



Research Article

Investigating the Correlation between Post-mortem Interval and RIN Values: A Pilot Study

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Abstract

In this human post-mortem autopsy study, 10 organ tissues from 4 post-mortem cases with different Post-Mortem Intervals (PMIs) were evaluated. Clinical history, agonal state, refrigeration time, and PMI of 10, 12, 16, and >24 hours were evaluated to determine histology and molecular markers of the post-mortem tissue. Results indicated PMI was not predictive of RNA stability (RIN values). We observed refrigeration time after death influenced body temperature, delayed post-mortem autolysis, and increased RNA Integrity Number (RIN) values. However, prolonged intervals of hypoxemia decreased RIN values. Clinical medical history and agonal state scores were two of the most important variables affecting RIN values. The 16 and > 24-hour PMIs for all cases had greater than 80% autolysis in the pancreas and small intestine compared to the 10- and 12-hour PMI cases with minimal to moderate autolysis (25-40%). Of the 10 tissues evaluated, the highest average RIN values were heart (7.1), lung (5.9), and skin (5.9). The observed changes in RIN values from refrigeration time during post-mortem collection positively affected the RNA integrity and delayed autolysis of histological samples. Therefore, it is necessary to minimize the time the sample is at room temperature, maximize refrigeration, and minimize hypoxemia intervals. RIN values in most post-mortem tissues, except the GI tract, were highly correlated with a medical history and agonal state but were not greatly influenced by the PMI in this small case study. Prompt refrigeration of the body after death may assist in preserving both post-mortem histology and RIN values.

Keywords: Autopsy; Histology; Post-mortem interval; RIN; RNA

Introduction

Human tissue specimens are one of the most valuable resources that researchers can use in the study of human disease and the development of new and improved treatments. Therefore, tissue biobanks, facilities that collect, store, and distribute tissue specimens, are essential for the advancement of both basic and translational research. Although studies using human tissue have been done for decades, the sensitivity and specificity of today's molecular assays are much greater than before. This has created a need for higher-quality tissue specimens for macromolecule

extraction. The molecular information contained in tissue samples can be heavily influenced by several factors, including the procedures used for sample collection and processing [1-3]. Due to this, it is critically important that the individuals responsible for developing and maintaining a tissue repository understand and implement best practices for biospecimen collection, handling, quality control, and storage. A number of professional organizations, such as the International Society of Biological and Environmental Repositories (ISBER) and the National Institutes of Health Office of Biorepositories have also developed best practices for bio banking that can be used as a guideline for the establishment of tissue banking policies, procedures, and workflows [4,5]. Following pre-established guidelines and

standard operating procedures will help ensure that tissue samples are uniformly handled in an optimum fashion, will minimize the influence of environmental variables on the quality and molecular profile of a sample, and will increase confidence in the analytical results obtained from that sample.

The quality of post-mortem brain tissue has been evaluated in many studies. Understanding neurological diseases in genomic research has been the focus when evaluating post-mortem brain tissue. These studies have found high RNA quality with much progress [6,7]. In these studies, the experimental variables recorded were post-mortem interval (time of death to the time of tissue removal), pH of brain tissue, temporal relation to the time of death (either sudden death or protracted illness), pre-mortem fever or sepsis, and other major health conditions prior to death [8]. In the past 10 years, RNA quality has been used as a determiner of overall tissue quality and integrity. RIN values and electrophoresis are currently used to analyze RNA quality [9]. Some reports have suggested that age, sex, time from death to tissue removal, clinical agonal state, and health conditions prior to death influence RNA tissue quality.

Until recently, little genomic data has been collected on the tissue quality of other post-mortem tissues. Traditional markers of post-mortem tissue quality have been descriptive with gross and histology analysis and have considered Post-Mortem Intervals (PMI), agonal condition, patient age, and disease state. The post-mortem neuropathology field has acquired chemical markers for tissue quality, starting with pH, and 28S/18S ratio, and now includes RNA quality determined by RIN values [8]. Procuring tissues from post-mortem organs has the potential to advance medical genomic research and increase the number of tissue samples available to researchers.

