ORIGINAL ARTICLE

ATP LEVELS AND ITS DEGRADATION PROCESS AS A POST-MORTEM INTERVAL INDICATOR

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Background: There are many scientific methods in practice to determine the time of death, but no methods alone will precisely state it. Researchers are trying direct and indirect methods to estimate the post-mortem interval (PMI) by gathering evidence to find a more accurate way out to measure time since death. Adenosine triphosphate (ATP) transfers energy within the body, and is a crucial indicator in estimating PMI. This study aims to find out the levels of blood ATP after death at certain periods to help determine the PMI. **Methods:** This experimental study was carried out at the Department of Forensic Sciences, University of Health Sciences, Lahore. Thirty-six rabbits with good health were taken and divided into six groups. Blood samples were obtained at regular intervals. The relationship between PMI and ATP degradation levels was analysed statistically. **Results:** At 0 hours, the mean ATP levels were recorded as 44.59 which declined linearly with the time recorded until 72 hours (1.51) post-mortem. The mean ATP levels at different post-mortem intervals were significantly different (p<0.001). **Conclusion:** ATP levels decline in blood post-mortem and can be used to determine PMI.

Keywords: Post-mortem, Post-mortem interval, ATP degradation,

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INTRODUCTION

The time of death holds great importance, especially in forensic sciences, as it is the most sought-after information that medical and legal experts try to find. In forensic sciences and criminal investigations, a tad bit of mistake or uncertainty of results may change the course of any murder case.[1] Soon after the death, the deceased body starts experiencing numerous physical as well as chemical variations. While there is no way to revert or evade these changes, it can be declared that these changes take place with order and can be somewhat predicted using constancy.[2] The rate at which expected changes will take place depends greatly on a variety of conditional and ecological attributes. The research lays its foundation on a solid understanding of how the time of death can be estimated as well as factors that can affect this interval.[3]

Many scientific methods include biochemical reactions and fluid concentrations levels that indicate time since death by changing their temperature and molecular concentration level in blood. Pericardial fluids, *p*H level, cerebrospinal fluid, vitreous humor, lipid concentrations in bone, synovial fluids, and Adenosine Triphosphate (ATP) levels are some indicators of post-mortem interval time since death.[4]

Adenosine Triphosphate is widely employed in determining the time elapsed since death. In a live body, ATP transfers the energy to muscles for voluntary and involuntary actions and to perform any physical activities.[5] When death occurs, metabolism in the body gradually decreases, hence the loss of ATP. It is crucial to take into account the significant role of ATP level to find a definitive method or technique to estimate Post-Mortem Interval (PMI) and hence our experiment was to determine the levels of ATP in blood after death at certain periods to help determine the PMI precisely.

Ninety-six percent of ATP comes from erythrocytes. When the death of human occurs, an imbalance of production and consumption of ATP starts. When the cause of death is unknown, then the breakdown of ATP is a very useful marker for measuring time since death because ATP gets utilized in metabolism and other chemical reactions actively. ATP level of blood starts to fall, and the equilibrium is disturbed after death.[6] The rate at which ATP changes into adenosine diphosphate (ADP) and then adenosine monophosphate (AMP) is the time since death when ATP unbalances the equation of production and decomposition of its molecules in the human body.[7]

Chinese researchers extensively conducted studies to measure the relation between ATP blood levels with the time passed since death. The process of breakdown of ATP molecules after death becomes slow as the time after death passes. Hence, it is found that there is a direct relation between blood ATP levels with the post-mortem interval time. In comparison with other research methods, the tendency of ATP to change and degrade may prove to be a better way for the estimation of PMI in medico-legal cases.[6] This study aimed to explore the correlation between blood ATP levels and post-mortem interval and to study this at different temperatures to come up with a reliable correlation between the two.

MATERIAL AND METHODS

This experimental study was conducted at the Department of Forensic Sciences, University of Health Sciences, Lahore. Adult, healthy rabbits of both genders were included in this study. Blood from the heart was taken for analysis.

The calculated sample size was 6 for each group. Thirty-six rabbits were used for the proposed study. The rabbits were sacrificed following the guidelines of the Ethical Committee of UHS and the international Public Service Guide for care and use of laboratory animals. All rabbits were placed in the supine position on the bench and sacrificed at the same time under anaesthesia with intraperitoneal injection.

