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Chapter 25

FORENSIC MEDICINE

Abstract

The knowledge and technique of medical science applied to assist in the resolution of crimes, legal disputes, etc., constitute forensic medicine. Establishing the identity of victims (in cases of murder, accidents, etc.), criminals (in case of rape, murder, etc.) and the father (in case of paternity disputes), etc., is critical in solving the problem of crimes and disputes. This field was completely revolutionized by the technique of DNA fingerprinting. Using this molecular diagnostic technique, the identity of a person can be traced with the help of bloodstain, semen stain, hair roots, tears, saliva and even perspiration.

Key words

Forensic medicine, DNA fingerprinting, pathology, autopsy, anthropometry, crimes ,pathology, PCR, RFLPs ,mtDNA ,sex determination ,Restriction fragment length polymorphism (RFLP) testing, single locus probe , SLP , DNA fingerprint , STR ,microsatellite, mitochondrial DNA analysis ,mtDNA, antemortem , DNA methylation, Clinical forensic medicine, post mortem

25.1 PROLOGUE

In the large majority of homicide trials, there is nothing controversial in the pathologist's testimony. This is not to say that the testimony is unimportant. It is just that, the medical evidence, usually does not establish the guilt or innocence of the defendant nor does it independently establish a degree of the guilt. What is really important , in the majority of instances, is to provide expert opinion based upon objective, indisputable facts which help to evaluate the reliability and credibility of other witnesses. The pathologist is a middle man between the facts of the crime and the testimony of persons whose version of the episode is at issue.

Trust and confidence in scientific evidence have undoubtedly improved over the past few years and forensic sciences themselves have progressed in reliability and effectiveness. Law enforcement agencies rely on scientific investigation to a constantly growing extent. Objectivity, reliability and completeness are the hallmarks of scientific evidence for it to be acceptable in courts. A scientific investigation should have pertinence to the problem under question. Irrelevant scientific investigation will only add to the confusion and may even lead to miscarriage of justice. The quality and reliability of scientific investigations depends on many factors. Failure to take notice of these factors may result in erroneous conclusions and wrong interpretation of the scientific observations. The

investigator, therefore, must take all necessary precautions to see that extraneous factors do not interfere with the scientific experiment and vitiate the findings.

25.2 CONCEPT

Forensic medicine and pathology have traditionally been regarded under the auspices of the medical examiner and/or coroner, with medico-legal investigation of unexpected, unexplained, mysterious and/or violent deaths being its principal application. The field of forensic medicine embraces not only traumatic and/or drug-related pathology, however, but also addresses issues of child and elder abuse, sudden and unexpected deaths of a broad constellation, public health and many issues within the overarching fields of the law, medicine and the judiciary. Applications of forensic medicine has impact in almost all fields of clinical medicine to an at least limited degree, with assessment of patient mortality and proper completion of the death certificate merely one of a number of examples which illustrate this relationship.

The knowledge and technique of forensic science help in solving cases of crimes, legal disputes, etc. This also helps in solving the problem of crimes and disputes for the identification of victims in cases of murder, accidents, etc., criminals in case of rape, murder, etc. and the father in case of paternity disputes. The scope of forensic medicine is boundless. The duties of the investigators include finding out the cause of death in suspected cases of homicide, infanticide, suicide and accidental deaths, to provide expert evidence on injuries regarding their manner of causation, their age and their ability to cause death or disability, to help the police in establishing identity of criminals and victims of crime with the help of medical knowledge to establish the time of death in medico legal deaths, to establish proof of foul play or rule it out.

The study of the above problems and scientific assembly of evidence for presentation to the legal authorities form the basis of this speciality. All branches of medicine, anatomy, pathology therapeutics and obstetrics provide the basic knowledge and applications of which shapes to conform to the needs of the law to form the body of the subject. The study of the above problems and scientific assembly of evidence for presentation to the legal authorities form the basis of this speciality. All branches of medicine, anatomy, pathology therapeutics and obstetrics provide the basic knowledge, application of which shaped to conform to the needs of the law form the body of the subject. The ultimate and sole aim is to arrive at the truth or the nearest approach to it so that justice may prevail on the cases that come up for trial. To attain this objective, an expert in forensic medicine should aim at thoroughness in his investigations, impartial reporting to the concerned authorities and he should depend on scientific truth in the formulation of his findings and conclusions.

