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Identification of Accused from Gutkha Spitting- Success of DNA Profiling

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Abstract

Gutkha is specifically manufactured in India which is prepared of crushed areca nut, tobacco, catechu, paraffin wax, slaked lime, and sweet flavourings. It is consumed by placing a pinch of it between the gum and cheek and gently sucking and chewing, similar to chewing tobacco. Though many states of India have banned gutkha, some people are using it illegally. The spitting of betel leaf juice or gutkha collected from the scene of crime plays important role in forensic DNA identification. Isolation of cell material from biological traces on forensic evidence is often a serious challenge to solving forensic cases. Successful extraction of DNA from such evidence having trace quantity of cellular portion even in critically low quantity could be achieved by proper observation and collection of evidence. We have analysed two different cases in our laboratory at Nashik, one of murder and another of burglary, in which traces of gutkha were used to solve the crime. In both cases, DNA was successfully isolated from gutkha spitting, and obtained DNA profile was tallied with DNA profile of reference blood of accused. This is one of the important pieces of evidence to prove the presence of the accused at the crime scene. It is supportive evidence for the judiciary while deciding to convict the accused along with other shreds of evidence.

Keywords: DNA; Polymerase Chain Reaction; Denaturation; Allele; Genotyping; Gutkha

Abbreviations: STR: Short Tandem Repeats; FSS: Forensic Science Services; PCR: Polymerase Chain Reaction.

Introduction

In the field of forensic, the recovery of traces of body fluids is one of the important pieces of evidence to connect the crime. There are different identification methods to identify the source. Blood grouping, enzyme grouping, DNA profiling, etc is common scientific methods for identification. In some medico-legal cases like murder, child abuse, rape, and robbery, blood may be absent at the scene, but saliva in some form may be procured. Besides blood, the blood group antigens are also secreted in various other body secretions, such as saliva, semen, gastric juice, nasal secretions, vaginal secretions, sweat, tears, urine, etc., from which blood groups can be determined [1]. This greatly helps in determining the blood group of a victim or a suspect. In such cases, saliva may be obtained in dry forms from crime scene articles like cigarette ends [2], bidi butts, tile, floor, wall, grass, glasses, cups, envelope flaps [3-7], bite marks, dental appliances, etc [8].

However, the presence of blood group substances in various body secretions depends on whether the individual is a secretor or a non-secretor, which can be determined

by the presence of Lewis antigen [9]. Stains of dried saliva are invisible, making its recognition and collection difficult. However, the presence of saliva can be confirmed by an amylase assay [10]. If the sample is in trace form, the best way to go is for DNA profiling which gives the exact identity of the perpetrator.

DNA profiling came into use around three decades ago, in the late 1980s. It was first developed in England in 1985 by Sir Alec Jeffreys [11,12]. It has been a useful tool in law enforcement as it works both ways, securing correct convictions and also exonerating the innocent. Furthermore, DNA profiling unlike other, forensic evidence can be collected easily and sustains for a long thereby increasing chances of accurate analysis by manifold. It has become an important method for human identification by introducing the study of microsatellite regions – Short Tandem Repeats (STR) Loci in criminal and civil cases [13-15]. The STR fragments are separated and detected by using capillary electrophoresis. Certain regions of DNA contained repeated DNA sequences. The regions with repeat units that are 2-6 base pairs in length are called Short Tandem Repeats. The first STR multiplexes developed was quadruply created by Forensic Science Services (FSS) that comprises four STR Loci [16]. Short tandem repeat (STR) analysis is regularly performed for generating DNA profiles from blood, saliva, and hair samples encountered as evidence to solve the crime [17,18].

Our forensic laboratory had received two different cases. One of murder and another of burglary. In the case, 'I' which was of murder, the accused brutally killed his cousin's father in law for the property issue. He tied one leg of the deceased to the banana plant with cloth and with the help of sickle he cut his neck separating the head from the body. Police seized the clothes of the accused and deceased. Our forensic team visited the crime scene and found gutkha spitting on nearby banana leaves. They collected those pieces of leaves for saliva detection and to ascertain the presence of the accused at that crime scene. Bloodstains on clothes were analyzed in biology division with blood grouping. But as the gutkha spitting stains were in trace quantity and it was difficult to obtain a blood group of saliva mixed with gutkha, we analyzed it using the DNA profiling technique to find out whether the accused is the source of it. We had received reference blood of the accused in a DNA kit provided by our laboratory for comparison. The DNA profile from gutkha and reference blood of the accused matched.

In the case 'II' which was of burglary, two suspects broken lock of the door, grill gate and three cupboards in the house and stolen gold and silver ornaments worth about 3 lacks. During the visit to the crime scene, our forensic team collected gutkha spitting on the bedsheet and wall of the bedroom where the cupboards were present. A DNA profile was obtained from gutkha spitting on a bedsheet matched with a DNA profile of reference blood of one of the accused.

