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Review Article

Putrified tissue analysis: A challenge for Forensic toxicologists

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ABSTRACT

The postmortem toxicology helps in determining the cause of death and information on events immediately before death. Forensic toxicologists receive various types of biological samples with putrefaction process which pose a greater challenge in chemical analysis. Interpretation of analytical toxicology results must incorporate pharmacokinetics and toxicology of the agents in question, the circumstances under which death occurred including the mechanism of exposure. The aim of present study is to describe the type of biological samples submitted for toxicological analysis in forensic toxicology section and factors associated in their analysis.

1. Introduction

The importance of toxicological results obtained from analysis of postmortem specimens is the substantial contribution to determining the cause of death and circumstances at the time of its occurrence. In agriculture economy easy availability and uncontrolled sale of insecticides, pesticides cause poisoning in the population. Forensic toxicologists analyse various types of biological samples among these biological specimens putrefaction occurs due to enzymolysis, autolysis, and bacteriolysis. The analytical methods are validated for the analysis of fresh biological samples, but degraded specimens creat severe problem of matrix interference during analysis therefore suitable exhumed samples can be selected for direct analysis using gas chromatograhic mass spectrometer for reliable results. Immunoassays designed simply for drugs of abuse testing are widely used for screening purposes and simply indicate the need for further analysis using a more selective method because of the risk of false-positive results apart from that many drugs are administered as either single enantiomers, or racemic mixtures, and yet achiral analytical methods are often all that is available for the analysis of biological specimen. In decomposed body generally vitreous humor is preferred which is less susceptible to postmortem change as compared to blood. The possibility of specimen contamination is always a great threat in chemical analyis moreover quite frequently specimen received at forensic laboratories are in advance state of decomposition. The main objective of forensic toxicologist is the separation of target analytes and to reduce possible interferences. The putrefiction tissue extraction procedures contribute in cost of analysis as well as methods accuracy and reliability. It should be note that forensic samples are unique and usually available in small amounts.[1–10]

1.1. Collection of biological samples for forensic toxicological analysis

The specimens available in postmortem toxicology investigations can be numerous and variable, and may be selected based on the case history, requests, legal aspects and availability in a given case. However, not all matrices are appropriate to the analysis of all drugs. Usually, during an autopsy, fluids and tissue samples are collected for carrying out several complementary analyses, including forensic toxicology. The postmortem samples collected from the corpse and the requested analyses in

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Table 1: In vivo samples

S. No	Sample	Quantity	Analysis
1	Exhaled air	As available	Volatile compounds
2	Oral fluid	1-2 ml	Drug of abuse
3	Breast milk	30 ml	Poisons and drugs
4	Sweat	As available	Poisons and drugs
5	Amniotic fluid	As available	Poisons and drugs
6	Meconium	2 gm	Poisons and drugs

these specimens are dictated by circumstances of the case and the condition of the body. So, although blood is the preferred reference matrix in the field of postmortem forensic toxicology, alternative matrices are required in case of limited volume, unavailable or unusable blood samples. There are several specific challenges to select and collect samples for antemortem and postmortem toxicologic al analysis. In several cases, the evidence found at the scene may represent the best guide for toxicological analysis and therefore cups, bottles, pipes, syringes, needles, cotton, spoons, silver paper and suspicious household products should be collected. Some of the biological samples and their required quantity are given in Tables 1 and 2.

1.2. Preservation of specimens

Putrefactive changes are influenced by several variables and influence the obtained concentrations for several analytes like blood ethanol concentrations may increase or decrease. Therefore, the preservation of samples and physical conditions during storage plays an important role. Samples should be stored in tightly sealed containers at 4°C or at −20°C; exceptions to this include hair and nail, which are stable at room temperature; hair samples should be stored at room temperature; if plasma or serum is needed for analysis, these are separated before blood frozen; sodium fluoride preservation of blood with a final concentration of 1%–5% by weight is mandatory for peripheral blood and facultative for other blood samples. It is mandatory that the collection procedure of pathologists be audited by toxicologists to avoid errors; absolute rules for the interpretation of toxicological results are absent since each case is unique.

