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Postmortem Time Affects Brain, Liver, Kidney and Heart DNA in Male Rat

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Abstract The aim of this paper is to evaluate the effect of post-mortem time (0, 24, 48 h) on the integrity of DNA extracted from the brain, liver, heart and kidney of male albino Sprague –Dawley rats. Random Amplification of Polymorphic DNA–Polymerase Chain Reaction (RAPD-PCR) followed by agarose electrophoresis were used to detect the correlation between DNA integrity and post mortem time. The results of the post-mortem DNA profile showed that DNA degradation was a time dependent process. DNA from brain, as compared to liver and kidney DNA, showed a slower degradation rate and therefore brain could serve as a valuable organ for studying degradation in longer post-mortem time. This study provides the first DNA profile analysis of the postmortem progress of DNA degradation in rats.

Keywords Postmortem time, DNA degradation, RAPD-PCR, Brain, Liver, Kidney

1. 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 [1]. 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 post-mortem interval (PMI) vary, but none can provide better than an 8-h window time estimate [1-5]. Deoxyribonucleic acid (DNA) analysis was applied as a possible method for post-mortem interval determination [6-10].

The determination of the quantity of DNA should be an objective and exact way to estimate the PMI [11-14]. Therefore, it is important to know which organ is most reliable for DNA extraction and also to know the effect of PMI on DNA degradation. Although *livor mortis, rigor mortis*, and to a lesser degree, *algor mortis* have been used to estimate the post-mortem interval, most experienced forensic pathologists agree that these characteristics provide at best, "post-mortem windows" [15-18].

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) [19]. Application of DNA analysis for the personal identification of decomposed body has had difficulties [20, 21]. Of these difficulties, the most important is the degradation of template DNA [22, 23], which is thought to be caused mainly by intracellular enzymes and decomposition through bacterial proliferation in the corpse [24-29].

The present study used a simple, easy, applicable and highly informative electrophoresis method that makes it ideal for the busy forensic laboratory. Liu *et al*. [2] 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 36h after death in rats. DNA degradation and the postmortem interval in combination with conditions such as temperature have been investigated in some studies [24-26]. However, the relation has not been investigated in sufficient detail. The objective of the present study is to investigate the postmortem time on DNA extracted from the brain, liver and heart of male Sprague - Dawley rats.

2. Methods

Twenty male Sprague - Dawley albino rats (174.53±6.93g) obtained from the Animal House of the National Institute for Medical Research (NIMR), Yaba, Lagos, were used in this study. The rats were divided into four equal groups, designated as Rat Groups A, B, C, and D. The rats were sacrificed via cervical dislocation, their brain; liver, heart and kidney were excised at point of death (0h) into a micro tube and labeled. Samples were also collected 24h and 48h later. The DNA extraction was carried out according to manufacturer's instructions of the QIAmp DNA Extraction Kit. DNA profiling was carried out by Random

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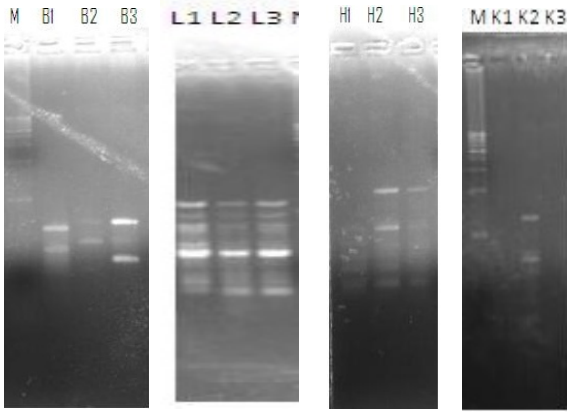
Amplification of Polymorphic DNA Polymerase Chain Reaction (RAPD-PCR). The RAPD PCR was carried out using RAPD primer-OPC-04: CCGCATCTAC [2].

