



Post Mortem Analysis of General Changes in Viscera

*¹Madhurima Kalsekar, ²O Sreeharshini

¹Amity University- Rajasthan, ²SRM University- Tamil Nadu
Email-¹ madhurima.kalsekar@gmail.com, ²harshinioruganti1@gmail.com

Received: 15th Aug 2016, Accepted: 25th August 2016, Published: 1st Sep 2016

Abstract

Postmortem changes begin soon after death and progress along a timeline. This study presents an analysis on the changes in the physical properties of goat organs and change in its DNA content after its death. Four organs of goat i.e liver, kidney, lungs and intestine were considered as working samples. The samples were cut into two halves so that one can be used for the study of general changes and the other can be utilized for DNA extraction at regular intervals of time. The techniques like Agarose gel electrophoresis and UV spectroscopy was performed to check the presence of DNA and to quantify the extracted DNA samples. The same protocol was performed for a period of 5 days and in the end agarose gel electrophoresis was performed to detect the presence of DNA, bands were observed confirming the quality of the DNA. Weight changes and changes in the color were also observed and recorded. This analysis can be useful to analyze the age of the dead bodies and the further investigative protocols.

Keywords

Post-mortem changes, goat organs, physical properties, Agarose gel electrophoresis, UV Spectroscopy

Introduction

A post-mortem analysis is the examination of a body after death and can also be called an autopsy. The interval between death and the time of examination of body is called post-mortem interval. This analysis is very crucial to know when the crime was committed. The most significant medico- legal issue in any post-mortem examination is to find out time since death. [1]

One of the most crucial longstanding problems in the field of forensic medicine is the determination of the time of death upon the detection of a possible homicide victim [2]. Postmortem changes begin soon after death and grows along a timeline. Two processes, putrefaction and autolysis, begin to transform the body; either one may dominate, depending on the situations surrounding death, as well as the climate. Putrefaction involves the action of bacteria on the tissues of the body. This process, ubiquitous in moist climates, is associated with green discoloration of the body; gas production with bloating combined; skin slippage; and a bad odor. Autolysis is the disruption of the body by

endogenous substances. It proceeds most speedily in organs such as the pancreas and stomach. It may predominate in more arid conditions and can ultimately result in mummification. In most conditions, autolysis and putrefaction occur in tandem. In temperate climatic conditions, they can proceed in rapid degradation of the tissues. These alterations may eventually produce great distortion of the body after death.[3]

In this study we extracted DNA from the organs of the goat. DNA content values can tell the post-mortem time interval (PMI) possibly.

Postmortem changes that occur in body are

Liver mortis - One of the early changes which is observed is livor mortis, also known as as lividity, post-mortem hypostasis, vibices and suggillations. This is a physical process. When death occurs, circulation stops and the blood begins to settle, by gravity, to the lowest portions of the body. This results in a discoloration of those lower, dependent parts of the body. Although beginning shortly, the first signs of livor mortis are normally observed after a period of almost 1 h following death with full development being observed 2–4 h following death.[4]

Rigor mortis- At the moment of death, the muscles relax completely, this is a condition called "primary flaccidity." The muscles then stiffen, maybe due to coagulation of muscle proteins or a shift in the muscle's energy containers (ATP-ADP), into a

condition that is known as rigor mortis. All of the body's muscles are affected. Rigor mortis begins within two to six hours of death, starting with the neck, eyelids, and jaw.[5]

Algor mortis- Once death has occurred, the body ceases to readjust its internal temperature and the internal temperature begins to approximate the ambient temperature. In most cases this involves a cooling of the body until ambient temperature is reached, most often in a period of 18–20 h [6]

Pallor mortis- Almost immediately after death a body of a person with light skin will begin to grow very pale. This is caused by a lack of blood in the Capillary region of the blood vessel. In a fully functioning body, the skin is the color it is because blood is flowing through all the veins and vessels that are woven throughout the body. In a dead body the blood no longer runs, and starts to give in to

gravity and to settle to the part of the body that is facing down, this is also the beginning of a later stage of liver mortis.[7]

Now a days, scope of forensic entomology has also been increased in forensic science to find out the time of death of a person.

Forensic entomology is the study of insects to determine the post-mortem interval (time since death) of a decaying human body. The insects found in, around, and near the body are collected and examined to generate a accurate time of death. Forensic entomology can be a great help in situations where decomposition is too extreme for a medical examiner alone to determine time of death [8]

Materials and Methods

Sample Preparation:

In order to study the general changes in the dead material Goat sample has been selected. The sample is obtained from the nearby slaughter house and has been processed for further analysis. The study was conducted by considering different organs of the sample. The organs selected for the study were Lungs, Liver, Kidney and Intestine. The parameters considered were Quantitative Analysis of DNA and morphological changes in the tissues.

