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Original article

Estimation of the post-mortem interval using microRNA in the heart in aluminum phosphide deaths

Manar A Ahmed [1], Sherein S galeb[1], Amr R Zaki[1] ،Abeer O Kamel[1], Ahmed G. Sobhi Abdalla[1]

Forensic medicine and clinical toxicology department, Faculty of medicine, Beni-suef university, Beni-suef

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Corresponding Author:

Abeer O Kamel

abeeromar619@gmail.com

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Abstract

Background: The assessment of the postmortem interval (PMI) represents one of the major challenges in forensic pathology. Because of their stability miRNAs, are anticipated to be helpful in forensic research. **Objective**: to see if estimation of PMI is possible using miRNA-122 expression levels in the heart samples from aluminum phosphide toxicity (Alpt). **Methods:** This was a cross sectional study on 60 post-mortem samples (heart tissues) collected at different intervals during forensic autopsies. The two groups were allocated equally according to the cause of death into Group I (control, n=30): Deaths caused by other than toxicity, and Group II (cases, n=30): Deaths due to Alpt. miRNA- 122 expression levels were measured in heart tissues at different PMI using RT-Q PCR. **Results:** miRNA-122 level in Alp deaths was up regulated with statistically significant difference. There was positive correlation between miRNA-122 with PMI.

Conclusion: The results of this study concluded that the PMI can be calculated using the degree to which particular miRNA-122.

1. Introduction:

In forensic medicine, estimating the postmortem interval (PMI) is a key area of study. In real life, PMI that occurs within 24 hours is typically considered early postmortem interval (EPMI), and PMI that occurs after that is referred to as PMI in advanced stage. Conventional techniques for calculating PMI typically rely on physiological alterations such supravital reaction, rigour mortis, algor mortis, and livor mortis [1].

These techniques work well with EPMI and only offer an approximate PMI estimation. With the delay in PMI, the aforementioned described changes might not be available for determining PMI in an advanced stage. In addition, the deceased body is too severely damaged by outside elements including temperature, humidity, and microorganisms [2].

Since they are kept safe in the cell nucleus, nucleic acids like DNA and RNA were thought to be less susceptible to outside damage, which could help with a more accurate assessment of PMI[3]. Because of their various qualities, particularly stability, miRNAs among the RNA species have the potential to be useful in determining TSD at advanced stages [4]

A common fumigant utilized extensively for the secure keeping of grains is aluminum phosphide (Alp). A1P, regrettably, has no known cure and is a significant cause of suicide in poor nations. Additionally, inadvertent phosphine exposure is possible. ALP suppress cytochrome c oxidase, oxidative phosphorylation by phosphine release, also it directly affects cardiovascular tissues and, in extreme situations, can lead to systematic failure and dying [5]

2. Materials and Methods:

This was a cross-sectional analytical study conducted from November 2022 to April 2023 at forensic medicine authority.

The study included 60 post-mortem samples (heart tissues)[6] collected at different intervals during forensic autopsies (PMI ranging from 2 to 96hours). they were allocated equally according to the cause of death into two groups. Group I (control, n=30): Deaths caused by other than toxicity,

and Group II (cases, n=30): Deaths due to aluminum phosphide toxicity.

Gene Expression

Monitoring the heart's miRNA-122 level:

The miRNA-122 level in the heart samples was tested using the All-in-OneTM miRNA qRT-PCR Detection Kit 2.0, Cat. number QP116, which is designed for the quantitative detection of mature miRNA. The All-in-OneTM miRNA qPCR primers are used in their entirety. Found in 9620 Medical Center Drive, #101 Rockville, MD 20850 USA, sourced by Gene Copoeia, Inc. Real-time PCR for miR-21 was made as described by ***(Wang et al.,2012)*[7]**.

Primers:

miR-122

Forward Primer (5′–3′): TGGAGTGTGACAATGGTGTTTG

Reverse Primer (5′–3′): Uni-miR qPCR primer

GAPDH (internal control)

5’ ACG GCA AGT TCA ACG GCA CAG 3’ as a forward order

5’ GAA GAC GCC AGT AGA CTC CAC GAC 3’ as a reverse order

Ethical consideration

The study proposal was confirmed by"Research Ethics Committee" of Faculty of Medicine of Beni-seuf University. **(Approval No: FMBSUREC/02102022/Sayed).** The requirement for informed consent was waived as samples were taken from dead persons who were anonymous.