Through these principles he seeks the constant improvement of the relationship between science and justice in the interest of personal rights, responsibilities and freedoms. In the large majority of homicide trials, there is nothing controversial in the pathologist's testimony. This is not to say that the testimony is unimportant. It is just that in and of itself, the medical evidence, usually does not

establish the guilt or innocence of the defendant nor does it independently establish a degree of the guilt. What it really is all about, in the majority of instances, is to provide expert opinion based upon objective, indisputable facts which help to evaluate the reliability and credibility of other witnesses. The pathologist is a middle man between the facts of the crime and the testimony of persons whose version of the episode is at issue. Trust and confidence in scientific evidence have undoubtedly improved over the past few years and forensic sciences themselves have progressed in reliability and effectiveness.

Law enforcement agencies rely on scientific investigation to a constantly growing extent. Objectivity, reliability and completeness are the hallmarks of scientific evidence for it to be acceptable in courts. A scientific investigation should have pertinence to the problem under question. Irrelevant erroneous conclusions and wrong interpretation of the scientific observations. The investigator, therefore, must take all necessary precautions to see that extraneous factors do not interfere with the scientific experiment and vitiate the findings. Proper collection and preservation of the materials used in the scientific experiment will ensure reliability in the results obtained. The next pitfall is in the transmission of material for scientific experiments by specialities in their chosen fields. To make sure that the material is not lost, misplaced or tampered with it is necessary that an unbreakable chain of custody is established. Each person in the chain should take responsibility for the proper transmission of the material to the next person and should maintain a record of the date and time of the recovery of the specimen, what was done with it, when it left possession and to whom given. Needless to mention that the chain should be as short as possible. Scientific observations, often allow more than one interpretation depending upon the circumstances of the situation. It is necessary, therefore, that the investigator is, provided with adequate information of the particular incident, regarding the background and circumstances surrounding the incident, so that, he may draw the correct conclusions from his scientific observations keeping in view the correct perspective of the situation as a whole.

Modern scientific investigation is not a one man's job. It is a team work involving several experts working individually or together with a common aim, namely to arrive at the truth. This concept of team work may be clearly understood if it is agreed that a single incident may present a number of different clues which require the employment of different specialists for their interpretation. Conversely, a single item of evidence may require the combined examination by several specialists. There is now growing recognition of the importance of team work of investigations, particularly in the investigation of murders, mass catastrophes, vehicular accidents, arsons etc. A well trained and experienced forensic pathologist at the scene of crime can be of great assistance to the investigating team to assure that the dead body is properly preserved and handled. Necessary photographs and sketches are made, all potential valuable evidence recognised and collected and marked by appropriate officials. This information will be of great value to the forensic pathologist in his procedures with respect to autopsy of the deceased in determining the cause of death and in forming other opinions concerning how death occurred (accident, suicide & homicide).

An autopsy report gives vital clues about the nature of crime and helps in solving the case. Though all doctors are qualified to perform autopsies, experts say vital clues can be missed by them. "A forensic medicine expert can pick up fine details during autopsy, which can be missed otherwise.

The time, cause and manner of death can be identified during autopsy. Though the aim and object of the system of justice is to discover the facts and to ensure that truth has its full sway on the judgements passed in the courts of law, it is not able to adhere to these aim and object because of some inherent shortcomings in the system. Though the aim of the court is to place responsibility upon the contending parties, in a litigation to present relevant facts and hopefully determine the truth, in reality, the adversary system often undermines the pursuit of truth with opposing sides seeking to win, at all costs, without obligation to reveal information which may be detrimental to them.

