Relationship between causes of death and early post mortem hepatic DNA degradation

Osama S. El Okle[1], Omnia I. El Euony [1], Sherif M. Nasr [2]

[1]Department of Forensic Medicine and Toxicology, Faculty of Veterinary medicine, Alexandria University, Egypt.[2]Department of Genetics, Faculty of Veterinary medicine, Damanhur University, Egypt

ABSTRACT

The pattern of postmortem DNA degradation is frequently used as indicator of postmortem interval, but it could be affected by many internal and/or external factors. The main objective of the present study is the analysis of postmortem hepatic DNA damage in relation to time passed since death under the impact of different causes of death in order to improve our understanding about the effect of cause of death on the characters of postmortem hepatic DNA fragmentation. For this purpose, our study was carried out on four groups of rats (*n=6*).Animals were experimentally killed by slaughtering, electrocution, drowning and zinc phosphide poisoning, respectively. Liver samples were collected at zero, 2 h, 6 h, 12 h and 24 h postmortem time points. Analysis of postmortem DNA denaturation was undertaken using gel electrophoresis. Obtained data revealed presence of marked effect of the cause of death on the pattern of DNA smearing after death. Hepatic DNA of slaughtered rats showed the highest resistant to degradation till 24 h after death, while the poisoning by zinc phosphide enhance the rate of DNA degradation over all other groups. DNA band smearing in drowned rats appeared relatively earlier than those electrocuted, but the both occupied average site between slaughtered and zinc phosphide-intoxicated animals. It can be concluded that cause of death must be taken into account as a major factor could modify the early postmortem hepatic DNA damage.

KEYWORDS: Cause of death, DNA degradation, Electrophoresis, Postmortem interval.

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1. INTRODUCTION

The determination of postmortem (PM) interval is still an important task for the forensic practitioner. Several traditional methods are currently used to estimate time since death roughly. These methods depends on postmortem changes which may be classified as purely physical processes as body cooling and hypostasis, physicochemical processes as rigor mortis, metabolic processes as autolysis, bacterial and insects processes as putrefaction and cadaver entomology(Madea, 2005).Scientific attempts were paid to replace these traditional methods by more accurate measurements. Some of these efforts were successful, for example the nomogram method which developed and described by Henssge (2002).Other methods such as ^{1}H-magnetic resonance spectroscopy have also recently been applied to identify metabolites emerging from brain tissue during putrefaction(Madea, 2016).

There were several earlier studies which suggested DNA degradation as an indicator for the early postmortem interval (DiNunno et al., 1998; El-Harouny et al., 2008). But the main problem, as in any biological process, that the postmortem denaturation of DNA may be influenced by several internal and/or external factors as type of examined tissue, ambient temperature, humidity, diseased conditions and bacterial or fungal contamination(McNally et al., 1989; Cina, 1994; Graw et al., 2000).To our knowledge, the cause of death as affecting factor on the rate of early postmortem DNA degradation is still poorly investigated. Therefore, the cardinal objective of the current study was to analyze the relationship between hepatic DNA degradation and time passed since death under the effect of many different causes of death in rats.

2. MATERIALS AND METHODS

2.1. Animals and experimental protocol:

A total of twenty-four healthy male albino rats, weighing between 150-160 g were randomly and equally divided into four groups (6 each group). Animals in the first group were killed by incised wound in neck region (slaughtering), while rats in the 2nd, 3rd and 4th groups were killed via electrocution (220 V), drowning and zinc phosphide poisoning at dose of 12 mg/kg b. wt. (lethal dose according to Albretsen (2004)). Immediately after death, small incised wound in abdominal region was opened using sterilized surgical scalpel and the liver was sampled for zero time. Then at 4 different postmortem times point (2, 6, 12, 24h) liver was resampled again from the same rats, under controlled surrounding temperature of 17±2 ºC, relative humidity of 60±5%(adjusted by incubator) and instrumental sterilization. Incised wound was closed using surgical forceps in between sampling process in order to prevent the environmental contamination. The PM progression in DNA denaturation was visualized using gel electrophoresis.

2.2.Extraction and quantification of the whole genomic DNA:

Whole genomic DNA was purified from liver samples using Thermo Scientific Gene JET Genomic DNA Purification Kit following manufacturer protocols. Isolated DNA was stored at -20°C till analysis. The concentration of DNA in each sample was measured by nanodrop spectrophotometer at A_{260} and A_{280}. The ratio between absorbance value 260 and 280 nm gives estimate DNA purity (Wilfinger et al., 1997)

2.3.*Assessment of postmortem DNA degradation using gel electrophoresis:*

The degree of hepatic DNA fragmentation was visualized on gel electrophoresis. 2% agarose was prepared by weighing 1 g agarose with 50 ml of 0.5X TBE buffer in a 250 ml flask. The mixture was cooked at microwave until agarose particles were completely melted. While agarose was cooking up, the gel bed was prepared on a level bench. Just agarose was cooked up to about 60 ºC, 0.5 mg/ml ethedium bromide was added with agitation. The agarose was then poured and any bubbles should be removed. When the gel had completely solidified, it placed in to the electrophoresis tank containing sufficient electrophoresis buffer 0.5X to cover the gel with 1 mm. DNA samples which mixed with DNA loading buffer were loaded in the gel using 20 µl adjustable micro-pipette. The samples were slowly loaded to allow it to sink to the bottom of the wells. Appropriate DNA molecular weight (50 bp) marker was loaded in adjacent well. The power supply was connected and adjusted to 90 Volt for 45-75 min. DNA fragmentations were visualized on the UV transilluminator and photographed by gel documentation system(Buitkamp et al., 1991).