Statistical analysis

Statistical methods:

We used SPSS version 28 to code and input the data (IBM Corp., Armonk, NY, USA) using an unpaired t-test, a non-parametric Mann-Whitney test [8]. A Chi-square test [9] and Spearman correlation coefficient [10].

3. Results:

Table (1): Demographic data

		Control		cases		P value
		Count	%	Count	%	
Sex	female	14	48.3%	14	48.3%	1
	male	15	51.7%	15	51.7%	
cause of death	Alpt	0	0.0%	29	100.0%	< 0.001
	brain trauma	8	27.6%	0	0.0%	
	hemorrhagic shock	15	51.7%	0	0.0%	
	mechanical asphyxia	6	20.7%	0	0.0%	
Residence	rural	15	51.7%	24	82.8%	0.012
	urban	14	48.3%	5	17.2%	

51.7% of aluminum phosphide deaths were male, 82.8% were from rural areas with mean age 27.83 ± 15.44. In the current study, the commonest causes of death in controls were hemorrhagic shock (51.7%) followed by brain trauma (27.6%) and only (20%) were mechanical asphyxia. most of them were males (51%) with mean age 29.41 ± 11.98 and 51.7% from rural areas. the mean PMI in no toxication control deaths was 29.19 ± 19.63 while that of aluminum phosphide deaths was 38.95 ± 31.88 as shown in table (1).

Table (2): Post mortem interval and mi RNA-122 in control and case groups

	Control					cases					P value
	Mean	SD	Median	Minimum	Maximum	Mean	SD	Median	Minimum	Maximum	
PMI (h)	29.19	19.63	23.00	6.00	72.00	38.95	31.88	24.00	2.00	96.00	0.514
miRNA-122	0.87	0.68	0.61	0.11	2.41	1.17	0.68	0.91	0.41	2.70	0.035

The mean PMI in no toxication control deaths was 29.19 ± 19.63 while that of aluminum phosphide deaths was 38.95 ± 31.88 with no statistically significant difference (P-value >0.05) as shown in table (2)

miRNA-122 expression level in aluminum phosphide deaths were up regulated when compared to no toxication control deaths with mean expression (1.17 ± 0.68, 0.87 ± 0.68 respectively) with statistically significant difference (P-value 0.035) as shown in table (2).

Table (3): Relation of micro ribonucleic acid -122 with post mortem interval in case and control groups

		PMI (h)	miRNA-122
Control	**Correlation Coefficient**	1.000	1.000
	P value	.	.
cases	**Correlation Coefficient**	1.000	1.000
	P value	.	.
	N	29	29

There was positive correlation between PMI with miRNA-122 in no toxication control group and aluminum phosphide deaths (P-value <0.001) as shown in table (3)

4. Discussion:

post-mortem interval (PMI), which defines the estimated period between the discovery of a cadaver and the time of death, is a focus of inquiry in the forensic discipline due to the potential for both civil and criminal consequences [11]. There are a variety of techniques to calculate PMI, including evaluation of physical (algor mortis, livor mortis), physicochemical (rigor mortis), biochemical (electrolyte concentration, enzyme activity), microbiological (decomposition), entomological, and botanical factors [12]

One of the agrochemical pesticides used to boost agricultural productivity is aluminum phosphide (Alp). Because it is readily available at a low cost, it is frequently used as suicide poison. Alp is becoming a common self-poisoning agent in Egypt [13]. Cardiovascular tissues accounted for 70% of Alp-related deaths [14].

In order to establish a more precise calculation of PMI, forensic pathologists have been able to carefully assess the rate of biological markers' degradation (such as proteins, DNA, and RNA). This has been

made possible by the advent of new scientific fields like molecular biology [15].

Since RNA is naturally more accurate than DNA and proteins, its degradation and the loss of particular RNA transcripts when an organism dies appear to be particularly sensitive in terms of speed and temporal correlation [16].

miRNA is more stable than longer RNAs like mRNA [17], so we used in our study miRNA expression to estimate PMI.

Cardiac patients had higher levels of numerous microRNAs, including microRNA-122. This early exosomal miRNA is linked to mitochondria-dependent apoptosis and oxidative stress at the cellular level. Nevertheless, it is still unclear if miR-122 influences heart function in addition to this apoptosis[18].

The purpose of this study to estimate PMI in aluminum phosphide deaths using miRNA expression levels in the heart.