The PCR was performed in 25 µl of a reaction mixture containing DNA (10-200ng), 200 µM of each deoxynucleoside triphosphates (dNTP) (Promega), 2.5 mM $MgCl_2$, 1X PCR Buffer, 20 pMol primer, 2.5 unit of *Taq DNA* polymerase (Promega) and sterile distilled water. Thermal cycling was conducted in an Eppendorf Master Cycler Gradient for an initial denaturation of 94°C for 5min followed by 40 amplification cycles of 1min at 94ºC; 1min at 28ºC and 1min at 72ºC. This was followed by a final extension step of 10min at 72ºC. The amplification product was separated on 1% agarose gel electrophoresis and visualized by ethidium bromide staining. 1kb DNA ladder was used as DNA molecular weight standard.

3. Results

The results of RAPD profiles of the brain, liver, heart and kidney of Rat Group A at 0h, 24h and 48h post-mortem are presented in Figure 1.

RAT A



(A) (B) (C) (D)

Figure 1. RAPD profiles of the brain, liver, heart and kidney of RAT A at 0h, 24h and 48h post-mortem

Where A = the profile of the Brain DNA at 0h (B1), 24h (B2) and 48h (B3) post-mortem is slightly inconsistent as the hours passed on.

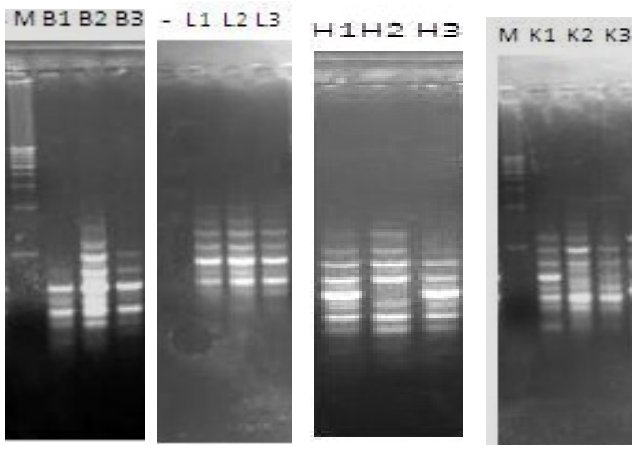
B = the profile of the Liver DNA is well defined at 0h (L1) PM, 24h (L2) PM, 48h (L3) PM. There is consistency in the banding pattern.

C = the profile of the Heart DNA is poorly defined at 0h PM (H1) but well defined with consistency in the banding pattern at 24h PM (H2) and 48h PM (H3).

D = the profile of the Kidney DNA at 0h (K1) is not well defined, DNA profile is slightly defined 24h (K2) PM and no definition 48h (K3) PM.

The results of RAPD profiles of the brain, liver, heart and kidney of Rat Group B at 0h, 24h and 48h post-mortem are presented in Figure 2.

RAT B



(A) (B) (C) (D)

Figure 2. RAPD profiles of the brain, liver, heart and kidney of RAT B at 0h, 24h and 48h post-mortem

Where A = the profile of the Brain DNA at 0h (B1), 24h (B2) PM and 48h (B3) PM is well defined with some of the banding patterns being consistent showing high integrity of DNA in the brain.

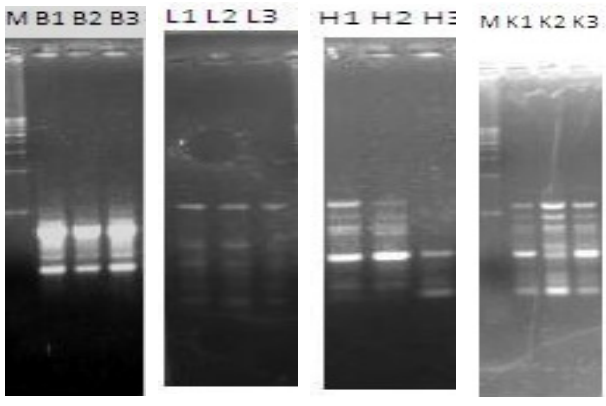
B = the profile of the Liver DNA at 0h (L1), 24h (L2) PM and 48h (L3) PM is well defined with some of the banding patterns being consistent showing high integrity of DNA in the liver.

