CASE REPORT

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DNA Analysis of Dental Pulp to Link Incinerated Remains of Homicide Victim to Crime Scene

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ABSTRACT: Teeth endure postmortem degradation and extreme changes in ambient temperature and pressure better than most human tissues. This ability to resist deterioration allows the teeth to be studied as a method of establishing the identity of a decedent. Additionally, dental hard tissues, and in some instances soft tissues, may provide investigators with other sources of forensic data. In this case, a female homicide victim was transported to a location where her remains were burned. The high temperatures of a gasoline fire effectively incinerated the body precluding deoxyribonucleic acid (DNA) analysis from conventional sites. However, most of the teeth survived the conflagration. They were used to identify the victim. Additionally, the dental pulps were found to be an excellent source of high molecular weight genomic DNA. This proved to be an effective method to link the victim's body to biological evidence recovered from the site of the murder.

KEYWORDS: odontology, human identification, human teeth, DNA, genetic typing

The teeth have been used for many decades to identify deceased victims of crime. Antemortem dental records can sometimes be recovered for a missing person or a suspected victim. Consequently, the unique dental traits and restorations of a decedent can be studied and compared to dental charts, radiographs, study models, treatment records and other information. This comparative analysis may show enough points of concurrence to confirm the identity of the victim [*1–3*].

The inert, mineralized structures of teeth are known to resist postmortem degradation [*4*]. Additionally, teeth can withstand extreme changes in environmental conditions such as those encountered when the body is immersed in water, buried in the ground, or incinerated in a fire [*5–7*]. A victim's body may be subjected to deterioration, decomposition, mutilation, or deliberate disfigurement [*8–10*] but usually the teeth will remain recognizable. This ability to survive deliberate, accidental, or natural change has led analysts to focus on the teeth as a possible source of other valuable forensic data [*11–13*].

Forensic identification using deoxyribonucleic acid (DNA) from trace biological evidence is becoming widely accepted by the justice system. DNA analysis is highly discriminating, sensitive, and specific. Studies of restriction fragment length polymorphisms (RFLPs), amplified fragment length polymorphisms (AMP-FLPs) and short tandem repeats (STRs), processes referred to as "DNA typing," allow investigators to obtain DNA allelic profiles from evidence recovered at the scene of a crime [*14,15*].

The specificity of these analytical protocols offers an advantage over other techniques used to determine biological markers. Other techniques, such as the analysis of human lymphocyte antigen (HLA) and blood group substances, are based on exclusion where investigators search for differences in test results. If no differences are found after a statistically acceptable amount of testing, the probability of a match can be determined [*15*]. Alternatively, in DNA typing, the genetic profiles of forensic samples are analyzed to determine if they match the genetic profiles from known exemplars. If the results of DNA typing from the exhibit are the same as those from the known source, analysts conclude that they either have a common origin or the results match by chance. Since the probability of a match by chance is extremely low, there is usually a high level of certainty that the exhibits come from the same source. Provided a suitable specimen can be obtained, DNA typing is the best test for including an individual in a crime or excluding a falsely associated suspect [*15–17*].

The case reported here demonstrates the difficulty often faced by investigators in establishing that the remains of a homicide victim originated from a specific crime scene. The burned body of a woman was discovered July 6, 1991 in Vancouver, British Columbia. A 29-year-old Caucasian cocktail waitress was apparently brutally beaten and shot to death. Her body was removed from the death scene and transported in the perpetrator's car to a second location. Subsequently, gasoline was used to incinerate the remains resulting in nearly total cremation.

Case Circumstances

A custodian discovered the victim's burned body in a garbage dumpster situated in a parking lot behind an office and warehouse

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complex. Fire investigators concluded the configuration of the two lids at the top of the dumpster, one being fully open and the other closed, created a vortex draft during combustion. Evidence obtained from examination of the dumpster indicated the accelerant used was most likely gasoline. Combined, the gasoline and vortex draft resulted in an extremely high internal temperature (approximately 2000°F) and an intense, destructive fire of 30 to 40 minutes duration. Virtually all of the physical evidence was destroyed. A mass of human tissues weighing approximately sixteen kilograms, consisting mostly of ash and charred debris, was recovered and transported to the crime morgue for forensic autopsy.

