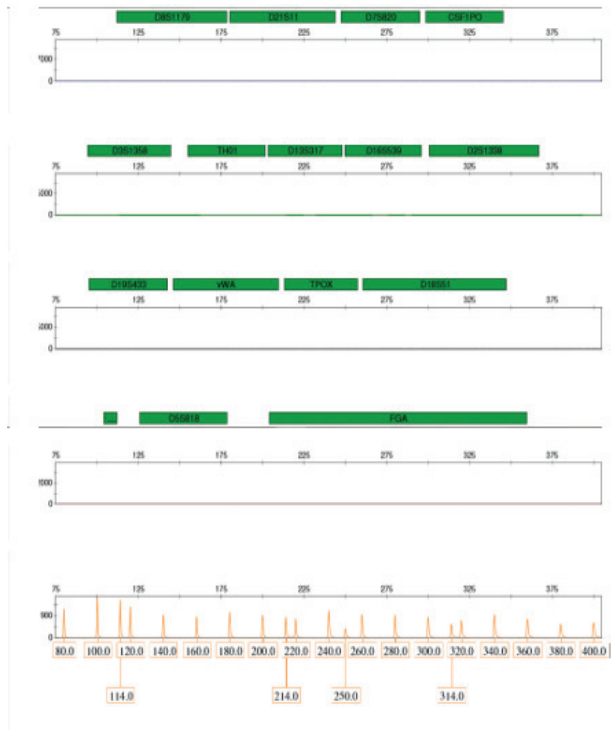


Figure 3a - Blank profile of negative control.

5. Discussion

The first stage in DNA fingerprinting is the identification of biological fluid on collected evidence material. In most crime laboratories, serological tests are used to screen evidence material for the presence of biological fluid of human origin. The key issue in serological analysis is the human specificity and sensitivity [25].

The detectable amount of acid phosphatase (AP) was preliminary attributed to the presence of seminal material [20]. The AP positive items were tested for prostate specific antigen (PSA) [21] using a rapid immunochromatographic device. The presence of seminal material was confirmed through kernechtrot picro indigo carmine staining of the smears [22]. The presence of semen on a prepubertal child’s body or clothes potentially indicates sexual contact and is mostly accepted as proof in court. DNA profiling revealed the presence of seminal material from the accused on anal swabs col-

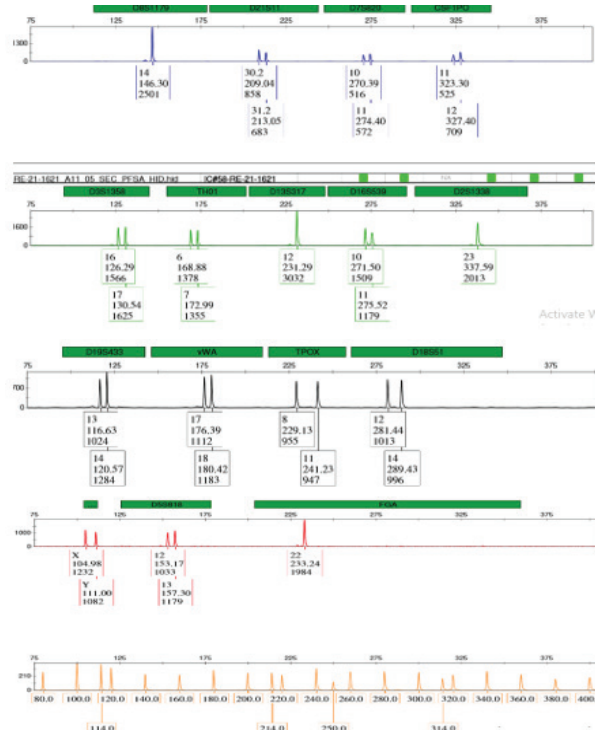


Figure 3b - DNA profile of known positive control.

lected from the deceased victim. The anal swabs are intimate samples and the most probative evidence item in the investigation of a case of sexual assault. The presence of seminal material on anal swabs is a direct indication that the child had been raped before being murdered.