The lawyer aims at winning in the fight, not at aiding the court to discover the facts. He does not want the trial court to reach a sound and educated guess if it is likely to be contrary to his scientific investigation will only add to the confusion and may even lead to miscarriage of justice. The quality and reliability of scientific investigations depends on many factors. Failure to take notice of these factors may result in client's interest. This is a special privilege enjoyed by lawyers. An expert witness has no such privilege and he has no axe to grind. Nor does he have a personal interest to protect a client. His evidence, therefore, is objective, unbiased and impartial as he is not concerned with the outcome of the judgement. A medical witness is not expected to take more responsibility upon himself that he can cope with.

Contrary to general belief, forensic medicine is not a static science. It is vital and is continuously growing, taking into its fold any new scientific discovery and effectively turn it to its own use. Many a scientific principle such as photography, neutron activation analysis, Coombs test, etc. has, from time to time, found a permanent and accepted place as significant armaments in the hands of the forensic pathologist in his relentless war against social injustice, misdemeanour and felony.

25.3 FORENSIC MEDICINE AND DNA FINGERPRINTING

DNA fingerprinting without doubt represents one of the most significant advances in forensic medicine in this century. Some of the commonly used presumptive test reagents for identification of blood and semen could potentially affect the recovery of intact high-molecular-weight deoxyribonucleic acid (DNA) from evidentiary samples (Samba *et al.*, 1994). DNA can be used not only for convicting the guilty but also for exonerating the innocent. It was demonstrated for the first time that a DNA fingerprint could be used to find a perpetrator from within a population. In 1985, a year after the development of DNA fingerprinting, the polymerase chain reaction (PCR) was discovered (Saiki *et al.*, 1985). This discovery has revolutionize the field of molecular biology, though the method would not come into routine use in forensic cases until the early 1990s, since new platforms and biochemical tools were needed in order to take full advantage of the potential of PCR. In particular, new automation technology is the key, and the advent of the automated fluorescent DNA sequencer in the early 1990s is a major step forward. More generally, forensic DNA analysis has benefited substantially from the Human Genome Project, for the genome could be sequenced only with automated equipment that permitted high-throughput processing. Because forensic science could use the same equipment and

biochemical tools that gene sequencing used, new methods were rapidly developed in the early 1990s that would have been considered impossible just a few years earlier.

Perhaps the best example of this adjunct benefit of genomics is the development of national DNA databases. Since its inception in 1995, the National DNA Database for England and Wales has expanded to include more than 2.75 million reference DNA profiles, against which all specimens obtained from the scene of a crime ("crime stains") are routinely compared (Werrett *et al.*, 1982). The likelihood that a match will be found is approximately 30 percent. Many other countries have since followed suit, and the benefits of such databases are considerable, since persons who commit serious crimes such as murder usually have a previous criminal record. The United Kingdom's policy permits the collection of DNA profiles from all convicted criminals, as well as from anyone suspected of committing a crime that could lead to a prison sentence and the law allows authorities to retain the DNA profile even if the suspect is found innocent. Consequently, persons who later commit more crimes can be identified and apprehended quickly (Gill *et al.*, 2000; Marchi 2004).

The analysis of DNA isolated from forensic biological evidence provides valuable information relating to the identification of the source of the sample. Sex determination from blood and bloodstains by polymerase chain reaction (PCR) was also performed (Semba *et al.*, 1994) from the viewpoint of forensic medicine (Fig 25.1). In the present PCR method, Y chromosome specific sequence (DYZ3) and X chromosome specific sequence (DXZ1) belonging to alphoid (alpha) centromeric repeat family were specifically amplified. The limit of detection of the specific sequences by PCR corresponds to 0.00001 microliter of the whole blood. In case of diluted blood, it was possible to detect X and Y