It is estimated that the victim's body was reduced to approximately 25% of its original size and weight (Fig. 1). The head, thorax, abdomen, and proximal portions of the legs could be distinguished but they were diminished to charred masses. The distal ends of the upper and lower extremities were totally incinerated. However, most of the teeth and dental restorations survived the heat of the fire and were in surprisingly good physical condition (Fig. 2). Radiographic examination of the skull revealed remnants of four bullets from a small caliber weapon.

Detailed clinical and radiographic examinations of the teeth were completed. Many unique dental characteristics were discovered including four unerupted third molars (wisdom teeth), numerous dental restorations including silver and gold fillings, a supernumerary tooth (mesiodens), bilateral mandibular tori, and other unusual anatomical traits. A survey of the current Canadian missing persons dental database for individuals fitting this description was unproductive.

FIG. 1—*Partially incinerated remains of homicide victim stabilized by pathologist for photographic documentation.*

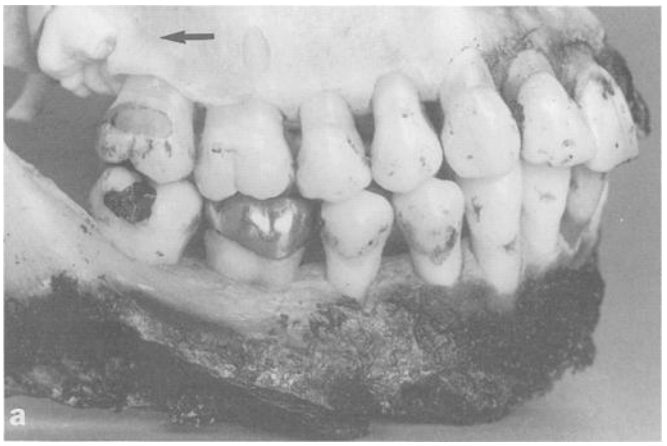

FIG. 2*a*—*Right aspect of victim's jaws, teeth and dental restorations following incineration. The lower jaw is charred and burned. Note the presence of an unerupted upper third molar* (arrow).

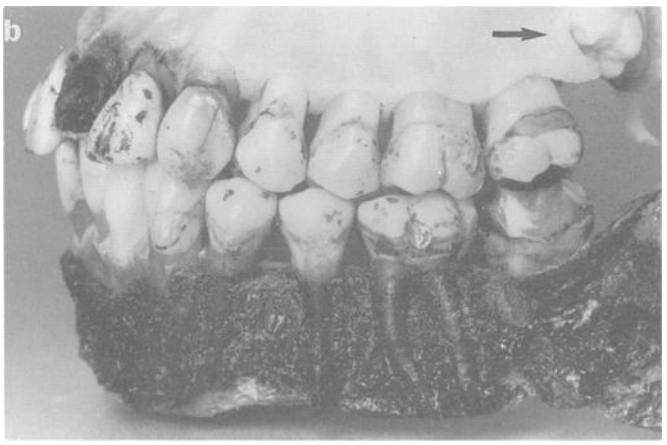

FIG. 2*b*—*Left aspect of victim's jaws, teeth and dental restorations. The lower jaw and an upper front tooth are charred and burned after incineration. Note the presence of an unerupted upper third molar* (arrow).

Four days after the body was discovered, a man requested that police attempt to locate his missing girlfriend who was later demonstrated to be the victim. Almost simultaneously, a clerk working at a Vancouver bank contacted police to report that an unknown woman attempted to fraudulently withdraw money from the missing woman's account. Detectives viewed the two calls, one involving a report of the missing person and the other about someone trying to withdraw money from her account, as a very strange coincidence. They surmised that she may be the victim of foul play and a search for the missing woman's dental records was started in accordance with section policy.

