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Original Research Article

DNA quantification as a determinant factor of postmortem time interval in different models of death

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ABSTRACT

The quality and quantity of DNA have a vital role in forensic medicine. As time elapsed since death of recovered body by drowning or submersion should be identified in homicidal or suicidal manner of death or animal sudden deaths. Sixty rats were used in this experiment in 3 models of natural death, drowning and submersion. The DNA quantity was determined by diphenylamine in the brain, heart and lungs. It was noticed that there was a correlation between reduction of DNA quantity and postmortem time interval in the natural model of death. While the submersion >freshwater drowning>saltwater drowning enhanced DNA degradation, especially between 24-48 hours after death. Brain DNA considers the most resistance for degradation than heart and lung. In conclusion, DNA concentration in tissues could correlate to the postmortem time interval in natural death and other models of deaths as drowning or submersion but with different rate of reduction of DNA quantity.

1. Introduction

DNA based technology is a routine technique for identification of unknown decomposed bodies, especially in disasters as DNA degradation could correlate to the degree of postmortem changes and postmortem time interval (PMI).[1] The determination of DNA quantity should be considered as a reliable method for PMI estimation.[2] Postmortem time interval is not an easy task and still controversial up till now in forensic medicine even advanced molecular techniques like single gel electrophoresis, flow cytometry and Feulgen staining imaging analysis that was used in DNA quantification in tissues after death as evidence of DNA degradation.[3,4] DNA amount was detected in various organs like heart, liver, kidney and spleen after death and it was found that spleen DNA was the best correlated to postmortem time interval as evidence of DNA degradation in human corpses,[5] rats[6] and mice.[7] Moreover, the using of various tissues as samples for DNA fragmentation analysis would indicate the best correlation between time since death and DNA fragmentation.[8] The correlation between PMI and DNA denaturation based on many factors, especially type of tissue, ambient temperature[9] and DNA degradation rates could be affected by other factors like pH value and disease**.** In drowning of rats, there was a linear relationship between the degradation rate of nuclear DNA and PMI at 0, 3, 6, 12 and 24 hours on tissues like liver, spleen, lung and muscle.[9,10] The aim of this study to investigate the

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correlation of PMI and DNA concentration in the heart, brain and lung in natural induced death and drowning and submersion in fresh and salt water at different time points.

2. Materials and Methods

2.1. Animal protocol

Experimental procedures was conducted on animals were done with the approval of the animal care committee of the ethics Board of the faculty of veterinary medicine, Mansoura university, Egypt.

Sixty male Sprague Dawley rats (weight, 230-260 g) were purchased from faculty of pharmacy, Mansoura University, EGYPT). All rats were maintained on a 12-h light/dark cycle with free access to food and water.

2.2. Experimental groups

2.2.1. First experiment

20 male rats were exposed to brain stem death that carried out according to Zhang et al.[11] Four dead rats were kept in fixed supine position at room temperature for each time points 0, 6, 24, 48 and 72 hours and tissues as heart, lung and brain were separated and stored at -20 for extraction of DNA.

2.2.2. The second experiment

Twelve male rats were drowned in fresh and other twelve male rats were drowned in saltwater and four drowned rats for each time points were extracted, kept in room temperature for 0, 24 and 48 hours. Tissues of heart, lung and brain were separated and stored at -20 for extraction of DNA.

The third experiment: sixteen male rats were exposed to brain stem death that carried out according to Zhang et al.[11] and then eight submerged rats under fresh or saltwater for 24 or 48 hrs. Tissues of heart, lungs and brain were separated and stored at -20 for extraction of DNA.

2.3. DNA extraction and measurement

The tissues were homogenized, and DNA extracted by the method described earlier by Munro and fleck[12] and DNA concentration (mg/gm of tissue) was measured by a spectrophotometer at wavelength 595 after diphenylamine reaction[13] and standard curve carried out by standard different concentration of DNA.

2.4. DNA quality and detection by using the Agarose Gel Electrophoresis

All DNA samples after extraction were preserved in DNA lysis buffer. Then 10 ng of each DNA sample and 0.5 μg/ml of ethidium bromide (EtBr) were running in gel electrophoresis (1 gm of agarose dissolved in TAE (40 mM Tris-acetate, 1 mM EDTA) and TBE (45 mM Tris-borate, 1 mM EDTA) for 15 to 30 min then imaging with UV.[14]

2.5. Statistical analysis

The one-way ANOVA and the student t-test were used to evaluate statistical significance.[15] (p-value cutoff determined as 0.05). All statistical analyses were done by SPSS Statistics 13.