Extraction of DNA from the sample:

The selected organs were further divided into two sections, one for the quantification and the other for quality changes. All the four kinds of tissues were used for extraction of DNA and purification. The extraction method uses high concentrated detergents like SDS for cell was and tissue breakage. Tris HCL for Buffering, EDTA for protection from nucleases and Alcohol for precipitation of DNA. All the extracted DNA samples were further purified using chilled ethanol precipitation. The purified DNA was further preserved at 4⁰ for further analysis. The quantification was performed for a week on daily basis and the data was recorded.

Study of Morphological Changes

Apart from the molecular analysis the general changes in the morphology was also the point under consideration.

Agarose Gel Electrophoresis for the detection of DNA bands.

To detect the presence of DNA in the extracted samples agarose gel electrophoresis was performed. Here 8% of Agarose was prepared in TAE buffer and was casted in a Gel tray. The wells were made and the gel was set in the buffer tank. The DNA samples extracted for all the days are loaded in the wells and run for a period of approximately half an hour. After the complete run the gel was observed in UV

Transilluminator / Geldoc system. The bands obtained were observed and recorded. A positive result indicates the presence of DNA in all the extracted samples.

Quantification of DNA using DPA Method

In order to comparatively analyze the DNA samples obtained from various tissues at various intervals of time Spectrophotometric method based on DPA protocol was employed. The protocol works on the principle that the purine and pyrimidine bases in the DNA react with the DPA in alkaline conditions to produce a green colored complex. The intensity of the color developed is read against spectrophotometer. The color developed is directly proportional to the quantity of the DNA present. The DNA was quantified in all the samples and the results are recorded.

Results and Discussion

Study of Changes in the Morphological features like Color and weight of all the four tissue type are shown below.

Table 1: Showing the changes in the Color and Weight of the Liver

	Color of the Tissue	Weight of the Tissue
Day 1	Brown	6.58
Day 2	Dark brown	3.30
Day 3	Dark brown	2.24
Day 4	Brownish Black	1.95
Day 5	Brownish Black	1.72

Colour and Weight changes observed in Goat Liver sample are depicted in the above table. It is observed that the weight of the liver sample reduces to almost half its original weight on the 2nd day. This weight loss can be attributed to water loss. The reduction in weight slows down after day 2 and only minor changes are noted.

Table 2: A focus on the Kidney sample for color and weight changes

	Color	Weight
Day 1	Brown	8.31
Day 2	Dark Brown	6.11
Day 3	Dark Brown	5.61
Day 4	Blackish Brown	5.43
Day 5	Blackish Brown	5.27

The above table depicts the Colour and Weight changes observed in Goat Kidney sample. Weight reductions in the kidney sample are minor and are almost constant after Day 3 (Only decimal changes are observed.)

Table 3: Changes in the Intestinal Tissue

	Color	Weight
Day 1	Creamish White	4.43
Day 2	Brownish White	1.33
Day 3	Light Brown	1.08
Day 4	Light Brown	0.95
Day 5	Brown	0.70

Table 3: Colour and weight changes observed in Goat Intestine sample for a period of 5 days has been depicted in the table. The intestine sample lost

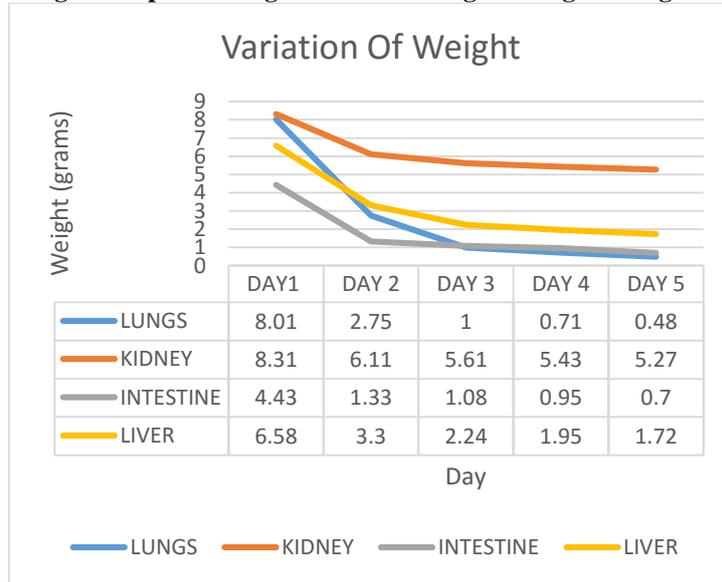
around 3 grams of weight on day 2. Thereafter the reduction in weight is nominal.

Table 4: Color and Weight changes in Lungs

	Color	Weight
Day 1	Pinkish White	8.01
Day 2	Greenish Pink	2.75
Day 3	Reddish Green - Cream	1.0
Day 4	Greenish Cream	0.71
Day 5	Greenish Cream	0.48

Colour and Weight of the samples showed the most drastic weight loss. The samples lost nearly 6 grams of its initial weight on day 2 where the values have come down from 8.01g to 2.75g. On day 7 the weight of sample was found to be 0.48 grams. The weight loss over 7 days was 16 fold or on an average the sample lost 1 gram weight per day.