This study aimed to determine whether PMI was an accurate predictor for RNA stability. We also analyzed factors such as

refrigeration time, transportation method, and hypoxemia interval for potential influence on RIN values. We hypothesized that PMI would be a predictive factor for RNA stability and that refrigeration would positively affect RIN values.

Materials and Methods

Informed Consent

All tissue samples were collected following a detailed Institutional Review Board (IRB) approved protocol, informed next of kin consent, and processed according to documented Standard Operating Procedures. Informed consent was obtained from the patient for allowing both the post-mortem examination and publication of this study.

Manner of Death

Manner of death is an important piece of data from an examiner's documents that gives a record of any causes or medical conditions related to death. There were five categories for the manner of death considered: natural, accidental, homicide, suicide, and undetermined cause.

Agonal State

An agonal state is a pre-mortem event that affects tissue quality. The agonal state must be considered when analyzing the quality of post-mortem tissue samples. Three categories considered for the agonal state are as follows (Figures 1-4): (i) rapid death due to violent or natural causes (usually unexpected death of healthy individuals); (ii) intermediate death (patients that were somewhat ill, but death was not expected); and (iii) slow death (death caused by an illness with a prolonged terminal phase). Medical documents were evaluated to determine the category of agonal state for each post-mortem case.

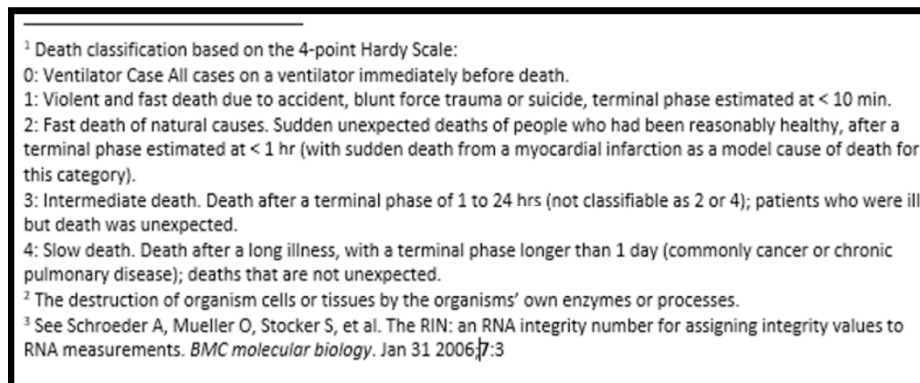


Figure 1: Description of 4-point Hardy Scale.

Patient	Agonal Score	Agonal Factors
A	3	Failed bone marrow transplant for multiple myeloma
B	1	CO ₂ Poisoning
C	2	Wound (more detail later)
D	3	48 hrs medically-induced hypothermia during brain death protocol

Figure 2: Agonal state score and factors of Patients A-D.

Tissue	A	B	C	D	Average
Liver	3	4.9	2.5	7.7	4.5
Kidney	7.2	2.9	3.4	4.1	4.4
Heart	8.2	6.8	7.7	5.8	7.1
Lung	5.6	5.8	5.1	7	5.9
Small Intestine	5.2	6.4	3.6	5.5	5.2
Pancreas	4.7	2.6	1.8	2.7	3
Skin	7.4	3.6	4.6	7.8	5.9
Brain	7.9	4.5	4.7	N/C	N/C
Cerebellum	8.7	3.9	N/C	5.3	N/C
Skeletal muscle	8.3	N/C	N/C	N/C	N/C
Bone marrow	2.7	2.9	N/C	3	N/C

Figure 3: Breakdown of Patients A-D RIN Values.

