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Review Article

Utilization of Brain Tissue as A Viable Postmortem Toxicological Specimen: A Review on Collection and Preservationof Samplefor Toxicological Analysis and Its Advantage Over Other Specimens

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Abstract:

Collection of proper autopsy specimen and preservation are essential stepsfor the toxicological analysis in Forensic Medicine. Faulty collection and preservation of the specimens/samples can greatly alter or negate forensic chemical or toxicologicalexamination. In forensic toxicology practicein Bangladesh, postmortem specimen that is subjected to toxicological examinations generally focusing on mainly blood and sometimes urine or other fuds from different body cavities. Analysis of blood from different anatomical sites and tissue samples and urine may assist in the interpretation of the postmortem results. However, in many postmortem cases, there is little or no blood for quantitative drug analysis, or there might be such traumatic injury which led to significant blood loss or there is possibility of contamination form contents of the ruptured stomach. Besides, analysis of urine reveals negative result, if death occurs closely the time of intoxication. Given the circumstances, brain tissue may be a valuable specimen in postmortem toxicological analysis. The position of the brain in the body secures a tremendous protection and isolation which can eliminates or at least attenuates many of the interpretive challenges with postmortem blood, urine or other fluid specimens.This review paper is an update on the standard methods of brain tissue specimen collection and preservationprocedures for toxicological analysis and its value as well as advantages over other specimens, which might be of possible interest for forensic professionals in the country.

Keywords: *Postmortem Examination, Autopsy, Toxicological Analysis, Brain tissue, Blood, Urine.*

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Introduction

In forensic medicine practice, finding out the manner of death and the causes behind rely upon several factors – sceneinvestigation, medical history, autopsy examination and toxicological analysis[1,2]. Forensic toxicology is mainly concerned with the determination of the presence or absence and role of alcohol, drugs and their metabolites as well as other toxic substances in biological fluids, and/or tissues to solve a medico legal problem[3,4]. Forensic toxicology can be divided into three main categories[4]:

1. *Workplace or pre-employment testing*– that deals with pre-employment drug screeningas required by the workplace/government authority;

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2. *Postmortem toxicology* – which deals with the toxicology testing on deceased individuals as a routine part of the autopsy process to establish the cause of death and clarifying its circumstances in postmortem investigation, e.g.determination of the presence and the amount of toxic substance in the postmortem body including its chemical change and dilution;
3. *Human performance testing or 'criminal toxicology'* – which is used to elucidate the absence or presence of substances modifying human performance or behavioure.g.criminal offenses, driving under the influence of alcohol or drugs, committing a crime while on a drug, or having a crime committed against an individual such as a sexual assault.

In the arena of postmortem toxicology in our country, specimens that are subjected to toxicological examinations range from bodily fluids to tissues, generally focusing on blood and urine[5,6]. Since extraction and analytical techniques were refined tremendously through decades, analysis of brain material is still neither standard nor popular procedure in forensic toxicology in our country to date. This review paper aims to discuss when and howbrain tissue can be utilized as a viable postmortem toxicological specimen and its advantages over other specimens.

Brain tissue as a viable toxicological specimen

In many postmortem cases, brain could be a more helpfulthan that of bloodor urine or other body cavity fluid sample, as we know that the central nervous system is the site of action for numerous drugs and delays postmortem redistribution of chemicals, due to its secluded location[7-10]. The interpretive challenges with urine results are that a positive finding only reflects recent exposure, since the bladder is pharmacologically outside the body[8].Besides,analysis of urine reveals negative result, if death occurs closely the time of intoxication[9]. Hence, it is challenging to get the accurate concentration in toxicological analysis of urine. Similarly, postmortem blood concentrations may not necessarily reflect the drug concentration at the time of death; as drug concentrations may change as a result of body storage conditions, time and site of blood sampling[6,9]. In many postmortem cases, there is little to no blood for quantitative drug analyses, traumatic injury may lead to significant blood loss or contamination from ruptured stomach contents[8,9]. Postmortem toxicological analysis of brain tissue has many potential advantages. It is anatomically sequestered; less and delayed putrefication occurs in the brain tissue, and metabolic activity is comparatively low[7,8]. As we have discussed earlier, the protected and isolated position of the brain may eliminate the challenges of postmortem redistribution (PMR) and delay or attenuate residual enzymatic activity on certain substrates artifactually altering their concentration and concentration of any substance in brain has been found to be more stable over time when compared with other sample organs (e.g., liver) and cavity fluid[7-10]. Thus, brain tissue has some advantages over other specimens collected at autopsy. Moreover, several studies have found that drug concentrations are homogenous throughout the brain tissue and usually reflect a consistent result in analysis[6,11-13].