3. RESULTS

In general, hepatic DNA showed PM time dependent degradation. DNA kept its intactness in all groups at zero and 2 h PM time point. As expected, cause of death clearly affects the rate of hepatic DNA fragmentation. Data revealed that hepatic DNA of rats died via slaughtering able to keep its intactness longer than other groups, as smearing doesn't appear till 24 h PM time point(Fig. 1). On contrast, zinc phosphide poisoning induced the most earlier and extensive PM hepatic DNA degradation as smearing began to visualize at 6 h PM time point, while DNA was clearly and markedly degraded at 12 and 24 h PM time point(Fig. 2). Despite there was a degree of similarity in the rate of PM genomic DNA degradation in those killed by drowning and electricity, it take place earlier in drowned animals at which smear could be detected at 12 h PM(Fig. 3), while the intactness of DNA in electrocuted group clearly affected after 24 h PM (Fig. 4).

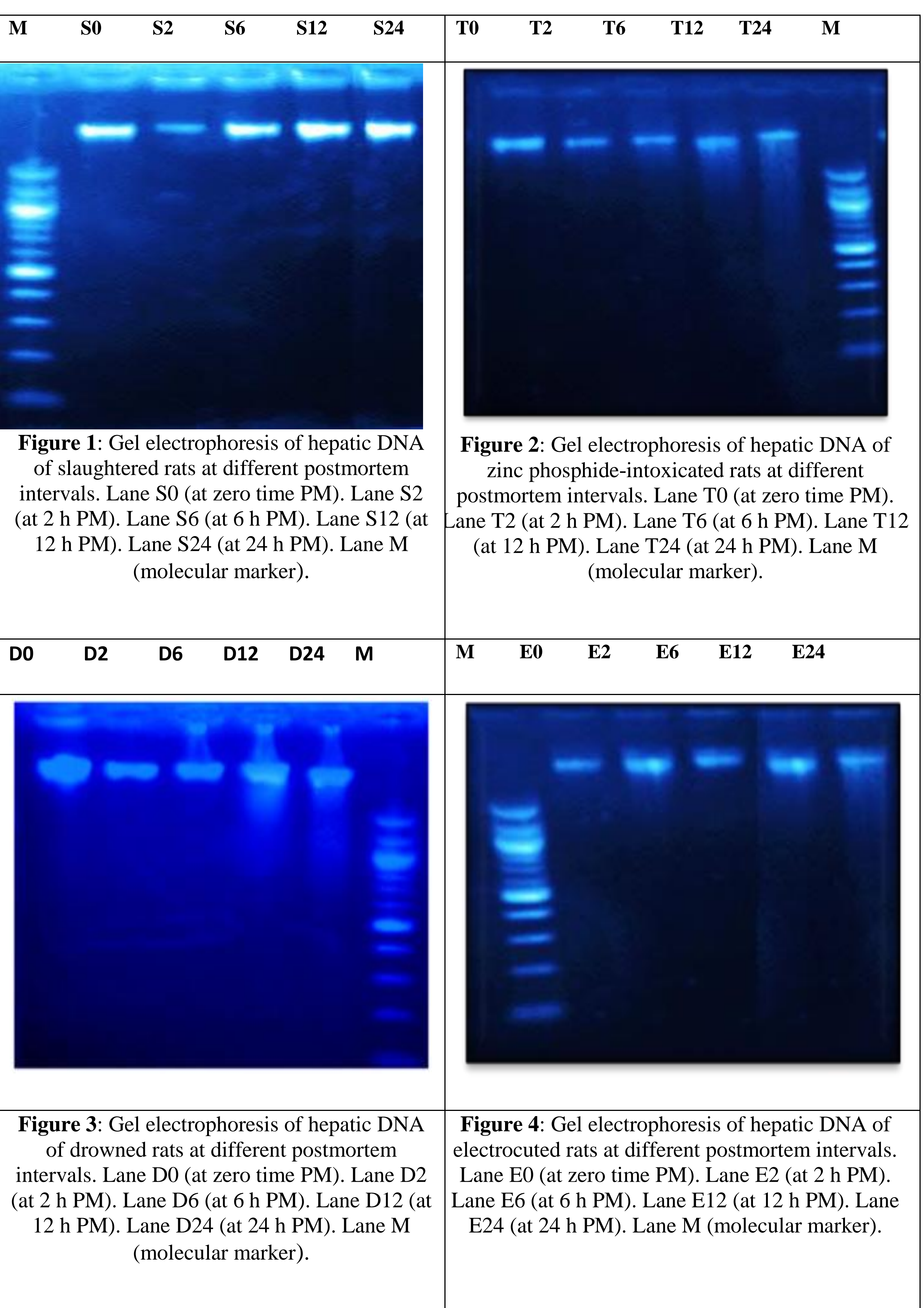

Figure 1: Gel electrophoresis of hepatic DNA of slaughtered rats at different postmortem intervals. Lane S0 (at zero time PM). Lane S2 (at 2 h PM). Lane S6 (at 6 h PM). Lane S12 (at 12 h PM). Lane S24 (at 24 h PM). Lane M (molecular marker).