3. Results

3.1. DNA degradation after natural death

It was recorded that DNA concentration was reduced along with time progress after death due to postmortem tissue degradation that resulted in DNA degradation. Heart and lung tissues showed a significant decrease in DNA quantity started after 6 hours of natural induced death and continued to the time of the experiment. Meanwhile, the brain showed a slower rate of degradation that was detectable at 24 hours after death. (Figure 1)

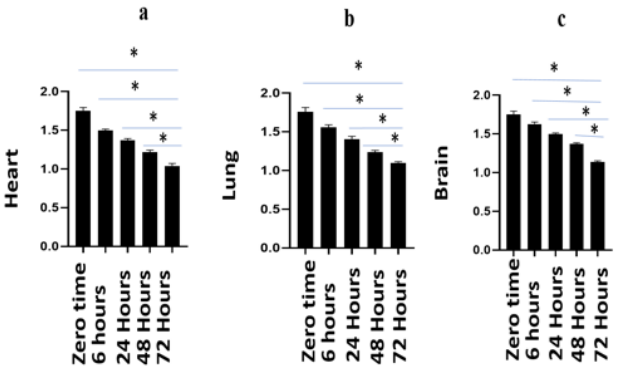

Fig. 1: DNA concentration (mg/ gm of tissue) atdifferent time points after natural death induction In; **a:** Heart; **b:** Lung and; **c:** Brain tissues at zero, 6,24,48 and 72 hours after rats death.

3.2. DNA degradation after both fresh- and/or saltwater drowning

DNA quantity in recovering freshwater drowned rats showed a high rapid downwards compared to natural death. Heart and lung tissue showed a significant reduction with time progress noticed at 24 hours after death. While brain DNA concentration reduced at 48 hours after death. Moreover, DNA degradation in case of recovered dead rats after saltwater drowning noticed to be slower compared to freshwater drowning and natural death due to the preservative effect of salt water on tissue that delay tissue degradation (Figures 2 and 3).

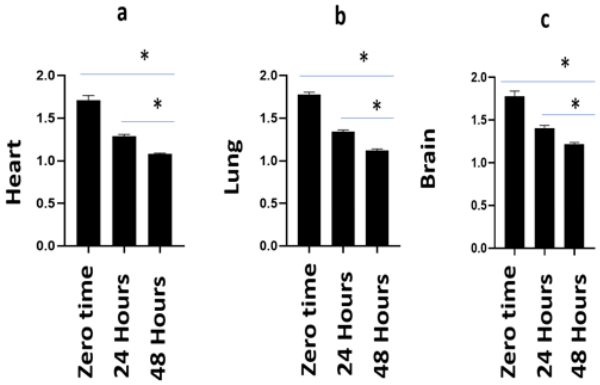

Fig. 2: DNA concentration (mg/ gm of tissue) atdifferent time points after freshwater drowning of rats in; **a:** heart; **b:** Lungand; **c:** Brain tissues at zero, 24 and 48 hours after death.

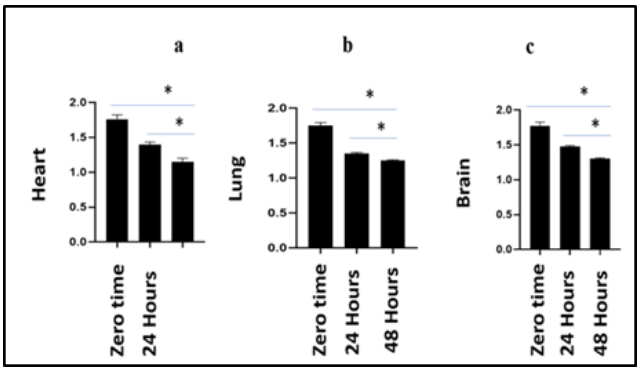

Fig. 3: DNA concentration (mg/ gm of tissue) at different time points after saltwater drowning of rats in; **a:** Heart; **b:** Lung and; **c:** Brain tissues at zero, 24 and 48 hours after rat's death.

3.3. DNA degradation after both fresh- and saltwater submersion

DNA quantity reported to be more decreased in freshwater submersion compared with saltwater submersion. This decrease depends on how much time the dead body remained in submersion state comparable to both fresh and saltwater drowning DNA concentration showed more decrease in submersion cases which noticed more in the case of freshwater submersion. Notably, submersion in either fresh or salt water accelerated the DNA degradation immediately after recovery from water when compared to the natural death model at different time points (Figures 4 and 5).

3.4. DNA quality test for detection of DNA degradation or loss in postmortem models of death.

DNA still appeared until 72 hrs. in natural model of death (lane1-12, 48 hrs. of fresh and salt drowning (lane 13-17). While DNA in submersion cases showed less quality

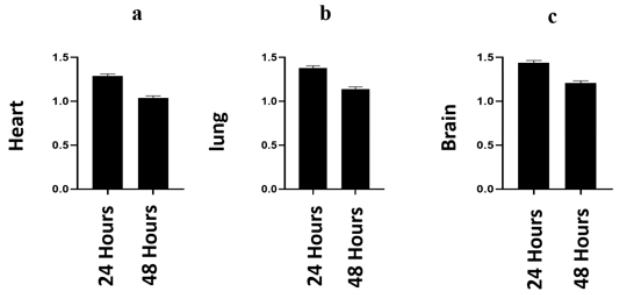

Fig. 4: DNA concentration (mg/ gm of tissue) at differenttime points after freshwater submersion In; **a:** Heart; **b:** Lung and; **c:** Brain tissues at 24 and 48 hours after induction of natural death in rats.