In this research 51.7% of aluminum phosphide deaths were male, 82.8% were from rural areas with mean age 27.83 ± 15.44.

According to ***Mathai and Bhanu (19),*** men with a mean age of 35.5 ±10.488 made up the majority of those who died from acute Alp poisoning. while ***Sheta et al. (20)*** noticed that females were the majority of those who died from acute Alp poisoning with a mean age of 21.15± 10.11 years possibly due to early marriage and 92.3% of Alp deaths were from rural areas. The rate of exposure to Alp in rural regions may increase due to the wide use in agricultural activities without restrictions.

In the current study, the commonest causes of death in controls were hemorrhagic shock (51.7%) followed by brain trauma (27.6%) and only (20%) were mechanical asphyxia. most of them were males (51%) with mean age 29.41 ± 11.98 and 51.7% from rural areas. the mean PMI in no toxication control deaths was 29.19 ± 19.63 while that of aluminum phosphide deaths was 38.95 ± 31.88.

Similarly, ***Lv et al. (16),*** the most prevalent cause of death was a hemorrhage (38.5%), followed by head injury (30.8%) and only (30.8%) were mechanical asphyxia. They were mostly female (46%), with age from 20 to 60 years. PMI range from 6 to 24 hours.

The current study showed that there was no statistically significant difference between gender, age and residence with PMI and other parameters in both groups.

Similar conclusions reported by ***Nagao et al. (21), Mundalil Vasu at al. (22), Agoro et al. (23), Fais et al. (24).***

In our study, miRNA-122 expression levels in aluminum phosphide deaths were

significantly up regulated when compared to no toxication control deaths with mean expression (1.17 ± 0.68, 0.87 ± 0.68 respectively).

As stated by ***Tong et al. (25),*** Doxorubicin treatment given repeatedly raises the levels of miR-21 in the murine heart. ***Wei et al. (26)*** shown that miR-21 expression is markedly increased in H 2 O 2 stimulated cardiomyocytes. Also, ***Hou et al., (27)*** noticed that following the administration of isoproterenol, a panel of miRNAs was shown to be, miR-21 elevated in rat hearts**.** ***Vacchi-Suzzi et al. (28)*** demonstrated that miR-21 expression could be useful indicators for detecting prolonged doxorubicin-induced heart damage.

The possible explanation of our data that Alp alters the fluidity of the mitochondrial membrane, causing the outer membrane of the mitochondria to rupture and inflate. This causes the release of pro-apoptotic proteins and lipid peroxidation, which in turn activates caspases to cause cell death [29,30]**.** phosphine can activate apoptotic pathways in a variety of organs, including the heart, kidney, and liver [31,32]**.**

The heart is particularly susceptible to damage due to rapid rate of oxygen intake and inadequate antioxidant defense systems [33]**.**

MiRNA-122 has been linked to oxidative stress-induced apoptosis in heart tissue [34]**.** NF-B and programmed cell death protein 4 (PDCD4) activation are possible ways of ROS-mediated miRNA-122 induction, as reactive oxygen derivatives have been found to induce miRNA-122 expression and functions [35,36]

The current study showed that there was positive correlation between miRNA-122 and PMI in both groups.

Long et al. (37) investigated miRNA's impact on the age-related mice's wound healing process. They discovered that miRNA expression remained high in the wound area and that it had increased more than threefold by day 7 compared to day 3 levels.

Similar conclusions reported by ***Ibrahim et al. (38)*** who assessed the expression levels of miRNA in albino rats with incisional wounds at three interval times: 0h, 24h, and 48h after infliction and found that miRNA expression levels significantly increased with increasing PMI.

A significant correlation between RNA degradation and the post mortem interval discovered by ***SampaioSilva et al. (39); Wang et al. (40).***

The explanation of our results that, miRNAs may be able to withstand ribonuclease degradation because there is

evidence that they continue to be produced long after the main transcript is silenced [41,42]. One explanation is that ribonucleases and other proteins are difficult to attach to mature miRNAs because they are so strongly connected to the active RNA-induced silencing complex, particularly through their 5′ and 3′ ends [43]. The methylation of miRNA, which also protects against ribonuclease attack, is one of the other hypotheses. However, only plants have been found to have miRNA methylation to this point [44].

5. Conclusion

The results of this study indicate that the PMI can be calculated using the degree to which particular miRNA-122

6. References

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