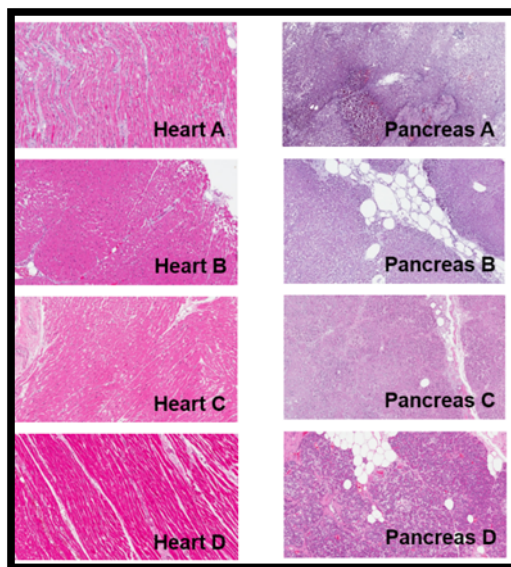


Figure 4: Histological analysis. H&E staining of Patients A-D heart and pancreas at 10x magnification.

Post-Mortem Interval (PMI)

Time intervals are major factors when considering the quality of post-mortem tissues. For this study, the time intervals considered were: (i) time of death to refrigeration; (ii) duration of refrigeration to collecting tissue; and (iii) time from tissue collection to tissue processing. The post-mortem intervals were recorded and considered for analyzing data.

Tissue Collection and Processing

Post-mortem tissue samples were obtained from what is now known as the IU Health Pathology Lab and Marion County Coroner's Office. There were approximately 10 tissues collected per case. The tissues collected included: the kidney, liver, heart, lung, pancreas, small intestine, skin, skeletal muscle, brain, and bone marrow.

Tissues were fixed overnight at room temperature in 10% NBF and then transferred through graded concentrations of alcohol to xylene inside a Leica Automated Vacuum Tissue Processor. They were embedded in paraffin before being cut into five-micron thick sections, mounted onto positively charged slides, and baked at 60 degrees celsius. The tissues were then stained with H&E.

Slide Evaluation

Two pathologists used bright-field light microscopy to evaluate and review the slides.

RNA Extraction and Quality Assessment

RIN values were used to determine the level of RNA degradation of the tissues collected and this value was used to determine the overall quality of the tissues collected.

RNA Extraction on Frozen Tissues

RNA was extracted using the pure script RNA isolation kit (Gentra) according to the manufacturer's recommendations. RNA integrity and yield were assessed by determining sample absorbance at 260 and 280nm and by analysis on the Agilent Bio analyzer using the RNA 6000 Pico kit (Agilent Technologies, Inc., Palo Alto, CA). The genomic specification for an adequate tissue sample was the following: a RIN value greater than 5.5 in the tissue specimens. Every case collected was evaluated via histology for a percentage of normal tissue and a percentage of post-mortem autolysis.

Results

Although the PMI was not predictive of RNA stability (RIN values), refrigeration time after death influenced body temperature, delayed post-mortem autolysis, and increased RIN values. However, the prolonged interval of hypoxemia decreased RIN values. Clinical medical history and agonal state scores were

two of the most important variables affecting RIN values. High agonal state scores (greater than 24 hours) lowered RIN values, which was similar to what has been published with pH evaluation on post-mortem brains in the past. Histology did help predict the RIN score for most organs, except for the pancreas and small intestine which autolyzed very quickly. The 16- and >24-hour PMI cases had greater than 80% autolysis in the pancreas and small intestine compared to the 10- and 12-hour PMI cases with minimal to moderate autolysis (25-40%). Of the 10 tissues evaluated, the highest average RIN values were heart (7.1), lung (5.9), and skin (5.9). The liver (4.5), pancreas (3), and kidney (4.4) displayed the lowest average RIN values out of all tissues collected across Patient A-D.