In both cases, traces of saliva present in gutkha with the help of DNA profiling concluded the presence of the accused at the crime scene.

Materials and Methods

Instruments and chemicals used for DNA analysis were as follows:

- PrepFiler Express DNA extraction kit. Lot No. 1807201.
- AmpFlSTR® Identifiler kit. Lot No. 1807261
- HiDi Formamide.
- Liz 600 Size standard.
- Quantifiler Duo DNA kit. Lot No. 1710101.
- AutoMate ExpressTM Forensic DNA Extraction System. Catalog number: 4441763
- PCR thermal cycler GeneAmp 9700. Catalog number: 4375786
- 3500 Genetic Analyzer. Catalog number: 4406017

Traditional workflow for generating DNA profiles includes the below steps:

1. Extraction of DNA from body samples found on different substrates.
2. Quantification of extracted DNA
3. Amplification of DNA using polymerase chain reaction (PCR) based STR reactions
4. Denaturation of amplified DNA
5. Genotyping using short tandem repeat (STR) technique
6. Analysis and comparison of generated DNA profiles.
7. Nowadays, advanced instruments such as AutoMate Express and EZ1 Advanced which work on robotic principles are helpful to minimize the analysis time. Commercially available kits for amplification of DNA allow the faster turn-around time [19,20].

Isolation of DNA

As the spitting sample on banana leaves in case I and on the bedsheet and wall in case II was in trace quantity, testing for saliva using traditional biological methods had been avoided. It had the possibility of a loss of sample in testing. Spitting sample from leaf, bedsheet, and the wall was carefully collected on a small portion of cotton swab soaked in PBS buffer. It was taken in a 2 ml centrifuge tube for DNA extraction. For extraction of DNA, the PrepFiler™ Forensic DNA extraction Kit (Applied Biosystems) was used. It enables the isolation of DNA from biological samples that contain small quantities of biological material in such a way that it removes the substances interfering with PCR. Additionally, the extracted DNA is having a sufficiently high concentration due to which the volume of extract for downstream analysis

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is minimal [21].

For the reference profile, a 40 µl blood sample of suspects in both cases was taken into another microcentrifuge tubes. 500 µl Lysis buffer from PrepFiler Express F DNA extraction kit [22] was added to all the sample tubes. The sample tubes were kept on a thermoshaker at 750 rpm at 70˚C for 40 min. The tubes were then centrifuged at 10,000 rpm for 2 min. Cartridges from the PrepFiler Express F DNA extraction kit were loaded to the cartridge rack in AutoMate Express DNA extraction system [23], Sample tubes, elution tubes, and tips were loaded as per machine guidelines and the machine program was run as per the recommended machine protocol. After completion of the program, elution tubes containing extracted DNA in highly pure form were stored at 4˚C till the next PCR amplification process.

Quantification of the Extracted DNA

Extracted DNA was quantified using Quantifiler® Duo DNA Quantification Kit [24] on an Applied Biosystems 7500 Real-Time PCR System according to manufacturer-recommended protocols. Quantified DNA was taken for downstream application.

PCR based STR Analysis

The quantified DNA extracted from the gutkha spitting samples from the crime scene and reference blood samples of suspects in both the cases was processed for Polymerase Chain Reaction using the AmpFlSTR® Identifiler PCR Amplification Kit (Applied Biosystems) [25] with the help of PCR thermal cycler GeneAmp 9700 [26] following the protocols recommended by the manufacturer. This kit contains a Reaction mixture, Primer set, and Taq Gold Polymerase enzyme. Primer Set contains locus-specific 6-FAMTM, VICTM, NEDTM, and PETTM dye-labeled and unlabeled primers in the buffer. The primers amplify the STR loci CSF1PO, D2S1338, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D19S433, D21S11, FGA, TH01, TPOX, vWA, and gender marker Amelogenin.

The reaction mixture used for PCR was prepared by adding Reaction mix 10.5 µl, Primer set 5.5 µl, and Taq Gold DNA Polymerase 0.55 µl. The extracted DNA sample of 10 µl was added to it. The DNA was amplified in 28 cycles using a PCR machine selecting 94.0 ºC, 59.0 ºC, and 72.0 ºC as temperatures of denaturing, annealing, and extension respectively (Table 1) (Figure 1).

Step	AmpliTaq Gold Enzyme Activation	PCR			PCR Final Step	PCR product till separation of STRs
	Hold	CYCLE (28 cycles)			Hold	
		Denaturation	Anneal	Extend		
Temp	95˚C	94˚C	59˚C	72˚C	60˚C	4˚C
Time	11 min	1 min	1 min	1 min	60 min	∞

Table 1: PCR protocol.