Chemical testing, the reaction with Kastle-Meyer (KM) reagent, was used as a presumptive blood test. A positive KM test indicated the presence of blood [23]. Human blood was confirmed through the use of a lateral flow immunochromatographic device, Seratic Hemedirect.

There is a direct transfer of blood between victim and suspect, if the injuries are inflicted by weapons other than firearms. The transfer of blood between suspect and victim is also proportionate to the level of physical struggle during the incident. The chances and amount of transfer further increase if blunt weapons are used [26]. The presence of the victim’s blood on the suspect’s clothing was a direct



Table 1 - FINAL Short Tandem Repeat (STR) DATA (Identifiler Plus).

Item	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	VWA	TPOX	D18S51	Amelo- genin	D5S818	FGA
SF (anal swabs)	12,13,14	28,30,33.2	8,9,10	10,12	15,16,18	7,8,9,3	11,12,13	8,10,11,12	18,20,24	14,15	14,15,16,17	8,9,11	14,16,20	X,Y	10,11,12	22,23,24
NS	12,13,14	28,30,33.2	8,9,10	10,12	15,16,18	7,8,9,3	11,12,13	8,10,11,12	18,20,24	14,15	14,15,16,17	8,9,11	14,16,20	X,Y	10,11,12	22,23 24,
V	12,13	28,33.2	8,10	12,12	16,18	7,8	11,13	11,12	20,24	14,15	15,16	8,9	14,20	X,Y	10,12	22,24
S	14,14	30,30	9,10	10,12	15,16	7,9,3	11,12	8,10	18,20	14,15	14,17	11,11	14,16	X,Y	10,11	22,23
Positive	14,14	30,2,31.2	10,11	11,12	16,17	6,7	12,12	10,11	23,23	13,14	17,18	8,11	12,14	X,Y	12,13	22,22
Negative	-,-	-,-	-,-	-,-	-,-	-,-	-,-	-,-	-,-	-,-	-,-	-,-	-,-	-,-	-,-	-,-

Note: SF: Sperm fraction; NS: Nail swabs from the victim; V: Victim; S: Suspect; Positive: Known positive control; Negative: Known Negative control



evidence that the accused person molested and murdered the child.

Evidence material indicative of physical contact can also accumulate in the fingernail hyponychium, which is an isolated area. The detection of the suspect's DNA from nail swabs taken from the victim indicated physical contact between them. This was also indicative of an intense struggle and resistance by the child during this brutal act. According to the post-mortem report, all the injuries were ante-mortem in nature; the accused sodomized and killed the victim through fatal injuries. Through forensic DNA profiling, it was proved that the person accused in this case was guilty of the crime.

Children are the most vulnerable group that fall prey to sexual abuse. Sexual homicide of children is influenced by the age of the victim [13]. The chances of becoming a victim to homicide outside the home increase as the child grows up [14]. The age group of the children when they start going out of their homes but are not mature enough are most suitable prey for sex offenders. Child sexual abusers may lack the necessary social skills or status to maintain lawful sexual relations and may therefore look for an alternative in the form of children [15]. The psychology, sexuality, history of self-victimization, disability, and family circumstances of the offender are key risk factors of CSA [16]. In another study, deviant sexual behaviour, convictions for non-sexual crimes, and social isolation were higher among sexual aggressors against children than sexual aggressors against women [17]. The inspection of a crime scene in such cases can be indicative of the personality problems associated with the perpetrator [18].

Throughout the world, laws relating to violence against children have been adapted [19]. To address the heinous crime of CSA effectively through more stringent legal provisions, the Pakistani Par-

liament passed a bill in 2020; however, there is a pressing need to ensure that children are educated properly about this issue by parents or through the school system and media.

6. Conclusion

The biochemical and genetic analysis together with the evidence collected from the crime scene confirmed that the suspect committed the rape and murder of the minor.

Conflict of interest

The authors declare there is no conflict of interest.

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