The largest dental insurance company in the province was contacted and any possible information about the missing woman was requested. The insurance company confirmed that she was a client and her dental records were obtained from the dentist of record. Detectives noted that an extra tooth, a mesiodens, was recorded as present in her mouth. They remembered this unique trait was also observed in a recent case involving an incinerated body. The

forensic odontologist was asked to compare the woman's dental records and the postmortem records from the homicide victim. He positively identified the remains recovered from the dumpster as those of the missing woman. The combination of a strange coincidence in the timing of two unrelated telephone calls and an astute observation by homicide detectives lead to the eventual identification of this victim. Given other circumstances, the body may have remained unidentified for a considerable period of time.

Detectives followed leads on the attempted robbery of the victim's bank account. A subsequent investigation led them to a woman in possession of the victim's personal identification. An interview led police to a friend of the suspect, a 25-year-old male exotic dancer. When contacted by police, the man voluntarily drove his car to headquarters and was questioned at length. As a result of this interrogation, the car was seized and the man became the prime suspect in the murder investigation.

A search of the car revealed several bloody articles of clothing and a bloodstained tire iron. The exterior of the car showed high- and low-velocity impact blood spatter. Transfer bloodstains were found in the trunk along with a small section of decomposing skin and connective tissue. An empty plastic gasoline container was also discovered in the trunk of the car. In the suspect's apartment, bloodstains were found on a pair of his blue jeans, a jacket and cowboy boots. Investigators concluded the car was either the actual murder scene or the murder occurred near the car and the body was placed in it immediately afterwards. The suspect was arrested and held pending charges.

It was determined that the blood found on the suspect's car and clothing was not his. There was much optimism that DNA analysis of the blood would either implicate or exonerate him in the crime. Unfortunately, after repeated attempts using various donor sites on the victim's body, it was determined that no source of undegraded DNA was available for comparison. Almost all of the muscles and organs and most of the large bones of the skeleton were totally consumed by the fire. Attempts to recover undegraded genetic material from all of these tissues proved unsuccessful due to the intensity of the postmortem incineration.

Methods

The odontologist learned that the investigation was not proceeding as expected because of the difficulty in finding undegraded DNA from the incinerated remains. Since the unerupted wisdom teeth were relatively well-protected by soft tissue and bone and had apparently survived the intensity of the fire reasonably well, the odontologist suggested there may be some utility in attempting to recover DNA from the soft tissues within the nerve chamber of these teeth. It seemed reasonable that remnants of soft tissue might be present inside the teeth and plans were initiated to isolate and recover these tissues.

Radiographic examination of the teeth and jaws revealed large pulp chambers present in the wisdom teeth (Fig. 3). The odontologist speculated that soft tissues, called the dental pulp, inside these teeth would experience the best chances of surviving the heat of incineration since they are located at the back of the mouth where the least amount of charring and external damage was noted. Others have previously found that dental pulps may be a valuable source of serological and biological evidence [*18–20*].

The wisdom teeth were protected from the fire by the large muscles of mastication and the tongue. Also, because the four wisdom teeth were impacted (embedded within the jaw bones) the dental pulp tissues inside these teeth were presumed to be even

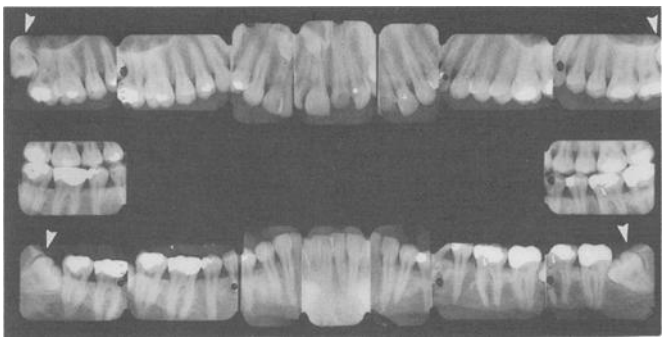

FIG. 3—*Radiographic survey of victim's teeth showing large pulp (nerve) chambers in posterior teeth and four unerupted wisdom teeth* (arrows).

better protected from the fire than the other molars. The wisdom teeth were fully formed with closed root apices.