specific sequences in the specimen

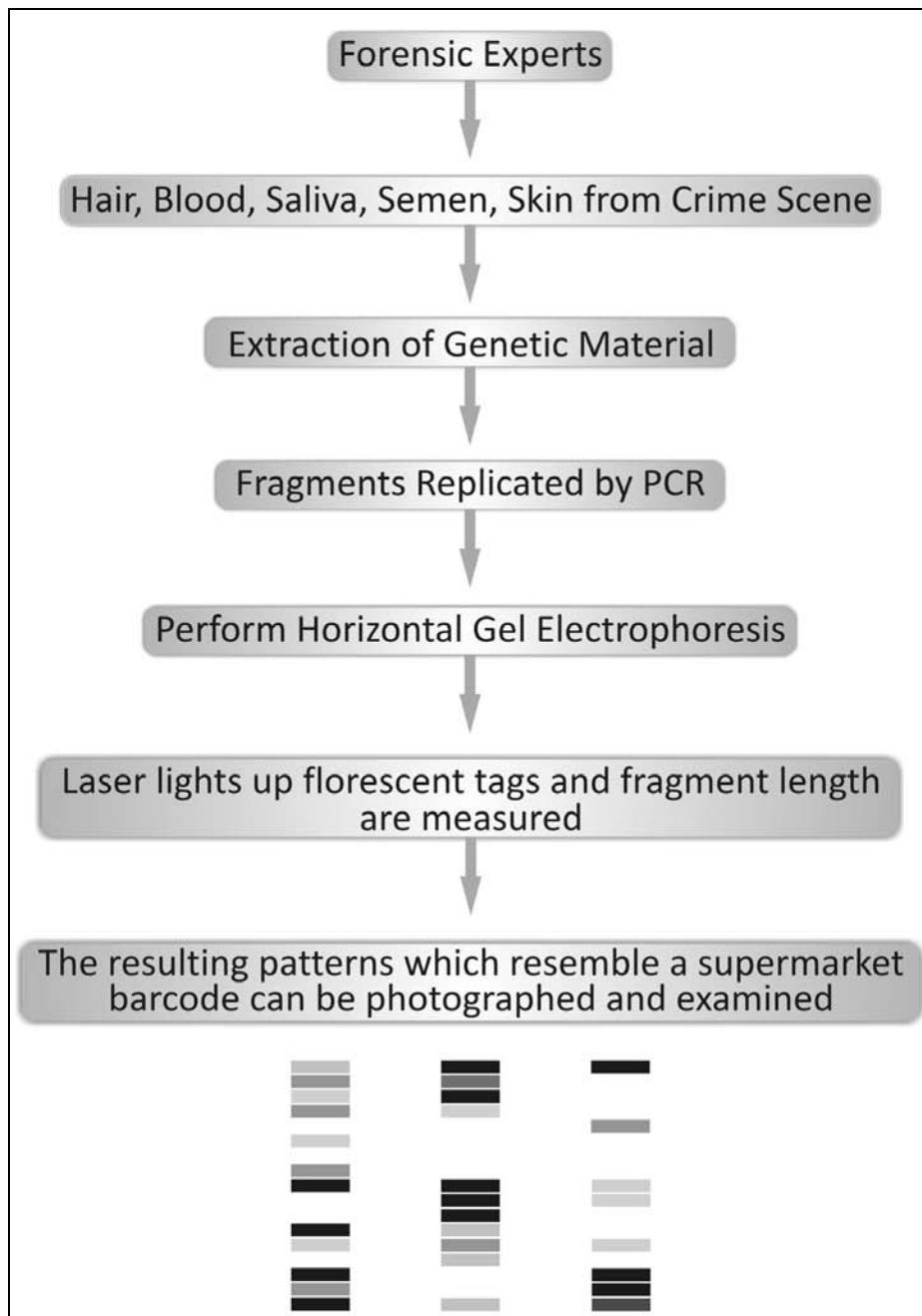


Fig 25.1 Method for analysis of DNA from forensic samples

diluted up to 100,000 times. X and Y specific sequences could be detected from a cotton cloth in size of 1 mm², on which blood diluted by 10,000 times had been attached. In case of bloodstains, X and Y specific sequences could be detected even from 1 mm of a single cotton fiber and sex could be determined. X and Y specific sequences could be detected even from blood specimen left at room temperature for 7 months, and from bloodstains left at room temperature for two years. Further, sex determination could be achieved from aged bloodstains preserved at room temperature for 22 years. X and Y specific sequences could be distinctly detected after blood specimen was heated in a water bath at 100 degrees C for 9 hours. After bloodstains were heated in an electric furnace at 150 degrees C for

30 minutes, both specific sequences could be detected. When male and female blood specimens were mixed together, X and Y specific sequences were amplified satisfactorily when male-female mixing ratio was from 100:1 to 1:1. From the mixing ratio of 1:10, amplified band of Y specific sequence began to gradually weaken, and only weak band was detected when the mixing ratio was 1:1000. These results reveal that the present sex determination method by PCR can be performed in simple and quick manner and has high detecting sensitivity, and sex can be determined even from putrefied, heated or aged specimens. Thus, it is expected to be one of useful examination methods in forensic practices.

