Relevance of sampling and DNA extraction techniques for the analysis of salivary evidence from bite marks: a case report

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ABSTRACT. Bite mark evidence has been repeatedly found in criminal cases. Physical comparison of a bite mark to the teeth of available suspects may not always be possible. Experimental studies have shown that the analysis of DNA present in the saliva recovered from bite marks might help in the identification of individuals. However, the application of this approach to an actual criminal case has been reported only once before in forensic literature. Therefore, there is very limited scientific and technical information available on this subject. The current study focuses on a woman found dead in her home; the autopsy ruled the death to be a result of manual strangulation. A bite mark was found on each breast. The single swab technique was used to collect evidence from these bite marks, and an organic extraction method was employed for DNA isolation. Short tandem repeat (STR) sequence typing was performed using a commercially available kit, and

the result was compared to the STR profile of a suspect. A full single-source STR profile was obtained from both bite marks, which matched the STR profile of the suspect. To the best of our knowledge, this is the second report on the analysis of DNA isolated from bite marks on the victim used to identify the crime perpetrator. Our results indicated that, contrary to most theoretical indications, a single swab technique for evidence collection and an organic method for DNA isolation could be very useful in solving this class of criminal cases.

Key words: Bite mark; DNA typing; Saliva

INTRODUCTION

In violent crimes, offenders are known to bite their victims as an expression of dominance, rage, or animalistic behavior. The resulting physical evidence can be compared with the teeth of any suspects in order to determine the origin of the marks. The use of bite mark evidence in criminal or abuse cases has been accepted by law enforcement agencies and courts since many years (Sweet and Pretty, 2001; Bruce-Chwatt, 2010).

However, most forensic dentists have not agreed on a standard explanation regarding the individuality (uniqueness) of the dentition, or the behavior of human skin upon being bitten. Furthermore, distortions may modify, complicate, or render impossible the interpretation of a bite mark (Bowers, 2006; Pretty, 2006, 2008). The use of bite marks as forensic evidence has an average false-positive error of 64% (Saks and Koehler, 2005). These difficulties have resulted in the use of salivary DNA evidence, deposited during the biting action, for the definitive identification of the perpetrator of the bite.

Studies conducted under controlled laboratory conditions have indicated that the DNA analysis of saliva placed on the skin of volunteers or cadavers may help in the identification of individuals (Sweet et al., 1997a; Anzai-Kanto et al., 2005; Kenna et al., 2011). However, the application of this approach to an actual criminal case has been reported only once before in forensic literature. Sweet and Shutler (1999) obtained a partial and mixed DNA profile from a bite mark on a body submerged in water. This information was mainly used to screen suspects and contributed, along with other physical evidence, to the resolution of that case.

Here, we have reported a homicide case, wherein a full single-source DNA profile of the perpetrator was obtained from bite marks on the victim, and have discussed the techniques employed, which could be useful in the solving of similar criminal cases in the future.

MATERIAL AND METHODS

Initial observations

A middle-aged woman was found dead in her home, lying partially clothed in the supine position on a bed. The arms and legs were in abducted position. No evidence of a fight was observed at the scene. Therefore, consensual sexual activity was surmised between the victim and the perpetrator.

The body was autopsied at the office of the coroner; the autopsy revealed that the victim died from manual strangulation. On the basis of postmortem changes, the postmortem

interval was estimated to be approximately twelve hours. Finger marks were observed on the neck, and a bite mark was observed on each breast (Figure 1).

Figure 1. Bite mark injuries on both breasts of the victim. Evidence was collected from the bite marks by the single swab technique, and DNA was isolated from the samples obtained using an organic extraction method. A full single-source STR profile was obtained, which matched the profile of the suspect.

Sample collection

The bite mark on each breast was swabbed by gently rolling a swab moistened with sterile distilled water on the bite surface. Cervico-vaginal samples were obtained from the body using sterile cotton swabs. All swabs were allowed to air dry at room temperature for 30 min, and were subsequently enclosed in cardboard evidence boxes. Finally, a blood sample was obtained from the victim.

During the course of the investigation, the police identified a man who was the last person to be seen with the victim; this man confessed to being a sexual partner of the victim for some time. Buccal swab samples were obtained from this suspect.