Figure 2: Gel electrophoresis of hepatic DNA of zinc phosphide-intoxicated rats at different postmortem intervals. Lane T0 (at zero time PM). Lane T2 (at 2 h PM). Lane T6 (at 6 h PM). Lane T12 (at 12 h PM). Lane T24 (at 24 h PM). Lane M (molecular marker).

Figure 3: Gel electrophoresis of hepatic DNA of drowned rats at different postmortem intervals. Lane D0 (at zero time PM). Lane D2 (at 2 h PM). Lane D6 (at 6 h PM). Lane D12 (at 12 h PM). Lane D24 (at 24 h PM). Lane M (molecular marker).

Figure 4: Gel electrophoresis of hepatic DNA of electrocuted rats at different postmortem intervals. Lane E0 (at zero time PM). Lane E2 (at 2 h PM). Lane E6 (at 6 h PM). Lane E12 (at 12 h PM). Lane E24 (at 24 h PM). Lane M (molecular marker).

4. DISCUSSION

Estimation of postmortem interval (PMI) is still a challenge in forensic medicine. With the development of molecular biological techniques, DNA quantification methods were widely applied in estimating PMI. After death, DNA, as any biological structure, rapidly decompose under the effect of nucleases and decaying bacteria, resulting in fragmentation, base alteration, cross-linking, and other forms of degradation. The degree of DNA damage was proportionally related to time since death (Boy et al., 2003; Lin et al., 2011).The intensive evaluation for the correlation between DNA fragmentation and time elapsed after death showed that it could be seriously affected by many factors as surrounding temperature, tested tissue (El-Harouny et al., 2008),salt mummification(Shved et al., 2014),Therefore, there is a great recommendation to investigate the impact of different causes of death on rate of PM hepatic DNA degradation in order to supply forensic practitioner with data must be taken inconsideration during using of DNA degradation as method for PMI determination. In the present study, rats were subjected to death via different methods of death. Slaughtering(as example for traumatic deaths and hemorrhage), electrocution (as example for electricity related deaths), drowning (as example for violent asphyxia), and zinc phosphide poisoning (as example for deaths caused by toxicants).

A variety of different tissues were used as a source of sample that would demonstrate the best correlation between DNA degradation and time since death. Although spleen have been recommended by several investigators (Cina, 1994; Boy et al., 2003), but hepatic tissue showed the best linear correlation between the time since death and the level of DNA degradation(Di Nunno et al., 2002). The outcome of other literatures showed that brain, lymph nodes and skeletal muscles could be a suitable source of DNA for up to 3 weeks, whereas liver lost high molecular DNA after 2 days due to the rapid decomposition rate and the relatively more ribonucleases activity (Finger et al., 1987; Alaeddini et al., 2010; Zaki et al., 2017). In the field of forensic medicine, the majority of cadavers usually discovered within 48 h PM. Therefore, in the current study genomic DNA was isolated from the liver at early PM period (within 24 h).

The analysis of the integrity of DNA after death was carried out using simple and quick electrophoresis method to become easily and routinely applicable in crime investigation lab. Results confirmed that hepatic DNA was degraded gradually in correlation to time passed after death in all groups, but the pattern of this fragmentation was clearly affected by the cause of death. Obtained data could be discussed in the light of that slaughtering usually associated with loss of large volume of blood. It was established that massive blood loss is considered as primary condition of decomposition retardation (Bardale, 2011)which is subsequently reflected as delayed DNA degradation in the present study.In contrast, hepatic DNA of zinc phosphide-intoxicated rats showed the most extensive and rapid damage among all experimental groups. Similarly, Muid et al. (2012) confirmed the ability of zinc phosphide to induce DNA damage in blood cells of the fowl by using comet assay.The ability of zinc phosphide to induce hepatic DNA damage seems to be related to the production of reactive oxygen species(Oghabian et al., 2016) and would be responsible for the rapidity of PM DNA denaturation in the current study. Our finding also was in agreement with El-Harouny et al. (2008) who observed that PM DNA degradation in the liver of drowned rats was mild till 6h after death and become moderate at 12 h then severe at 24h after death. Moreover, obtained data revealed that the hepatic DNA lost its intactness in drowned rats earlier than those electrocuted. This result may be related to the saturation of the

drowned bodies with water which gets optimum condition for the growth of putrefactive microorganism and accelerate decomposition rate once extracted from the water(Nandy, 2012).

5.CONCLUSION

It can be concluded that despite the well-established proportional relationship between PM DNA degradation and time since death, cause of death has the potential to change its rate of degradation which could affect interpretation of DNA analysis when used as a method of PMI determination.

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