The overlaying bone was removed using a surgical carbide bur rotating in a high speed dental turbine (Fig. 4). Profuse water irrigation during this procedure was used to avoid increasing the temperature of the bone and teeth to ensure any remaining pulp tissues were not overheated. After bone removal, the four teeth were gently elevated from their sockets and closely examined. A large piece of follicle, a soft tissue that lines the crypt where the crown of the tooth develops, was found attached to one of the lower teeth. This tissue, still relatively soft and pliable despite the heat of the fire, was gently removed and stored in a sterile glass vial. Like the dental pulps, it was speculated that this follicle tissue may provide a good source of genomic DNA. It was submitted for further analysis.

Based on the authors' previous clinical experience and discussions with another investigator [*21*], a decision was made to expose the pulp chambers by sectioning each tooth. Using a surgical carbide disc and water irrigation, the four teeth were longitudinally sectioned from crown to root tip. Pliable dental pulps were found in the chambers within the two upper wisdom teeth and were removed for analysis. In one lower wisdom tooth, the pulp was found to be harder and drier than the others but it was recovered for analysis as well. In the other lower wisdom tooth, the pulp chamber was devoid of soft tissues.

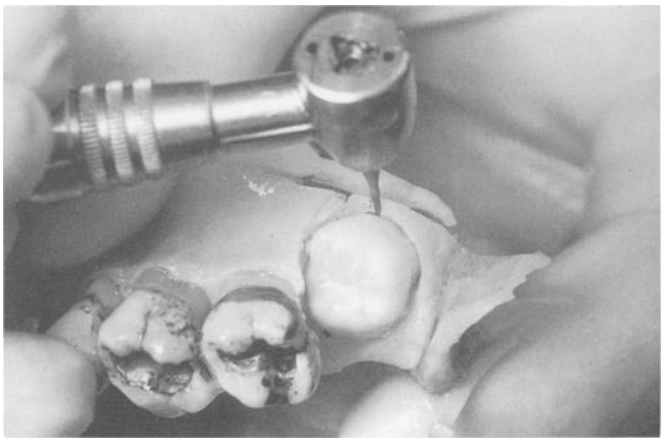

FIG. 4—*Surgical removal of bone to expose wisdom tooth prior to removal and sectioning.*

The recovered tissues were placed in separate sterile glass vials. A small sample of each exhibit was removed from the main sample and subsequently divided into two sections of equal size. Each sample was placed in 10% buffered formalin phosphate for storage. The following day, one section was fixed in Bouin's Fluid, processed on a Technicon Processor, and embedded in wax. Sections of 7 μm were cut, dried overnight, and stained with hematoxylin and eosin for histological examination. Using 100× magnification, numerous individual cells containing nuclei were observed. It was concluded that genomic DNA was present in the samples because the nuclei were intact and easily identified.

The other section of the exhibit was stained in hematoxylin for 2 minutes, "blued" in lithium carbonate for 30 seconds, and snap frozen by immersing it in isopentane (−70°C). Subsequently the tissue was embedded in Histo Prep and 10 μm sections were produced using a Reichert-Jung cryostat. The sections were air dried and stained with hematoxylin and eosin for light microscopy. At 100× magnification, cells containing nuclei were again observed. All of the exhibits were packaged and transported to the Biology Section of the Royal Canadian Mounted Police (RCMP) Forensic Laboratory Vancouver for DNA quantification and profiling.

Discussion

A detailed analysis of the biological, circumstantial, and physical evidence of the case was undertaken. The dental exhibits were transferred to the RCMP Central Forensic Laboratory, Ottawa, Ontario where they were found to contain large amounts of high molecular weight genetic material. Genomic DNA extracted from the dental pulp was quantified at approximately 1.35 μg DNA. This amount was considered ample since the RFLP protocol employed by the RCMP typically uses 200 to 500 ng. Considering the high yield of DNA from the dental pulps, the exhibit containing the dental follicle was not analyzed at this time.

The DNA from the victim's teeth became the "known" DNA source. The DNA extracted from the blood found on the suspect's car, the blood on the tire iron, the piece of tissue found in the trunk of the car, and the bloodstained clothing recovered from his apartment became the "questioned" DNA source.