DNA extraction and amplification

DNA was extracted from the cervico-vaginal samples using a differential extraction procedure, as detailed by Yoshida et al. (1995). DNA was isolated from all other samples using the standard phenol-chloroform extraction and ethanol precipitation (Sambrook and Russel, 2001) methods.

The DNA extracts obtained from the samples were subjected to polymerase chain reaction (PCR) using the commercially available multiplex kit AmpFLSTR Identifiler (Applied Biosystems, Foster City, CA, USA), according to the manufacturer protocols. This kit simultaneously amplified 15 short tandem repeat (STR) loci, in addition to the Amelogenin gender-determining marker.

Capillary electrophoresis

The samples were subjected to capillary electrophoresis using an ABI PRISM 310 genetic analyzer (Applied Biosystems). The samples were run on a capillary containing the POP-4 polymer; the Genescan-LIZ 500 standard was used to size alleles, and the data was analyzed using the GeneMapper ID analysis software v.3.2 (Applied Biosystems). STR allele frequency population data (Cerda-Flores et al., 2002; Rubi-Castellanos et al., 2009) and the DNA-View software (Brenner, 2012) were used to calculate the frequency of the genotypes in our population.

RESULTS

The genetic profiles generated from the samples are summarized in Table 1. A full single-source DNA profile was obtained from each bite mark, which matched the profile of the suspect. Calculation of the genotype frequency in our population provided a value of 6.17×10^{-20}.

Analysis of the cervico-vaginal swabs revealed that sperm cell (male) fractions resulted in 8 loci, which matched the corresponding loci obtained from the suspect. The calculated genotype frequency in our population was 2.96×10^{-9}. Epithelial cell (female) fractions rendered a genetic profile identical to that of the victim.

Table 1. Short tandem repeat analysis of the samples.

Loci	Victim	Suspect	Bite marks swabs	Vaginal swabs[a]
Amelogenin	XX	XY	XY	XY
D8S1179	12/14	10/14	10/14	10/14
D21S11	29/32.2	32.2/33.2	32.2/33.2	32.2/33.2
D7S820	11/11	9/10	9/10	-[b]
CSF1PO	12/12	7/11	7/11	-
D3S1358	14/14	15/16	15/16	-
TH01	6/9.3	7/9.3	7/9.3	7/9.3
D13S317	8/9	11/13	11/13	-
D16S539	10/11	11/11	11/11	11/11
D2S1338	17/19	17/23	17/23	17/23
D19S433	13/14	13/13.2	13/13.2	-
vWA	16/16	16/16	16/16	16/16
TPOX	8/11	8/8	8/8	8/8
D18S51	14/16	12/13	12/13	12/13
D5S818	9/11	11/12	11/12	-
FGA	23/26	21/21	21/21	-

[a]For simplicity, only the results corresponding to the sperm cell (male) fraction are shown. [b]No result was obtained.

DISCUSSION

Bite mark injuries have been observed in some of the most serious crimes, and may often be the only physical evidence available. Saliva has been shown to be recovered, and

the DNA subsequently typed from bite marks. However, to the best of our knowledge, the application of this approach to an actual criminal case has been reported only once before in forensic literature (Sweet and Shutler, 1999). Therefore, there is limited scientific and technical information available on this subject. In this paper, we have reported the identification of the perpetrator of a homicide through the analysis of DNA isolated from bite marks found on the victim, and have discussed the techniques employed.

Remarkably, a complete STR profile of the perpetrator was obtained. The amount of saliva deposited on the skin is usually very small in bite marks; in addition, environmental factors, such as heat, bacteria, moisture, and mold, can render the DNA unusable (Wick, 2000). Partial profiles were obtained even under controlled experimental conditions, wherein the saliva was placed on the skin of volunteers (Anzai-Kanto et al., 2005).

However, research has revealed that varying qualities and quantities of DNA are extracted from different individuals from the same site, even under similar conditions (Muruganandhan and Sivakumar, 2011). Therefore, the DNA recovered from the evidence may have (by chance) been of sufficient quality and quantity to facilitate STR typing in this case. Furthermore, the time elapsed between the crime and sample collection was approximately 12 h; Kenna et al. (2011) have reported that salivary DNA persists on the skin for a minimum of 96 h.