RFLPs and 9-bp deletion type of mtDNA may suggest the characteristics of the human races (Misawa, 1994). In a study sex determination using polymerase chain reaction (PCR) on tooth material was evaluated from the viewpoint of forensic medicine by Murakami *et al.*, (2000). Using the method of DNA analysis, Restriction fragment length polymorphism (RFLP) testing, using a combination of single locus probe (SLP) that vary highly among individuals, produces a DNA fingerprint or profile. The PCR method using STR (microsatellite) and mitochondrial DNA analysis (mtDNA) is suitable for examination of the forensic biological samples (bloodstains, hairs, seminal stains, bones, tooth). For sex identification of bloodstains, bleached skeletons and teeth, Southern blot hybridization with Y-chromosome specific probe (pHY10; 3.4 kb) and PCR amplifying with sex chromosome specific fragments, can be used. Mitochondrial DNA bones and teeth can also help in sex determination (Harihara *et al.*, 1990). The antemortem dental information including dental records and radiological records provided by families of the victims, play a major role in speedy identification (Yamamoto, 1996).

Central to this technology, which is based on the analysis of the genetic component of cells, is the use of DNA probes to regions of the human genome that exhibit great variability between individuals. These probes fall into two main categories. The first group comprises those that can detect a large number of these “hyper variable” loci simultaneously, namely multilocus probes (MLPs). On autoradiography, these give rise to a band pattern that is reminiscent of the bar code on supermarket goods, the main advantage of which is that a single such test provides a lot of information very rapidly. MLPs are, therefore, the probes of choice when the amount of material for testing is not limiting, e.g., a blood sample for paternity testing. In many forensic cases, however, the material evidence available for testing is minute, such as a few hair roots or a tiny semen stain, and the situation is often complicated by the presence of tissue from more than one person.

25.4 APPLICATIONS OF DNA METHYLATION MARKERS IN FORENSIC MEDICINE

The methylation pattern of human genome is space-time specific, sex-specific, parent-of-origin specific and disease specific, providing us an alternative way to solve forensic problems (Zhao and Yang 2005). DNA methylation profiles represent a more chemically and biologically stable source of molecular diagnostic information than RNA or most proteins. Recent advances attest to the great promise of DNA methylation markers as powerful future tools in the clinic. DNA methylation is a post-replication modification that is predominantly found in cytosines of the dinucleotide sequence CpG. Epigenetic

information is stored in the distribution of the modified base 5-methylcytosine. In the past decade, DNA methylation analysis has been revolutionized by two technological advances viz. bisulphite modification of DNA and methylation-specific polymerase chain reaction (MSP).

25.5 FORENSIC MEDICINE AND ANTHROPOMETRY

Forensic medicine is an interdisciplinary science which in everyday practice applies all the knowledge that medical sciences, have accepted as reliable and scientifically solid facts or processes, and qualitative and quantitative definitions with the help of which accurate and reliable statements can be made. The use of anthropometry in the field of forensic science and medicine dates back to 1882 when Alphonse Bertillon, a French police expert invented a system of criminal identification based on anthropometric measurements. His system was based on three fundamental ideas- the fixed condition of the bone system from the age of twenty till death; the extreme diversity of dimensions present in the skeleton of one individual compared to those in another; the ease and relative precision with which certain dimensions of the bone structure of a living person can be measured using simply constructed calipers. This system of identification spread rapidly through much of the world but the system was not accepted much in view of some major drawbacks and discovery of other identification systems e.g. dactylography .