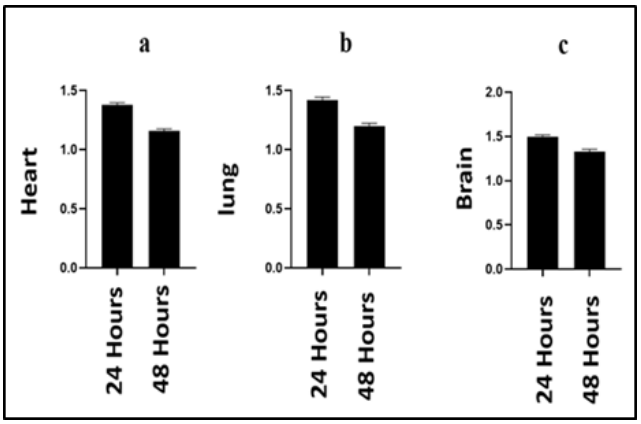

Fig. 5: DNA concentration (mg/ gm of tissue) at different time points after saltwater submersion In; **a:** Heart; **b:** Lung and; **c:** Brain tissues at 24 and 48 hours after induction of natural death in rats.

when compared to other model of natural death (lane 17, 18,19)(Figure 6)

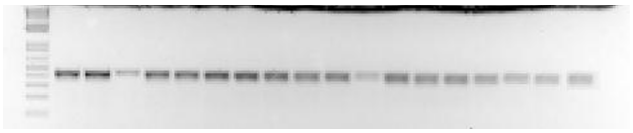

Fig. 6: Showed the DNA quality and degradation in natural model of death (lane 1-12), drowning and submersion (lane 13-17) and submersion (lane 17,18,19). Also, lane 3, 11 showed a little faint band of DNA concentration and complete loss of DNA in lane 19 in case of one sample of submersion rats.

4. Discussion

The estimation of the PMI is critically important in various human death investigations and is similarly important in some animal forensic medicine investigations.[16] To better understand the effects of different causes of deaths on

relation to PMI, we compared natural model of death with drowning or submersion in both freashwater or saltwater at different time points. As DNA is more stable, its extraction and characterization are very important for measurement of DNA quantity, quality and validations studies[17] and also measurements of DNA quantity is important in forensic medicine to identify present or absent of DNA and degradation status of DNA in the sample for identification of unknown cases.[18] Notably, the DNA degradation was more enhanced in the submersion >freshwater drowning>saltwater drowning respectively and could correlate to PMI (Figures 1, 2, 3, 4 and 5). The DNA degradation in tissue after death resulted from chemical alteration, strand breakage, and microbial attack. These autolysis processes reduced the yield of high molecular DNA quantity and reduced the chance of subsequent PCR identification.[19] There were many factors that affected the DNA degradation, one of the biggest factors in aqueous environments that enhanced the tissue damage were hydrolysis, or the breakage of chemical bonds through the addition of water.[2] The hydrolysis resulted in damage of the DNA through deamination, depurination and or depyrimidination.[2] The DNA of brain tissue considered the most resist the degradation process.[9,20–22] While the DNA degradation of heart could be a hallmark for early PMI estimation as rate of DNA degradation in first 6 h after death had a linear correlation with postmortem interval (Figure 1).[2,5] The degradation DNA was greater and sometimes rapidly in the setting of rats recovered after submersion and decomposition when compared to control zero or the natural model of death (Figures 5 and 6).[23] Also, the temperature of water that stored the drowned rats or submerged ones could delay the DNA degradation[7] and then the degradation was accelerated in rats recovered bodies due start of autolysis and putrefaction. Moreover, the DNA quantity and quality of recovered bodies from different tissues was affected by both the duration of immersion and also by the type of water that the remains were submerged in[24] and in drowned rats, the DNA could still be identified from clothes exposed to water for more than 1 week in winter and 4 hours in summer.[25] Similarly, there was a correlation between DNA quantity or degradation in different tissues like brain, heart and other tissues and PMI[8,21–31] and more specific in drowned rats.[9,20] The limitation of total DNA quantity and PMI correlation as it cannot be able to differentiate between human DNA and bacterial or fungal DNA.[23]

5. Conclusion

DNA concentration in tissues of brain, heart and lung could correlate to PMI in the natural death model and other models of death like drowning or submersion but the rate of reduction of DNA was different when the cause of death, circumstantial and storage of cadaver were different. The current findings need more investigation in human corpse to find constant PMI for each model of death.

6. Conflict of Interest

The authors declare that there is no conflict of interest.

7. Source of Funding

None.

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