These samples were examined by restriction fragment length polymorphism analysis using the restriction endonuclease Hae III and Southern blot hybridization (Royal Canadian Mounted Police, Biology Methods Manual, 1993). Profiles were obtained by successive hybridization with probes corresponding to five polymorphic VNTR loci (D1S7, D2S44, D4S139, D10S28, and D17S79) and the monomorphic locus D7Z2 for gender determination. The DNA profile derived from bloodstains left at the scene of the crime matched the tooth pulp DNA across five loci (Fig. 5). The estimated frequency of occurrence of the four loci match (excluding D4S139 due to RFLP bands being outside the RCMP upper bin boundary) was 1 in 540 million using the RCMP Combined Caucasian Database and the fixed-bin statistical analysis method [*22*].

A match was confirmed between the dental pulp and the other biological evidence. It was concluded that the victim's body had at one time been in the suspect's vehicle and was moved to the dumpster where it was discovered. The suspect was charged with first degree murder and was subsequently tried by judge and jury.

The recovery, preservation, and submission of the DNA exhibits from dental tissues and the conclusions reached from the subsequent analysis were entered into evidence at the preliminary hearing. These conclusions, and by extension the recovery of DNA from these exhibits, was the basis of the case presented by the Crown against the accused. After the preliminary hearing, defence counsel realized the case was very strong against his client and elected to stipulate to virtually all of the forensic evidence.

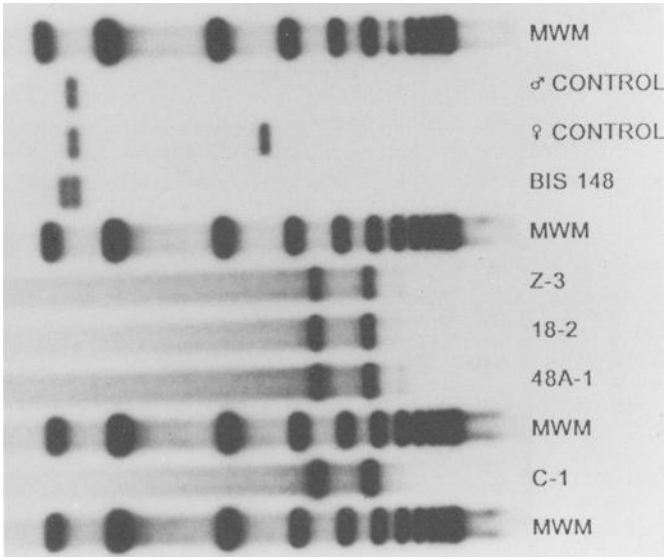

FIG. 5—*Example of an autoradiograph from this case demonstrating genetic profiles obtained following amplification of the D10S28 locus. (Note: C-1 is known sample from tooth pulp; Z-3 and 18-2 are samples from bloodstains in the suspect's vehicle; 48A-1 is sample from bloodstain on suspect's boot; MWM is 1 kb molecular weight marker; BIS 148 is bloodstain internal control).*

During the trial, the jury was confronted with the fact that the accused admitted killing the victim. Therefore, they were only faced with decisions about the premeditation of the murder. The forensic evidence introduced in the preliminary hearing proved to have a profound effect on the case. On November 19, 1993 the accused was found guilty of second degree murder. Approximately one week later, he was sentenced to life in prison without the possibility of parole for eighteen years.

Conclusions

The comparison of antemortem dental records to postmortem dental traits is recognized as a reliable scientific method to establish a positive identification for the deceased victims of crime. As in this case, the teeth are known to withstand postmortem degenerative changes and the effects of extreme variations in ambient temperature, pressure, and humidity very well [*23*]. Because of this ability of dental structures to survive postmortem deterioration, it is speculated they may offer a valuable source of other forensically significant data [*15,23,24*].

In this case, the teeth were the primary material used to positively identify the victim. However, the teeth also became important as a source of high molecular weight, undegraded genetic material which allowed comparison of the genetic profile of the victim to biological evidence found at the scene of a murder. With the increasing use of DNA analysis in resolving forensic casework, police investigators and other forensic experts are recognizing the potential for teeth to be used as a source of genetic material.

Acknowledgments

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