The animals were divided into 6 groups of 6 each at different time intervals 0 hrs, 6 hrs, 12 hrs, 24 hrs, 48 hrs and 72 hrs respectively. Blood was drawn from the hearts of the rabbits by direct laparotomy/ autopsy procedure.

The interaction of the fluorescent reagent with ATP in the blood sample leads to fluorescence, whose intensity was luminometrically detected and recorded as Relative Light Unit (RLU). The ATP concentrations corresponding to the RLU were calculated from the standard equation involving RLU values and ATP levels.

The concentration of ATP (ηmol/µL or µmol/mL or mM) in the test samples was calculated as:

ATP concentration= (BV×D)×DDF

Where B=amount of ATP in the sample well calculated from the standard curve (ηmol or mM).
V=sample volume added in the sample wells (µL).
D=sample dilution factor if a sample is diluted to fit within the standard curve range (before the reaction well set up)
DDF=deproteinization dilution factor

Statistical analysis of data was performed with SPSS-20 and MATLAB software for data interpolation, fitting and curve plotting, testing the regression equations and interpolation functions.

RESULTS

The mean ATP levels at each post-mortem interval are mentioned in table 2. At 0 hours, the mean ATP levels were recorded as 44.59 which declined linearly with the time recorded at 6 hours (32.35), 12 hours (28.35), 24 hours (22.34), 48 hours (1.98), and 72 hours (1.51) post-mortem.

Data were normally distributed as assessed by the Shapiro Wilk test. A one-way ANOVA test was applied to compare the mean ATP levels among different post-mortem interval. It was found that the mean ATP levels at different post-mortem intervals were significantly different (p<0.001) (Table-1).

Table-1: Comparison of ATP levels in the blood at different post-mortem intervals (ηmol/µL)

PMI (Hrs)	ATP (Mean±SD)	Range	*p*
0	44.59±3.38	40.62–48.48	<0.001
6	32.35±2.30	29.67–35.31	
12	28.35±1.99	26.26–31.10	
24	22.34±1.56	20.61–24.74	
48	9.03±1.98	6.36–11.66	
72	1.51±0.92	0.26–2.62	

DISCUSSION

This study was done to determine the levels of ATP in blood at different time intervals at room temperature. Our results found a linear decrease in blood ATP level starting at 0 hrs to 72 hrs post-mortem. Interpolation of ATP levels in the blood is an appropriate choice for PMI estimation which is weakly influenced by ambient temperature.[8] Sun T, *et al*[8] experimented to find a relationship between ATP changes of rabbit blood and post-mortem interval. Blood ATP levels were measured by using the ATP fluorescence rapid detection technique, every 4 hours till 72 hours. They found that the blood ATP levels slightly increased early after death and then declined constantly at all temperatures. The ATP levels in blood showed relatively stable and regular degradation changes within 72 hours after death. Our results also showed a linear regression in ATP levels in blood being highest at 0 hrs (mean 44.59) and lowest at 72 hrs (mean 1.52). We didn't counter any hike in ATP levels in early hours, may be because our first-time interval was 6 hrs, not 4 hrs.

Sun TY, *et al*[5] also found that blood ATP level decreased with the extension in PMI. There are many studies available on determining the PMI by levels of ATP in muscles and its degradation products but quite a few studies available to discuss ATP levels in blood making our discussion minimal and hence limiting our study to this conclusion only.

In forensic medicine, determination of time of death has always been challenging to determine the time of death, both theoretically and practically as post-mortem changes are certain and they keep on progressing influenced by various external factors. Among all these factors, the most important factor is environmental temperature, affecting the estimation of PMI.[9] Our experiment was done at ambient room temperature and no temperature changes were recorded.

CONCLUSION

Under controlled experimental conditions, ATP level in blood degrades post-mortem, following a certain pattern. ATP levels in blood decline as the PMI extends. This decline in ATP level in blood may be used as a potential marker for the determination of PMI more accurately. The temperature change may have a lesser effect on ATP level decline and is linear in change.

LIMITATIONS

In animal studies, data available on PMI is very limited or restricted. Interpretation of the results obtained from different animal data is of substantial disparity in terms of research methods and species studied, making it hard to generalize the conclusions regarding the relativity of the findings of the experiments performed on animals.

FUTURE ASPECTS AND UTILIZATION

ATP detection in blood for estimation of PMI is convenient and reliable and may be easily taken up by the forensic scientists to use in real-time scenarios. Further studies are required to establish a set of average value drop in ATP at certain time periods.

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