C = profile of the Heart DNA at 0h (H1), 24h (H2) and 48h (H3) PM is well defined with great consistency in the banding patterns indicating high integrity of DNA in the heart of the organism.

D = the profile of the KidneyDNA at 0h (K1), 24h (K2) PM and 48h (K3) PM is well defined with some of the banding patterns being consistent showing high integrity of DNA in the kidney.

The results of RAPD profiles of the brain, liver, heart and kidney of Rat Group C at 0h, 24h and 48h post-mortem are presented in Figure 3.

RAT C



(A) (B) (C) (D)

Figure 3. RAPD profiles of the brain, liver, heart and kidney of RAT C at 0h, 24h and 48h post-mortem

Where A = the profile of the Brain DNA at 0h (B1), 24h (B2) PM and 48h (B3) PM is well defined with the banding patterns being consistent showing high integrity of DNA in the brain

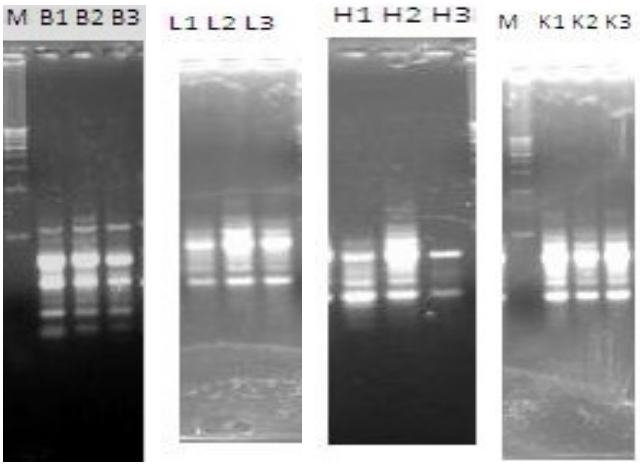
B = the profile of the Liver DNA at 0h (L1), 24h (L2) PM and 48h (L3) PM is well defined with banding patterns consistent at this hours, showing high integrity of DNA at this hour in the liver.

C = the profile of the Heart DNA at 0h (H1), 24h (H2) PM and 48h (H3) showed disappearance of bands with later PMI indicating reduction in the integrity of DNA in the heart of the organism.

D = the profile of the Kidney DNA at 0h (K1) and 48h (K3) PM is slightly defined with banding patterns alike at this hours, at 24h (K2) PM there is great appearance of bands showing high integrity of DNA at this hour in the kidney.

The results of RAPD profiles of the brain, liver, heart and kidney of Rat Group D at 0h, 24h and 48h post-mortem are presented in Figure 4.

RAT D



(A) (B) (C) (D)

Figure 4. RAPD profiles of the brain, liver, heart and kidney of RAT D at 0h, 24h and 48h post-mortem

Where A = the profile of the Brain DNA at 0h (B1), 24h (B2) PM and 48h (B3) PM is well defined with all the banding patterns being consistent showing high integrity of DNA in the brain

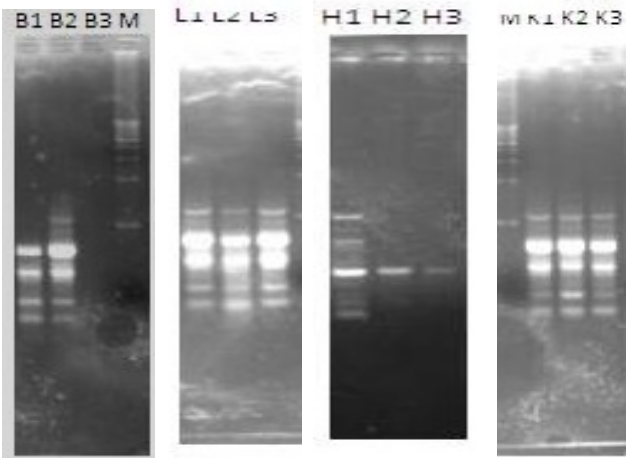
B = the profile of the Liver DNA at 0h (B1), 24h (B2) PM and 48h (B3) PM is well defined with all the banding patterns being consistent showing high integrity of DNA in the liver.

