The Relationship Between Postmortem Interval And Dna Degradation In Different Tissues Of Drowned Rats
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Citation

Abstract
Upon the death of an organism, internal nucleases contained within the cells should cause DNA to degrade into smaller fragments over time, if these fragments can be isolated and visualized, and if the fragmentation is proved to be measurable and quantifiable, it can be a good indicator of the postmortem interval (PMI). This study aimed to evaluate the effect of PMI on DNA degradation in different tissues of drowned rats through quantitative analysis of DNA degradation by easily applicable method. METHODS: To profile postmortem degradation of DNA, it was extracted, at different PMI (0, 3, 6, 12, 24 hours), from the brain, lungs, spleen, liver and skeletal muscles of drowned rats. Electrophoresis method was used to detect the relationship between the amount of degraded DNA and PMI in different tissues. The present research used a simple, easy, applicable and highly informative electrophoresis method that make it an ideal for the busy forensic laboratory. RESULTS: The postmortem DNA fragmentation observed in this study, reveals a sequential, time-dependent process with the potential for use as a predictor of PMI in cases of drowning. CONCLUSIONS: There is linear relationship between the degradation rate of nuclear DNA and PMI in some studied viscera like liver. Some organs like brain showed slower degradation rate of DNA. So, it is considered as a valuable organ for studying DNA in longer PMI. This result shows a potential for use as a future applied method of evaluating time since death.

INTRODUCTION
One of the most important longstanding problems in the field of forensic medicine is the determination of the time of death upon the discovery of a possible homicide victim. With a majority of homicide victims discovered within the first 48h, it is critically important to be able to determine time of death quickly, and with accuracy and precision. Current methods of determining postmortem interval (PMI) vary, but none can provide better than an 8-h window time estimate (Johnson and Ferris, 2002).

The time of death of an individual can easily be determined if the postmortem interval can be assessed. Although livor mortis, rigor mortis, and, to a lesser degree, algor mortis have been used to estimate the postmortem interval, most experienced forensic pathologists agree that these characteristics provide, at best, "postmortem windows" (Cina, 1994).

During postmortem autolysis, cellular organelles and nuclear DNA break down into their constituent parts. DNA analysis was applied as a possible method for postmortem interval determination (Boy et al., 2003). Determining the quantity of DNA should be an objective and exact way to estimate the PMI (Liu et al., 2001). So, it is important to know which organ is most reliable for DNA extraction and also to know the effect of PMI on DNA degradation.

Several methods have been developed to quantify DNA, from basic UV spectrometry, through gel-based techniques, to dye staining, blotting techniques, and, very recently, DNA amplification methods (polymerase chain reaction, PCR) (Nicklas and Buel, 2003). The present search used a simple, easy, applicable and highly informative electrophoresis method that make it an ideal for the busy forensic laboratory. Liu et al. (2007) suggest that computerized image analysis technique CIAT is a useful and promising tool for the estimation of early PMI with good objectivity and reproducibility as quantitative indicator for the estimation of PMI within the first 36 h after death in rats.

ANIMALS AND METHODS
Animals: The study included forty Albino rats that were classified into 5 groups; first group rats were sacrificed immediately after drowning (as a method of inducing death), while the 2nd, 3rd, 4th, 5th groups were sacrificed at 3, 6, 12, 24 hours postmortem respectively. Animals were dissected to obtain organs (lung, liver, spleen, muscle and brain). DNA of rats’ viscera were detected by gel electrophoresis and the amount of DNA were measured by detecting its optical density (OD) employing an image analysis program.


Equipments: 1-Eppendorf tube (Naser company). 2-Blue tips. 3-Deepfreezer-20ºC (Ideal company). 4-Microcentrifuge (Beckman). 5-UV trasilluminator (Biometra). 6-Horizontal electrophoresis (Biometra standard power PACKP25). 7-Polaroid camera (Gelcam electrophoresis HOOD 0,7x). 8- Micropipette (Finn pipette 40-200 ul). 9-Incubator (Bst 5010).

**METHOD OF DNA STUDYING:**

Gel preparation: Gel was prepared using 1.8% electrophoretic grade agarose (BRL). The agarose was loaded with tris borate EDTA buffer (1xTBE buffer, 89mM Tris, 89mM boric acid, 2mM EDTA, pH 8.3) and then, 0.5 microgram /ml ethidium bromide was added to agarose mixture at 40ºC. Gel was poured and allowed to solidify at room temperature for 1 hour before samples were loaded.