On the other hand, the good results obtained in this study might have been a consequence of the sampling and DNA extraction techniques employed. Although several techniques, including the use of surgical gauze pads and cigarette papers, have been employed to recover saliva and other trace evidence from the skin with variable success, the use of sterile swabs is the most widely used technique that has received international endorsement. In fact, the double-swab technique is the recommended method for the recovery of dried stains or possible cellular material from the skin (Rogers and Newton, 2000). The DNA collection was maximized by collecting the saliva from the skin surface using two swabs: the first swab was moistened with sterile distilled water, and the second dry swab, used to collect the moisture remaining on the surface after the first swab. These two swabs are subsequently pooled together into a single sample (Sweet et al., 1997b).

Only one swab was used in this study to recover saliva from each breast of the victim; each swab was processed separately. We theorized that this might reduce the risk of obtaining a mixed STR profile of male and female origin, as the less contact with the injuries present on the victim (Figure 1), the less probability of presence of female material in the samples.

Effectively, a single profile, which matched the suspect's profile, was obtained. Previous experimental studies have shown that the double-swab technique allowed for the identification of pure DNA profiles (Sweet et al., 1997a,b; Anzai-Kanto et al., 2005). However, the use of the single swab technique may be more appropriate in actual cases, wherein the skin surface is disrupted and the sample might be contaminated with the DNA of the victim. In addition, despite the use of the single swab technique in this study, the quantity of recovered DNA from the bite marks was sufficient for STR typing. In fact, the difference in the DNA recovering capacity between the single and double swab techniques was only 9.3% (Sweet et al., 1997b), with such a difference not being significant at least in this case.

Another relevant aspect of this study is the use of an organic extraction method for the isolation of DNA from the breast swabs. A study compared the results of DNA extraction from saliva deposited on human skin among three techniques: organic, classical Chelex, and

modified Chelex (the latter was considered to be more efficient than the others; however, all methods could be applied for this purpose) (Sweet et al., 1996). Overall, the Chelex methods displayed certain advantages over the organic method, including higher DNA recovery, non-usage of toxic products, and less time-consuming (Lijnen and Willems, 2001). However, in forensic cases, wherein the substrate for analyses is decomposed biological material, the organic method was observed to provide better results than the Chelex method (Hoff-Olsen et al., 1999); in addition, a comparative study comparing the isolation of DNA from saliva deposited on stamps revealed that extraction with an organic technique provides much better results than the Chelex method (Fridez and Coquoz, 1996). Taken together, our results and those of others suggest that organic extraction may be a technique of choice for the analysis of relevant forensic samples with a suspected high level of decomposition (but sufficient DNA for typing).

Sweet and Shutler (1999) also reported a case wherein the single swab technique was used to collect evidence, and an organic method to isolate DNA; however, only a partial and mixed STR profile was obtained in that study. This may have been because the victim was recovered after 5.5 h from a river with slow-moving current; under such circumstances, the DNA may have undergone a process of degradation, resulting in an incomplete profile. On the other hand, a total of four swabs were taken from the bite mark injury on the victim, and the extracts from individual swabs were pooled into a common exhibit for STR typing, while we used only one swab to recover saliva from each breast of the victim, with each swab being processed separately. This fact supported our assumption that the purity of the DNA profile obtained in this case was a function of the degree of contact between the swab (used for sampling) and the injuries on the victim.

Finally, DNA was isolated from cervico-vaginal samples taken from the victim using a differential extraction procedure. The STR profile obtained for the perpetrator was incomplete. Other techniques may be used for the isolation of DNA in sexual assault cases with variable success, including laser microdissection and flow cytometry. However, a majority of the forensic laboratories use differential DNA extraction, which does not require expensive equipment and can be performed quickly (Vuichard et al., 2011). Also, many factors influence the success of DNA typing in cervico-vaginal samples. Only a partial STR profile may be obtained in case of severe degradation of the genomic DNA, or presence of PCR inhibitors (Butler, 2010). These factors are beyond the control of the investigators, as when possible alternative sources of the DNA of the perpetrator must be considered (the bite marks found on the victim in this case).

In conclusion, this is the second case in forensic literature reporting the analysis of DNA isolated from bite marks present on the victim, in order to identify the perpetrator of a crime. Our results indicate that, contrary to most theoretical indications, a single swab technique for evidence collection and an organic method for DNA isolation could be very useful in solving this class of criminal cases. Saliva deposited on bites must always be considered as a potential source of evidence, especially if DNA cannot be extracted from other sources (difficult or not possible).

Conflicts of interest

The authors declare no conflict of interest.

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