MJMR, Vol. 33, No. 2, 2022, pages (26-29).

Open Access
ISSN:2682-4558

Research Article

Pituitary Luteinizing Hormone Analysis for Estimation of Time Passed Since Death: Biochemical Study (An Experimental Study)

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Abstract

Background: The assessment of the time since death is one of the most prevalent and challenging concerns that a forensic practitioner tackles. While accurate determination is not always attainable, it is frequently possible to specify merely a broad temporal frame. By identifying a time range within which the death might have happened, determining this period assists in the examination of the circumstances surrounding death. Furthermore, a thorough understanding of postmortem processes and the factors that influence them will assist in determining the postmortem interval, often known as the period since death. The study was done to estimate the PMI through biochemical changes in pituitary luteinizing hormone at 0, 1, 5, 10, 15 days postmortem intervals. **Methods:** One hundred adult albino rats were dissected to measure changes in levels of LH hormone in pituitary gland tissues by the enzyme-linked immunoassay technique. **Results**: the mean of luteinizing hormone levels in the pituitary by ELISA revealed a statistically significant difference in all examined postmortem intervals (0, 1, 5, 10, 15 days) when compared all intervals with each other. They decreased statistically with increased PM period. **Conclusion:** Biochemical analysis of pituitary luteinizing hormone is helpful in estimation of time passed since death.

Keywords: Postmortem Interval, Luteinizing hormone, Enzyme-linked immunoassay**.**

Introduction

One of the most crucial responsibilities for forensic professionals, especially in criminal situations, is determining the period since death. The human body goes through a series of modifications after death. Although these changes occur in a very orderly manner, several environmental conditions, such as temperature, air humidity, and the kind of habitat, as well as the deceased body's intrinsic qualities, may influence decomposition {1}.

The postmortem interval refers to the phases of autolysis that have elapsed since an individual's death. The forensic pathologist can more correctly estimate the postmortem interval by understanding frequent postmortem alterations and the variables that influence them {2}.

"The master gland" is the pituitary gland (hypophysis cerebri). This small gland has two lobes: an anterior lobe called adenohypophysis and a posterior lobe called neurohypophysis, both of which are located in the sella turcica of the basisphenoid bone body in the skull {3}.

LH, also known as lutropin and occasionnally lutrophin, is a heterodimeric glycolprotein generated by gonadotropic cells in the anterior pituitary gland. Each monomeric unit is a glycoprotein molecule with

one alpha and one beta component that are non-covalently bonded to create a fully functional protein. There are 92 amino acids in the alpha subunits and 120 in the beta subunits. The LH beta subunit is responsible for the specificity of the interaction with the LH receptor, as well as the biological impact it has. This beta subunit's amino acid sequence is quite like that of hCG (human chorionic gonadotropin), and they both activate the same receptor[4].

LH hormone is a glycoprotein that induces ovulation in females and controls Testosterone production by extra tubular Leydig cells in males[5].

This study was designed to estimate the time passed since death through pituitary luteinizing hormone changes at five different postmortem intervals.

Materials and methods

Within the investigation, 100 adult albino rats (weight 150-200 gm; age 8 weeks) were employed. They came from the university's growth facility for research animals in Minia, Egypt.

The animals were kept in clean plastic cages with proper ventilation and cleanliness, and they had free access to a well-balanced standard diet pellet food as well as tap water. They were kept at a consistent humidity and temperature and treated to a 12-hour light/12-hour dark cycle.

Experimental design

The rats were separated into five groups (each with 20 rats): Group I, Group II, Group III, Group IV, and Group V. (0, 1, 5, 10, 15 days PM respectively). Under ether inhalational anesthesia, the rats were slaughtered by cervical dislocation. Each time, the pituitary gland was dissected and processed for biochemical analysis using the ELISA method.

Luteinizing Hormone (LH):

Rat LH (Luteinizing Hormone) ELISA Kits, Elabscience Biotechnology Incorporated Company, United states America USA (Catalog No: E-EL-R0026 96T). The measurement unit is mIU /ml.

Sample collection:

Pituitary gland tissues were chopped into minute pieces and thoroughly washed in ice-cold Phosphate buffered Saline (PBS) (0.01M, pH=7.4) to eliminate excess blood, which might affect the results. Tissue slices were weighed and subsequently homogenized in PBS using a glass homogenizer. By sonicating the suspension and freezing and thawing it, the cells were disturbed. The supernatant was obtained by centrifuging the homogenates at 5000g for five minutes.

Test principle:

The Sandwich-ELISA technique was utilized to determine the hormone's concentration. This kit came with a micro-ELISA plate that was pre-coated with a rat LH antibody. Standards and samples were combined with the antibody in the micro-ELISA plate wells. Then a biotinylated detection antibody specific for rats LH and an Avidin-Horseradish Peroxidase (HRP) conjugate were added to each microplate well and incubated. The components that were no longer needed were rinsed away. Only the wells containing rat LH, biotinylated detection antibody, and Avidin-HRP conjugate became blue after receiving the substrate solution. The solution was introduced to the enzyme-substrate reaction after a halt, and the color changed to yellow.

Results

Measurement of Luteinizing hormone among different groups:

The range and the mean of luteinizing hormone levels in the pituitary by ELISA revealed a statistically significant difference in all examined postmortem intervals (0, 1, 5, 10, 15 days) when compared all intervals with each other.