1.3. Interpretation of analytical results

Interpretation of postmortem toxicology results is a complex area. It is to be presumed that concentrations of drugs and poisons measured in blood obtained at autopsy reflected the situation at the time of death, hence interpretation of results could be made simply by comparison with 'normal' or 'therapeutic' plasma concentration data. However, we know that interpretation of postmortem toxicology results must take into consideration the clinical pharmacology and toxicology of any agents in question, the age of the individual, the circumstances under which death occurred including the mechanism of exposure and other factors such as whether prolonged resuscitation was attempted, how the body was stored prior to sampling, and how the samples were collected. Dehydration may have resulted from exposure to heat during a fire, or dilution may have occurred in bodies recovered from water, a phenomenon perhaps more apparent in bodies recovered from fresh water than from sea water. If discovery of a body is delayed, the extent of decomposition can make not only specimen collection, but also the interpretation of qualitative let alone quantitative results very difficult. Tolerance cannot be measured in retrospect, although hair or nail analysis can sometimes be employed in an attempt to assess exposure to toxic metals, illicit drug use, or adherence to prescribed medication in the weeks or months before death. Although hair is well preserved even after burial, analysis gives no information pertaining to acute poisoning and qualitative information on exposure may be all that can be gleaned. Moreover, there is always the possibility of external contamination from, for example, skin secretions, of passive contamination, and of removal of analyte through either excessive washing, or cosmetic hair treatment, or of distributing analyte from the surface to the matrix of hair during sample preparation. Measurement of poison concentrations in a representative specimen of gastric contents can sometimes give an estimate of unabsorbed dose if the total volume of contents is known. However, simply detecting a basic drug in gastric contents does not prove recent ingestion. Endogeneous metabolism and decomposition involving both autolysis and putrification may increase or decrease drug metabolite levels in postmortem analysis. Drugs and poisons can change concentration of postmortem due to poor or unequal quality of blood and other specimens, anaerobic metabolism and redistribution which makes forensic toxicology largely handicap in the interpretation of postmortem results. Proper interpretation of toxicological findings requires integrating the clinical setting and findings with the toxicological results in a way that maes medical sense. The interpretation of toxicology results in postmortem specimen requires the toxicologist and pathologist to be cognizant of drug-drug interactions. In the recent trend the interpretation of toxicoloical results should accont autopsy finding, crime scene information and related medical history. The absence of reference data of drug and poisons concentration in

Table 2: Post-mortem samples

S. No	Sample	Quantity	Purpose
1	Cardiac blood	30 ml	Post mortem redistribution of compoun ds
2	Blood from thoracic or abdominal cavities	30 ml	Drug of abuse
3	Brain	30 gm	Poisons and drugs
4	Vitreous humour	2-5 ml	Poisons alcohol and drugs
5	Spleen	30 gm	Poisons and drugs
6	Lung	30 gm	Poisons and drugs
7	Liver	30 gm	Poisons alcohol and drugs
8	Bile	10 ml	Poisons and drugs
9	Kidney	30 gm	Poisons heavy metals and drugs
10	Heart	30 gm	Poisons and drugs
11	Bone	30 gm	Poisons and drugs
12	Synovial fluid	1-2 ml	Postmortem redistribution
13	Bone marrow	10 ml	Accumulating poisons
14	Fly larvae (maggots)	10 no	Pesticides and drugs
15	Adipose tissue	30 g	Accumulating poisons
16	Skin	2-4 cm	Drugs and animal poisons
17	Skeletal muscle	30 gm	Poisons and drugs
18	Urine	30 ml	Poisons, alcohol and drugs
19	Head hair	150–200hairs	Drug of abuse and metallic poison
20	Stomach and Gastric content	30 ml	Poisons, alcohol and drugs
21	Cerebrospinal fluid	10 ml	Poisons and drugs
22	Finger nails	Clippings from fingers	Drug of abuse and metallic poison

the putrified tissue poses a problem to meaningfully and reliabily conduct toxicological testing.