DNA extraction and apoptosis detection in tissue: Nucleic acids extraction and detection of apoptosis was done according to (salting out extraction method) of Aljanabi and Martinez, (1997) and modification introduced by Hassab El-Naby,(2004). Where, a piece of 10 mg of liver, spleen, brain, muscle and lung tissues was squeezed by blue tips and lysed with 600 microlitre lysis buffer (50 mM NaCl,1 mM Na2 EDTA,0.5% SDS, pH 8.3) and gently shaken. The mixture was incubated overnight at 37ºC then, 200 microlitre of saturated NaCl was added to the samples, shaken gently and centrifuged at 12,000 rpm for 10 min. The supernatant fluid was transferred to new eppendorf tubes and then DNA was precipitated by 600 microlitre cold isopropanol. The mix was inverted several times till fine fibers appear , and then centrifuged for 5min. at 12000 rpm. The supernatant fluid was removed and the pellets were washed with 500 microlite 70%ethyl alcohol, centrifuged at 12000 rpm for 5min .After centrifugation, the alcohol was decanted or tipped out and the tubes blotted on Whatman filter paper, till the pellets appeared to be dry. The pellets were resuspended in 50 microliter or appropriate volume of TE buffer (10 mM tris, 1mM EDTA, and pH8) supplemented with 5% glycerol. The resuspended DNA was incubated for 30-60 min. with loading mix (Rnase+ loading buffer) and then loaded directly into the gel-wells.

Electrophoresis: Electrophoresis was performed for 2 hours at 50 volt in gel buffer (1 X TBE buffer) at room temperature with buffer level 2 mm cover the gel. Gel was photographed using a Polaroid camera while the DNA was visualized using a 312 nm UV transilluminator. Electrophoretic pattern of nucleic acids determined total genomic damage of DNA. The intensity of DNA nucleoprotein was measured by Gel-Pro computer program as maximum optical density values (max.O D).

Statistical Analysis: These data were run on an IBM compatible personal computer by using Statistical Package for Social Scientists (SPSS) for windows 11 (SPSS Inc., Chicago, IL , USA). Data were compared by using two types of statistics; Descriptive statistics: e.g., mean (x) and standard deviation (SD) and analytical statistics: e.g., student’s t-test (to compare two groups) was used to test association between variables. P value of <0.05 was considered statistically significant.

**RESULTS**

Liver (Plate 1 and gel Proanalyzer curve 1); there is intact DNA at zerotime , mild DNA damage at 3h and 6h PM, moderate DNA damage at 12h PM, severe DNA damage at 24h PM. Table (1) show comparison of optical density of liver DNA at different PMI.

Spleen (Plate 2 and gel Proanalyzer curve 2); intact DNA at zerotime , mild DNA damage at 3h PM, moderate DNA damage at 6h and 12h PM and severe DNA damage at 24h PM. Table (2) show comparison of optical density of spleen DNA at different PMI.

Lung (Plate 3 and gel Proanalyzer curve 3); intact DNA at zerotime , moderate DNA damage at 3h and 6h PM, severe DNA damage at 12h and 24h PM. Table (3) show
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comparison of optical density of lung DNA at different PMI.

Brain (Plate 4 and gel Proanalyzer curve 4); intact DNA at zerotime, 3h and 6h PM with mild DNA damage at 12h and moderate DNA damage at 24h PM. Table (4) show comparison of optical density of brain DNA at different PMI.

Muscle (Plate 5 and gel Proanalyzer curve 5); there is intact DNA at zerotime, mild DNA damage at 3h, moderate DNA damage at 6h PM and severe DNA damage at 12h and 24h PM. Table (5) show comparison of optical density of muscle DNA at different PMI.

**Figure 1**
Plate (1): Gel electrophoresis of extracted DNA from rat liver at different PMI after drowning.

**Figure 2**
Plate (2): Gel electrophoresis of extracted DNA from rat spleen at different PMI after drowning.

**Figure 3**
Plate (3): Gel electrophoresis of extracted DNA from rat lung at different PMI after drowning

**Figure 4**
Plate (4): Gel electrophoresis of extracted DNA from rat brain at different PMI after drowning.
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Figure 5
Plate (5): Gel electrophoresis of extracted DNA from rat muscle at different PMI after drowning.