As anthropometry is an important part of biological/physical anthropology, hence the persons specializing in anthropometry are familiar with range of biological variability present in the human populations and its causes, and are well trained in comparative osteology, human osteology, craniometry, osteometry, racial morphology, skeletal anatomy and function. They are well aware of the knowledge of archaeological field techniques and methods which serve well in crime scene recoveries involving buried and surface remains. The term 'forensic anthropometry' can be coined for this branch of applied physical anthropology, involving the use of methods/techniques of anthropometry in forensic/legal context. In other words, "forensic anthropometry is a scientific specialization emerged from the discipline of forensic anthropology dealing with identification of human remains with the help of metric techniques".

Anthropometric characteristics have direct relationship with sex, shape and form of an individual and these factors are intimately linked with each other and are manifestation of the internal structure and tissue components which in turn, are influenced by environmental and genetic factors. Anthropometric data are believed to be objective and they allow the forensic examiner to go beyond subjective assessments such as 'similar' or 'different'. With measurement data, the examiner is able to quantify the degree of difference or similarity and state how much confidence can be placed in this interpretation.

The main aim of an anthropometrist employed in the forensic medicine/medico-legal department, working with unknown variables, is to describe the remains in such terms so that one can achieve the goal of estimating age at the time of death, sex, stock/race/ancestry/ethnicity, stature, body weight/body build, details of individualizing characteristics i.e. amputations, fractures, ankyloses, deformities and

bone pathologies and to some extent the cause of death if reflected in the remains/bones. The objective is to enable the law enforcement agencies to achieve the ultimate goal of personal identification.

Forensic anthropometry incorporates most of the techniques originating with the analysis of human skeletal material from archaeological sites; the two disciplines have been closely linked. A good forensic anthropologist must, by definition, be a good skeletal biologist. He helps a forensic pathologist to reconstruct the biological nature of the individual at the time of postmortem examination, and sometimes giving clues and reconstructing the circumstances surrounding death. He is prepared for this by his training in describing the prehistoric skeletons from archaeological sites and usually by special experience in identifying unknown modern skeletons .

25.6 CLINICAL FORENSIC MEDICINE

Clinical forensic medicine (CFM) is "the application of appropriate forensic practices and principles, reserved for use by the pathologist at autopsy, to living patients in a clinical setting." "Living forensic" patients include survivors of trauma and potentially catastrophic experiences resulting in injury. CFM arose from "clinically" affirming that not all abuse or assault victims sustain fatal injuries. Appropriate medical documentation and interpretation of physical findings may aid law enforcement and/or social services in the legal evaluation of a case or situation. Additionally, timely collection of pertinent evidence may be performed as the case necessitates. (Recktenwald *et al.*, 2005)

Criminal violence and its associated trauma comprise a critical health problem throughout the world. Clinical forensic medicine represents a new discipline of medical practice that is evolving in direct response to the sequelae of criminal and interpersonal violence. The application of the principles and standards of the forensic specialist has been increasingly recognized as playing a crucial role in trauma care; the results of the extremes of human behaviour-abused children, individuals suffering from blatant neglect and maltreatment, or self-inflicted injury, and victims of road-traffic accidents, firearm injuries and other assaults. These cases must be reported to a legal agency for investigation and follow-up. As trends in crime and violence change, new antiviolence legislation is likely to be implemented; consequently, new personnel resources are required to ensure that these legislative mandates effectively meet the needs of society (Sharma, 2006).Recently it was reported that post-mortem histopathological investigations of the bone marrow in forensic medicine is an important issue for both the forensic and clinical pathologist (Roll *et al.*, 2009).

25.7 CONCLUSION

The technological advancement in forensic medicine will be imposing challenges, including those concerning genetic manipulation and the keeping of confidentiality regarding electronic files. On the other hand, old problems will recur, the most important being the vital question of research with

prisoners and the use of torture by agents of the state. There is a need for international legislative mechanisms that would establish a set of explicit rules of behavior, thus diminishing the possibility of ethical dilemmas. The subject of forensic medicine has grown from the days when it was taught as part of pathology, to being a specialty in itself. However, forensic medicine has to advance to keep pace with new developments and we need the committed efforts of all doctors in this profession to meet the expectations of society.

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