General Examination of Postmortem Brain

After examination of the dura, the brain is removed,weighed, and its weight recorded[14].The surface of the brain is inspected, lightly palpated for abnormalities, and the vasculature is examined.Abnormal findings are described, measured, and, whenappropriate, photographed[14-16].Next comes the part of sample collection for toxicological analysis.Sampling is of the utmost importance for a successful systematic toxicological analysis. Thereliability and accuracy of any toxicological result is usually determined by the nature and integrityof the specimen providedfor analysis. Furthermore, proper specimen selection and collection isof paramount importancefor the analytical results to be accurately interpreted with scientificvalidity, particularly whenthe results are to be used in the judicial system.

Procedure for Collection for Postmortem Brain Tissue Sample During Autopsy[1,14-17]

1. 25-30 g of brain tissue is to be collected for a plastic container with screw cap (without preservative);
2. It is basically relevant for drugs that act on the central nervous system (e.g.amphitamines, phenobarbitone, etc.);
3. However, also collected for lipophilic (e.g. drugs of abuse, organochlorated insecticides, etc.) and volatile xenobiotic analysis;

4. Nonetheless, the high lipid content may cause sometimes analytical problems;
5. In most cases, the brain tissue was found only useful for qualitative analysis.

Preservation in containers, labelling, toxicological request form and storage[1,14-17]

There is considerable variation in the types of kits used by different forensic institutions and laboratories. Regardless the format, it is key to the successful collection and consequent toxicological result to have necessary sample containers, to ensure that they are adequately labelled and that chain of custody is respected.

1. Containers should be new and preferably rinsed with distilled water and sterilized before use, unless the manufacturer's states it unnecessary;
2. If volatile xenobiotics (e.g. solvent abuse or intoxication with anaesthetic gases) are to be analysed, samples should be promptly collected and glass containers sealed with polytetrafluoroethylene (e.g. Teflon) or aluminium foil-lined lids are preferable to avoid greater losses by diffusion registered through plastic containers;
3. Containers should be filled (but not overfilled) to minimize headspace and therefore losses due to evaporation (e.g.volatiles such as ethanol);
4. Containers should be open at the time of analysis and only when cold at 4 °C;
5. A self-adhesive tamper-resistant stickers should be placed over container lids to avoid any adulteration of the specimen;
6. The labelling paper/sticker should include the following information: institutional case number identifier or request number; name of the victim or other identifier; sample type (e.g.brain), signature of the examiner; date and time of collection;
7. Toxicological analysis request forms should be filled as complete as possible, placed with samples inside a sealed plastic opaque bag and submitted to the forensic/toxicology laboratory for analysis;
8. A chain of custody report should be completed and signed asto maintain evidence of integrity of the sample/specimen.
9. Samples should be stored in tightly sealed containers at 4°C (short-term) or at "20°C or preferably at "80 °C (long-term).

Conclusion:

Since the brain is the primary site of action of many drugs, it becomes a useful specimen particularly for lipophilicsubstances such as halogenated hydrocarbons, narcotics, and antidepressants. This article aims to inform some advantages of brain tissue over other specimens like blood, urine or body fluids for toxicological analysis and general procedures for its sampling, labelling and storage. It is expected that this short review may help forensic professionals to accomplish their mission, since the toxicological result is first influenced by the quality and quantity of the sample available for analysis.

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