2. Results and Discussion

The success of postmortem toicology analysis depends on pathologist and toxicologist working as team. The pathologist mainly relies on the analytical skills of the toxicologist however fate of the results grossly depends on the specimen dispatched for analysis. The role of forensic toxicologist is to identify poisons, drugs and toxins which contribute in death of the subject. In the current scenario pathologist and forensic toxicologist must work closely as team to ensure that poisoning is not escaped. Hair, nail, teeth and putrefied materials are the most commonly submitted exhumed samples in addition to soil samples. Selection of soil samples is vital in the absence of hair and nail samples where suspicion of heavy metal poisoning cannot be over ruled. Control soil samples are then used to rule out the natural presence of heavy metals in soil samples. Hair is the first choice for the detection of heavy metals and certain drugs along with their metabolites as they may accumulate in hair after chronic use. Quantitation of drugs is not possible in exhumed specimens because of the sample degradation, except in appropriately collected hair samples where section wise analysis provides reliable results. Certain in-vitro studies have shown that most drugs can be detected both in blood and bone tissues, but there is a need to develop the correlation of drug concentrations in the tissue and the blood. In some cases however, drugs have been found only in bones but not in blood, and the correlation of drug concentration in both tissues cannot be developed. Owing to the availability of very limited research on bones, detection of any drug in bone can only be corresponded as the evidence of exposure to that drug. A large number of new psychoactive substances are available in the market. These substances are purposefully marketed as replacements for illegal drugs. These substances are very closely structurally related to controlled psychoactive molecules in order to create alternative psychoactive compounds. The abuse of these substances have been a matter of concern for human health, many cases of death because of intoxication with these chemicals have been recently reported. The rapidly increasing number of constantly varying psychoactive substances makes their identification and the study of their analytical and toxicological profile an extremely difficult task, especially when standards are not available. It is estimated that 20 or more new psychoactive substances appear annually. Designer drugs are usually synthesized, distributed and used in low levels and within small sub-populations, which makes their detection and control challenging for authorities and scientists. Due to this rapidly appearance of new psychoactive substances, and mainly the lack of standard references, the development of new analytical methods to detect these drugs is a significant challenge for the forensic toxicologist. Forensic toxicological analyses have traditionally focused on the use

of blood, body fluids, and certain organs in examinations of deaths due to intoxication. However, in putrefaction and contamination proper sampling from tissues becomes impossible, such as in exhumation cases. In these cases, bone marrow might be useful as an alternative specimen since it is a potential source for drugs. It is widely known that assessing the toxicological significance of a substance in the cause of a death is an extremely difficult task. This difficulty lies in the fact that, in the majority of the drug-induced deaths, several substances were consumed. With the new psychoactive substances this is even more evident because the amount and type of compounds that are present in the formulations vary considerably. As a consequence, the existence of simultaneously fast and sensitive methods for routine use in forensic toxicological laboratories becomes fundamental so that searching for these substances in biological samples is possible. Considering that forensic toxicology focuses mainly on postmortem analysis, it is crucial to study alternative specimens to assist death investigations.

3. Conclusion

The evidence and information obtained by the toxicologist is only as good as the quality of his specimens. The proper specimens must not only be obtained uncontaminated, but must also be preserved in their original condition for the toxicological analyses to be meaningful. Chemical examiner cannot refuse to examine visceral material if sent by the police or doctor even if cause & manner of death is determined. There are times when the FSL report is negative despite there being clear signs of death due to poisoning. One of the reasons for this is that there may not be any poison left in the body by the time death has occurred. This may happen due to vomiting, purging or elimination from the system by the kidneys or due to prolonged stay in the hospital immediately prior to the death. Certain vegetable poisons may not be detected in the viscera, as they have no reliable tests, while some organic poisons especially the alkaloids and glucosides maybe changed or split into other compound by oxidation during the life or by putrefaction after death, which have no characteristic reactions sufficient for their identification Even though several guidelines lay down that in cases of suspected poisoning, stomach wash and vomit along with viscera should be sent for examination, in many cases this procedure isn't followed; thereby reducing the chances of detection of poison. All the available evidence must be taken into account when an attempt is made to interpret postmortem toxicology data. An overall knowledge of the circumstances, time course, clinical and perhaps postmortem observations, substances thought to be involved in an incident and their pharmacology are important, together with knowledge of the specimens available for analysis and the analytical methods used. Bringing together the necessary information may not be easy, especially as many individuals with different backgrounds may be involved: investigating authorities, emergency treatment personnel, former medical providers, postmortem examiners, and analysts. Despite the problems listed above, toxicological and sometimes biochemical analysis is an essential component of many types of enquiries and can often provide evidence of exposure to drugs and other substances and may assist in estimating the extent and timing of the exposure. Extension beyond this requires full knowledge of the case under consideration and appreciation of the pharmacology of the agents in question.

4. Conflicts of interest

All contributing authors declare no conflicts of interest.

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