Fig 1. Graph showing variation of weight changes in organs



The above graph clearly depicts the changes in the weights of the four types of tissues with time

Measurement of DNA using Spectrophotometer

All the above DNA samples obtained are subjected for quantitative estimation to determine the effect of time on the concentration of DNA.

Table 5 Shows the Absorbance values of DNA obtained for all the samples

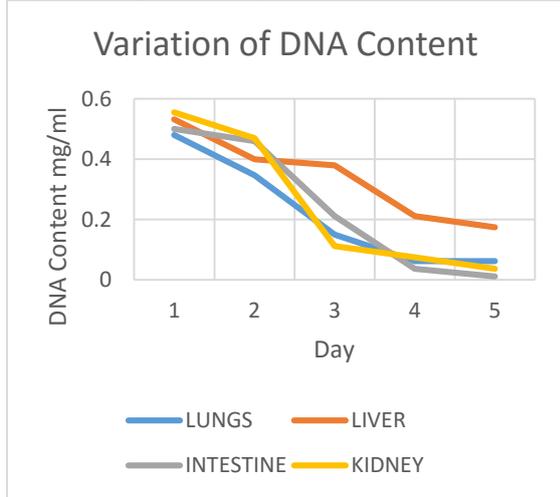
	Lung	Liver	Intestine	Kidney
Day 1	0.192	0.213	0.200	0.222
Day 2	0.139	0.160	0.184	0.188
Day 3	0.060	0.152	0.085	0.045
Day 4	0.025	0.085	0.015	0.030
Day 5	0.025	0.070	0.005	0.015

The above table shows the OD values obtained for the samples in spectrophotometer.

Table 6 shows the Concentration of DNA obtained in all the samples for a period of 5 days

	Lung	Liver	Intestine	Kidney
Day 1	0.480	0.532	0.500	0.555
Day 2	0.347	0.400	0.460	0.470
Day 3	0.150	0.380	0.212	0.112
Day 4	0.062	0.212	0.037	0.075
Day 5	0.062	0.175	0.012	0.037

Fig 2: Graph showing variation of DNA content



Intestine Day 1:



D: Day 5 Intestine



Fig 5 showing some of the physical changes in the samples observed during the study:

A)



B)

Lugs after 3 to 5 days:



E: Day 1 Kidney



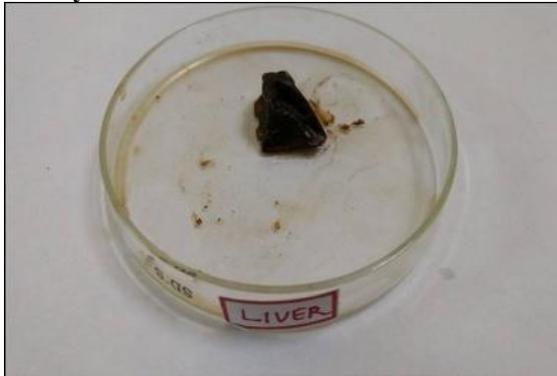
F: Day 5 Kidney



G: Day 1 Liver



H: Day 5 Liver



Department of Pathology, Medical University of South Carolina College of Medicine, 2015.

[4] Nashelksy M, McFellely P (2003) Time of death. In: Froede RC (ed) Handbook of forensic taphonomy, 2nd edn. CAP, Illinois

[5] Oever, R. van den. "A Review of the Literature as to the Present Possibilities and Limitations in Estimating the Time of Death." *Medicine, Science and the Law* 16 (1976):269–276.

[6] Fisher BAJ (2007) Techniques of crime scene investigation, 7th edn. CRC Press, New York

[7] Megyesi, M.S., Nawrocki, S.P., and Haskell, N.H. (2005). Using Accumulated Degree - Days to Estimate the Postmortem Interval from Decomposed Human Remains. *J Forensic Sci.* 50, 618-626.

[8] Anderson, D. G. (n.d.). *Forensic Entomology: The use of Insects in Death Investigation*. Retrieved from Simon Fraser University

Conclusion

It has been a major problem in the field of forensic science to find out the post-mortem time interval i.e time since death. PMI can be a very important factor to find out the criminal in case of murder. It gives an initiative to the police to start the investigation. The current work involves the study of various tissues after its death of the tissue. At times when no other method can be used to find out the time since death this approach can be a useful method. The determination of the quantity of DNA should be an objective and exact way to estimate the PMI.

References

[1] Eckert, William G. "Timing of Death and Injuries." *Medico-Legal Insights*. In *Inform Letter*, 1991.

[2] Joseph Sambrook and David W. Russel (2001). *Molecular Cloning: A Laboratory Manual* (3rd edition). Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press

[3] S Erin Presnell, MD Professor, Co-Director of Medical and Forensic Autopsy Section,