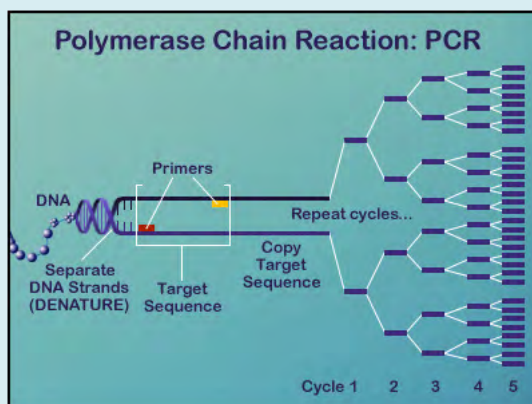

Figure 1: The amplified DNA samples were kept at 60.0 ºC for an hour and then at 4.0 ºC till the separation of STRs. PCR produces millions of DNA fragments of different sizes. Amplified products were separated and detected using 3500 Genetic Analyzer [27] and analyzed using GeneMapper® ID-X Software V 1.5. The separation of different fragments of DNA molecules based on their sizes was achieved by capillary electrophoresis. Simultaneous amplification of 16 STR Loci was completed and analyzed [28,29]. DNA profiles obtained were interpreted by comparing them with each other.

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Results and Discussion

The extracted DNA from all the samples were typed at 15 STR Loci and gender-specific Amelogenin locus using the PCR amplification technique. Identifiler^{TM} PCR kit of applied biosystems was used for DNA profiling. The following table shows 15 STR loci and one amelogenin locus which shows the gender of a person. Alleles obtained at every locus of crime scene sample are compared with alleles obtained from a reference blood sample of suspects (Case I-Table 2) and (Case II-Table 3).

STR Locus	GENOTYPE	
	Pieces of banana leaf with reddish color stains of gutkha spitting (Crime scene)	Blood (Suspected accused)
D8S1179	13,14	13,14
D21S11	30,32.2	30,32.2
D7S820	8,11	8,11
CSF1PO	12,13	12,13
D3S1358	16,17	16,17
THO1	8,9	8,9
D13S317	12,12	12,12
D16S539	9,9	9,9
D2S1338	19,24	19,24
D19S433	14,16.2	14,16.2
vWA	15,16	15,16
TPOX	8,10	8,10
D18S51	12,16	12,16
AMELOGENIN	X,Y	X,Y
D5S818	10,12	10,12
FGA	22,26	22,26

Table 2: DNA profiles obtained from saliva in gutkha spitting on a banana leaf (Case I) and reference blood of suspected accused.

STR Locus	GENOTYPE		
	Gutkha stains from Bedsheet (Crime scene)	Blood (Suspected accused I)	Blood (Suspected accused II)
D8S1179	13,15	13,15	14,14
D21S11	31.2,32.2	31.2,32.2	29,32.2
D7S820	8,9	8,9	10,12
CSF1PO	11,12	11,12	10,11
D3S1358	15,15	15,15	14,16
THO1	6,8	6,8	6,6
D13S317	11,11	11,11	12,12
D16S539	11,12	11,12	9,11
D2S1338	19,24	19,24	23,24
D19S433	15,15.2	15,15.2	13,14.2
vWA	17,18	17,18	16,18
TPOX	8,11	8,11	8,10
D18S51	14,14	14,14	16,26
AMELOGENIN	X,Y	X,Y	X,Y
D5S818	11,13	11,13	12,12
FGA	20,23	20,23	20,21.2

Table 3: DNA profiles obtained from saliva in gutkha spitting on Bedsheet (Case II) and reference blood of two suspects.

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In case I, the DNA profile obtained from saliva in gutkha spitting on banana leaf at crime scene found to be of male origin having XY alleles at Amelogenin locus matched at every locus with DNA profile obtained from a reference blood sample of male suspected accused.

In case II, the DNA profile obtained from saliva in gutkha spitting on a bedsheet at the crime scene was found to be of male origin having XY alleles at Amelogenin locus matched at every locus with DNA profile obtained from a reference blood sample of male suspected accused I.

Conclusion

Mostly the reasons behind the murder are property issues, money matters, or illegal relationships. In the case, I, accused brutally murdered his cousin father in law for their property matter. He separated the head of the deceased from the body with sickle and body found to be tied with a banana plant with a cloth. Such a heinous crime should get proved in a court of law to punish the accused strongly. To control such crimes, it is necessary to provide strong evidence in the court to increase the conviction rate. In case II, both the accused had a habit of stealing as they had thus looted a lot of places. It was necessary to provide evidence in court to prove them accused. It is the skill of the analyst to get the DNA profile from the available biological samples without wasting it. Further, if the sample is received to the laboratory in proper condition, it becomes somewhat easy to perform the analysis. This is one of the most helpful techniques to solve complicated heinous crimes. As there were no eyewitnesses, DNA profiling from gutkha spitting proved the presence of the accused at the crime scene. This is one of the important pieces of supportive evidence to strengthen the conviction of the accused in court.

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