C = the profile of Heart DNA at 0h (H1), 24h (H2) PM and 48h (H3) showed disappearance of bands with later PMI indicating reduction in the integrity of DNA in the heart of the organism.

D = the profile of the Kidney DNA at 0h (B1), 24h (B2) PM and 48h (B3) PM is well defined with all the banding patterns being consistent showing high integrity of DNA in the kidney.

The results of RAPD profiles of the brain, liver, heart and kidney of Rat Group A at 0h, 24h and 48h post-mortem are presented in Figure 5.

RAT E



(A) (B) (C) (D)

Figure 5. RAPD profiles of the brain, liver, heart and kidney of RAT E at 0h, 24h and 48h post-mortem

Where A = the profile of the Brain DNA at 0hr (B1) and 24hrs (B2) PM is well defined with some of the banding patterns being consistent showing high integrity but 48hrs (B3) PM disappearance of the band occurred.

B = the profile of the Liver DNA at 0hr (B1), 24hrs (B2) PM and 48hrs (B3) PM is well defined with all the banding patterns being consistent showing high integrity of DNA in the liver of the rat.

C = the profile of the Heart DNA at 0hrs (H1), 24hrs (H2) PM and 48hrs (H3) showed disappearance of bands with later PMI indicating reduction in the integrity of DNA in the heart of the organism.

D = the profile of the Kidney DNA at 0hr (B1), 24hrs (B2) PM and 48hrs (B3) PM is well defined with all the banding patterns being consistent showing high integrity of DNA in the kidney of the rat.

4. Discussion

The 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 [26-29]. DNA decays after death, in biological samples, and the ensuing damage is manifested in many forms. In postmortem studies, no previous researchers

studied the integrity of the DNA in these organs using this Random Amplification of Polymorphic DNA-PCR method.

Generally, results of this study revealed gradual degradation of intact nuclear DNA in the brain, kidney and heart, with increasing PMI. These findings coincide with those of Luo *et al*., [32-34] who showed gradual decrease of bone marrow DNA with prolongation of PMI. In addition, El-Harouny *et al*., 2009 [35] showed that using DNA maximal optical density, there was significant lower mean values in the concentration of brain, spleen, lungs, liver and skeletal muscle, with increasing PMI than control group at zero time at intact DNA which was prominent in the lungs beginning from 3 hours post mortem and in the spleen beginning from 6h post mortem. There is a significant ($P<0.01$) 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 [36] reported that in tissues, such as liver and kidney, enzymes tend to be more active and accelerate DNA decomposition.

In the present study, the RAPD method was useful in viewing the profile of random segments of genomic DNA. In addition, Lin *et al.,* [37] observed that the DNA degeneration rate of liver cells had a linear relationship to early postmortem period in rats. Considering the brain, liver, heart and kidney DNA degradation, the brain DNA degradation occurred at a slower rate than the other organs. The liver also showed a little regularity in their PMI DNA profile. The bands in the RAPD profiles of the brain and liver were fairly similar showing that the DNA in the brain, and the liver was more intact as compared to the heart and kidney, where disappearance of bands were more prominent. [38-40]

According to these results of this study, it can be concluded that the degradation of DNA shows a relationship with PMI 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 suggests that at a later PMI the brain, and then liver is a preferred organ for forensic studies than the heart and kidney.

The present study used a simple, easy, applicable and highly informative PCR method that makes it ideal for the busy forensic laboratory. These photographic images therefore were able to show that after 48hrs of decomposition Brain DNA can be used to get intact amount of DNA, then liver, which can then be used to determine PMI at later times. Therefore, the RAPD method can be used for a reliable and sensitive analysis of PMI and future human studies should be considered.

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