Discussion

The main results of this small post-mortem pilot study were as follows: (i) PMI was not predictive for RNA stability; (ii) PMI did not influence RIN values; (iii) prompt refrigeration after death delayed post-mortem autolysis and increased RIN values; (iv) prolonged intervals of hypoxemia decreased RIN values; and (v) medical history and agonal state were highly correlated to RIN values.

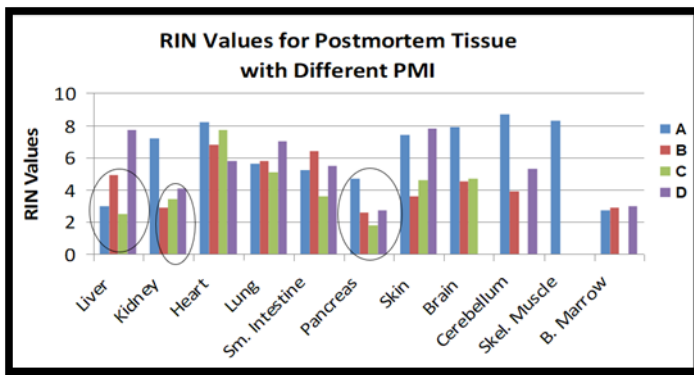
Contrary to previous observational studies, PMI was not an accurate indicator of RNA stability. This study served as a pilot study for the Genotype-Tissue Expression (GTEx) Normal Human Tissue Bio banking Study [10]. This study led to the National Cancer Institute (NCI) beginning to use Organ Procurement Organizations (OPO) for the collection of 42 normal tissues across the country. The OPOs were collecting normal tissue at about 2.5 hours post-mortem, compared to universities that had an average of 16 hours PMI.

Further analysis of human tissue specimens on a genetic and molecular level lays the foundations for further studies and advances in human genomics. Investigating the relationship between PMI and RIN values could lead to a deeper understanding of RNA degradation and its influencing factors. Being able to control these factors such as shortened time from death to refrigeration and storage of the body at cool temperatures until tissue removal has the potential for vast improvements in RIN values for post-mortem tissue collections.

This study served as a pilot for the following ongoing human post-mortem genomic studies: Suicidality and INBRAIN Project [11], COVID-19 Lung and Toxicology Study [12], Tissue Collection Skin Project [13], and Small Cell Lung Cancer Rapid Autopsy. Because of the pilot study, multiple studies involving genomic expression in esophageal carcinoma, lung adenocarcinoma, and breast tumors have been conducted [14-16] (Table 1) and (Graph 1).

Patient	A	B	C	D
Refrigeration (hrs)	4.80	2.10	9.80	0.13
Refrigeration Time (hrs)	23.55	14.50	2.17	9.17*
Refrigeration to Autopsy (hrs)	0.33	0.25	0.23	0.25
Total time (hrs)	28.68	16.85	12.20	9.55

Table 1: Comparison of Patients A-D PMI intervals. *Removed for 0.83 hours during this period.



Graph 1: Patients A-D RIN values for each tissue collected.

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Conclusion

As with previous post-mortem brain banking studies, the RIN values in most post-mortem tissues, except the GI tract, were highly correlated with a medical history and agonal state but were not greatly influenced by the PMI in this small case study. To mitigate the effects of RNA degradation and sample autolysis, there is a need to improve rates of refrigeration of the body after death, which may help preserve both post-mortem histology and RIN values. This pilot study paved the way for multiple studies to further investigate genomic expression in post-mortem human tissue.

Author Contributions

Conceptualization, G.E.S. and D.A.H.; methodology, G.E.S. and D.A.H., and C.F.S; validation, G.E.S, D.A.H, and R.H; formal

analysis, G.E.S., D.A.H., and R.H; investigation, G.E.S; writing-original draft preparation, G.E.S., M.S., M.R, and J.J.; writing-review and editing, G.E.S. and M.S.; supervision, G.E.S.

Data Availability Statement

Data supporting the study results can be provided followed by a request sent to the corresponding author’s e-mail.

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