They decreased statistically with increased PM period (Table 1) (figure 1).

Table (1): One Way ANOVA statistical analysis of LH hormone levels in the pituitary gland tissues from the dead rats at different PM periods.

		PMI					P value
		Day 0	Day 1	Day 5	Day 10	Day 15	
		N=20	N=20	N=20	N=20	N=20	
LH *(mIU/ml)*	*Range* *Mean ± SD*	(2.16-3.98) 3±0.57	(1.43-2.88) 2.3±0.44	(0.9-1.78) 1.3±0.23	(0.68-0.97) 0.8±0.08	(0.15-0.24) 0.2±0.03	***<0.001****
P value between each 2 groups							
Day 0			***<0.001****	***<0.001****	***<0.001****	***<0.001****	
Day 1				***<0.001****	***<0.001****	***<0.001****	
Day 5					***<0.001****	***<0.001****	
Day 10						***<0.001****	

- ***Data expressed by range, mean ± SD***
- ***One Way ANOVA test for quantitative data between the five groups, followed by post hoc Tukey's analysis between each two groups***
- ****: Significant level at P value < 0.05***
- ***SD: standard deviation***
- ***PM: postmortem***

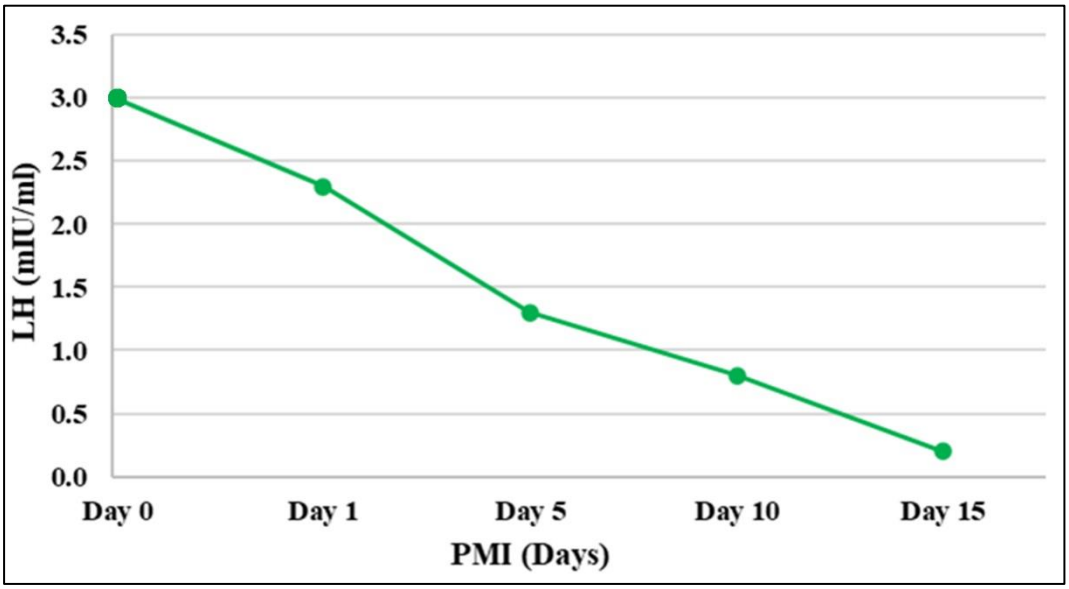

Figure (1): Relationship between the PM periods and LH levels in the pituitary gland tissues from the dead rats by ELISA.

Discussion

One of the most common and chall enging challenges a forensic practitioner faces is estimating the time since death. True, precise determination is not always possible, and most times just a broad period is provided. After death, the body undergoes a range of post-mortem changes, which are often used in forensic science to determine the post-mortem interval (PMI)[6].

Dissection of the pituitary gland was done at 0, 1, 5, 10, and 15 days. The organ was then processed and examined biochemically. This experiment was carried out

in accordance with the Minia University Faculty of Medicine's Ethical Committee's rules for laboratory animal care and use.
The study results on LH hormone levels in pituitary gland tissue revealed a significant difference between groups, with a significantly strong negative correlation with PMI, and a gradual decrease with increasing PMI, ranging from high levels at the time of death to weak non-significant values 15 days later.

According to Ishikawa et al.,[7] who studied Between 6 hours and 20 days PM, LH levels were measured in serial forensic autopsies instances. They detected hormone leaking from the cytoplasm after 2 days postmortem, as well as the persistence of LH immunopositivities in the adenohypophysis in all instances up to 15 days postmortem. PM favorable findings were not found after roughly 20 days. They justified their findings by the structural durability of secretory granules against autolysis for such a long period that PM immunopositivities of the LH hormone were seen, which might be useful in determining the duration after death.

Conclusion

According to the findings of the current study, changes occur after death in a time-dependent way based on distinct postmortem durations.

In pituitary gland level of LH hormone exhibited a statistically strong negative connection with PMI, and levels of LH hormone decreased with increasing time after death.

As a result, LH hormone may be utilized to predict PMI in the examined organ.

Consequently, it was determined that biochemical alterations in the hormones LH in the pituitary gland may be employed as good predictors of correct PMI evaluation.

Further research on postmortem biochemical changes in LH hormone, as well as other hormones in other organs, is strongly required.

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