Figure 6
Chart (1): Computer chart by gel Pro-analyzer shows the intensity of DNA against the molecular weight of each specified base pairs in rat liver at the studied PMI after drowning

Figure 7
Chart (2): Computer chart by gel Pro-analyzer shows the intensity of DNA against the molecular weight of each specified base pairs in rat spleen at the studied PMI after drowning

Figure 8
Chart (3): Computer chart by gel Pro-analyzer shows the intensity of DNA against the molecular weight of each specified base pairs in rat lung at the studied PMI after drowning

Figure 9
Chart (4): Computer chart by gel Pro-analyzer shows the intensity of DNA against the molecular weight of each specified base pairs in rat brain at the studied PMI after drowning

Figure 10
Chart (5): Computer chart by gel Pro-analyzer shows the intensity of DNA against the molecular weight of each specified base pairs in rat muscle at the studied PMI after drowning
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DISCUSSION

Determination of the PMI is one of the most valuable subjects in forensic practice. However, it is often very difficult to accurately determine the PMI in daily practice. Forensic DNA technology has recently been used to estimate the PMI (Hao et al., 2007). DNA decays after death, in biological samples, and the ensuing damage is manifested in many forms (Gilbert et al., 2003). So, this study aimed to profile postmortem degradation of DNA in relation to PMI. DNA was extracted from the brain, lungs, spleen, liver and skeletal muscles of drowned rats at different PMI (0, 3, 6, 12 and 24 hours postmortem). Total genomic damage of DNA was determined by gel electrophoresis and its intensity was measured by software Gel Pro analyzer computer program as maximum optical density.

In drowning cases, no previous researches studied the relation between PMI and DNA amount in these organs by using this modified electrophoresis method.

Generally, results of this study revealed gradual degradation of intact nuclear DNA in the studied organs with increasing PMI. These findings coincide with those of Luo et al. (2006) who showed gradual decrease of bone marrow DNA with prolongation of PMI. Concerning DNA maximal optical density, it showed a significant lower mean values in the studied organs with increasing the PMI than control group at zero time at intact DNA which was prominent in the lungs beginning from 3 hours PM and in the spleen beginning from 6 hours PM as seen in their computer charts. While, there is a significant higher mean value of maximum optical density than control group at 600, 400 and 200 base pairs which is prominent in the liver. In agreement with these findings, Johnson and Ferris (2002) reported that in tissues such as liver and kidneys, enzymes tend to be more active and accelerate DNA decomposition. In the present study, the used method was useful in detection of fragmented DNA in the liver up to 24 hours PM. Also, Lin et al. (2000) observed that the DNA degeneration rate of liver cells had a linear relationship to early postmortem period in rats.

Regarding spleen, there was descendent trend of the amount of intact DNA at the different PMI. This was similar to conclusion of Liu et al. (2004) by using flow cytometry while the method used in this study is much easier in application. Chen et al. (2005) showed also a good relationship between spleenic DNA degradation and PMI. The fragmentation in DNA had begun in lungs and skeletal muscles at 3 and 6 hours respectively; this may be also attributed to the presence of many enzymes in these organs. Also, postmortem skeletal muscles up regulate proteolysis related genes (Sanodou et al., 2004). Considering brain DNA degradation, it occurred at slower rate than other organs and become prominent at 24 hours PM. Leonard et al. (1993) concluded that human postmortem brain collections will continue to be valuable resources for the study of gene expression and isolation of nucleotide sequences.
According to the previous results of this study, it can be concluded that the degradation of DNA shows a well relationship with early PMI (up to 24 hours) in the studied organs. This degradation revealed sequential time dependent process with the potential for use as a predictor of PMI. The slower degradation of brain DNA invites more research use of molecular genetic techniques for the study of PMI from this organ.

The present study used a simple, easy, applicable and highly informative electrophoresis method that make it an ideal for the busy forensic laboratory. So, this method can be used for a reliable and sensitive analysis of PMI and future human studies should be considered with more prolonged PMI. It is also recommended to study DNA degradation and PMI in different causes of death for revealing if there is any effect of the cause of death on DNA degradation rate.

References


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