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Estimation of Postmortem Interval using microRNA in the liver in aluminum phosphide deaths in rat model

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Abstract

Background: One of the main challenges in forensic pathology is determining the postmortem interval (PMI). The autolysis mechanism is exploited in recent PMI enhancements. **Objective:** To determine if the assessment of PMI can be achieved by utilizing the expression of the liver's microtubule-associated protein light chain3 (LC3) and Serine/threonine-protein kinase (mTOR) levels in liver specimens from aluminum phosphide toxicity. **Methods:** This was a cross-sectional study that included 70 rats, which were divided equally into 7 toxicity groups. The toxicity groups were administered aluminum phosphide suspension via oral lavage, after which the rats died within six hours. Immediately after death, liver tissues were collected from the groups at (0, 1, 2, 3, 4, 5, and 6 days postmortem). MDA (Malondialdehyde),

CAT (catalase), and mTOR levels were measured in liver tissues using ELISA. The LC3 level was monitored using the GoTaq® 1-Step RT-qPCR. Organ samples were used for histopathological examination. **Results:** MDA and mTOR were upregulated with a statistically significant difference between the groups, while CAT and LC3 showed a statistically significant decrease between groups (P value <0.05). There was positive correlation between PMI and MDA and mTOR (P-value <0.001), while there was a negative correlation between PMI with CAT and LC3 (P-value <0.001). The linear regression analysis revealed that LC3, MDA, CAT and mTOR were strong predictors of PMI. **Conclusion:** According to the study's findings, the levels of LC3 and mTOR expression in liver tissue can be utilized to calculate the PMI. LC3 was a strong independent indicator for PMI estimation.

1. Introduction:

One of the most challenging problems in forensic pathology and death investigation is estimating the PMI. The majority of deaths occur suddenly and without witnesses, while some forensic investigations involve known PMI, such as those of hospitalized patients or witnessed casualties. The PMI employed for forensic medicine can be assessed using a variety of techniques, ranging from the more traditional ones—livor mortis, rigor mortis, and frigor—to those that rely on biochemical alterations, including changes observed in vitreous fluid [(1)].

Predictable morphological along with chemical changes in cadavers are first employed as PMI indicators; however, as the amount of time after death rises, the methods mentioned above lose their utility because they are only able to approximate the precise moment of death. PMI calculation methods are becoming more accurate thanks to new study areas like protein, RNA, as well as DNA degradation [(2)].

However, the seek for more accurate methods led to the creation of post-mortem genetics, which emphasizes on gene expression and mRNA degradation. One

exciting new area is the application of molecular biology to forensic science. After death, autophagy is observed in every tissue. More unbiased data on TSD may be obtained by examining the expression patterns of the main autophagy genes [(3)].

A popular and inexpensive rodenticide for grain preservation is aluminum phosphide. Due to its extensive market reach and unrestricted accessibility, it is one of the substances that can cause self-poisoning that is frequently utilized in many developing nations [(4)].

2. Materials and Methods:

This was a cross-sectional analytical study that included 70 rats. The rats were divided equally into 7 toxicity groups. The toxicity groups were administered aluminum phosphide suspension via an intragastric tube or oral lavage then rats died within six hours. Immediately after death, Group 0 rats' liver tissues were taken out. Group 1's liver tissues were obtained after a day. Group 2's liver tissues were collected after 2 days. Group 3's liver tissues were removed after 3 days. Group 4's liver tissues were taken after 4 days. Group 5's liver tissue was taken after five days. Group 6's liver tissue was extracted after 6 days. The obtained organ samples were used for biochemical, genetic and histopathological examinations.

Biochemical Analyses

Oxidative stress biomarkers in liver tissues homogenates:

1- Utilizing MDA test kits provided by BIODIAGNOSTIC, malondialdehyde (MDA) in tissue homogenate was measured photometrically following the method described by [(5)].

2- Assessment of catalase (CAT) in tissue homogenate: CAT was measured photometrically utilizing the BIODIAGNOSTIC CAT Colorimetric Assay Kit in accordance with the methodology outlined by [(6)].

Serine/threonine-protein kinase (mTOR):

Liver tissue samples used to assay the m TOR level in the sample using Rat MTOR (Serine/threonine-protein kinase mTOR) ELISA Kit supplied by (ELK biotechnology) catalogue number (ELK9209) accordance to the manufacturer's guidelines. This test detects Rat MTOR with good sensitivity as well as excellent specificity. No discernible interference or cross-reactivity between Rat MTOR and analogues was observed. Detection rang: 0.32-20 ng/mL and Sensitivity: 0.125 ng/mL. m TOR level was measured as described by [(7)].

Gene Expression

Monitoring the liver's microtubule-associated protein light chain 3 (LC3) gene expression:

LC3 gene expression was detected using quantitative real-time polymerase chain reaction (qRT-PCR). Briefly, total RNA was extracted from frozen tissue samples using the TRI Reagent® (Molecular Research Center, Inc. cat. No TR-118) following the manufacturer's protocol. RNA integrity was assessed using agarose gel electrophoresis and ethidium bromide staining. The extracted RNA was quantified by spectrophotometry. The resulting value was used to dilute the samples in nuclease-free water, aiming to equalize the parameters for gene expression analysis. Using the diluted samples, the quantification of LC3 gene expression was carried out using RT-qPCR, along with GAPDH quantification, the latter being used as an internal control to validate the reaction. Amplification was performed using a BRYT Green dye assay with GoTaq® 1-Step RT-qPCR System (Cat. No. A6020, Promega, Madison, Wisconsin, USA®) and a StepOnePlus Real-Time PCR System (Applied Biosystems, San Francisco, CA, USA), in addition to specific primers, following the manufacturer's protocol. The level of expression of target gene; LC3 was normalized relative to the expression of GAPDH mRNA in that sample using the ΔCt method. Relative differences in gene expression among groups were determined using the comparative C_t ($\Delta\Delta C_t$) method and fold expression was calculated as $2^{-\Delta\Delta Ct}$, where $\Delta\Delta C_t$ represents ΔC_t values normalized relative to the mean ΔC_t of control samples [8].

Primers:

LC3 PRIMER:

Forward: 5' –CAT GCC GTC CGA GAA GAC CT -3'

Reverse: 5' –GAT GAG CCG GAC ATC TTC CAC T -3'

GAPDH PRIMER:

F: 5′- TCA CCA CCA TGG AGA AGG C -3′

R: 5′- GCT AAG CAG TTG GTG GTG CA -3′

Histopathological examination

Liver samples underwent buffered formalin fixation, alcohol dehydration, xylene cleaning, and paraffin embedding. Hematoxylin and eosin (HE) staining was applied after the paraffin blocks were cut to a 5 μm thickness [9,10].

Ethical consideration

The Beni-Suef University "Research Ethics Animal Committee" gave its approval to the study's concept with Approval Number 022-379.

Statistical analysis

SPSS version 28 (IBM Corp., Armonk, NY, USA) was employed to code then enter the data. The data was summarized utilizing the mean and standard deviation. Groups were compared via the ANOVA with multiple comparisons post hoc test (Chan, 2003a)[11]. Quantitative variables were compared utilizing the Pearson correlation coefficient (Chan, 2003b)[12]. Through a variety of characteristics, PMI was predicted using linear regression analysis (Chan, 2004)[13]. Statistical significance was defined as P-values below 0.05.

3. Results:

As shown in table (1) MDA and m TOR were up regulated with difference between groups that was statistically significant (P value less than 0.05), while CAT and LC3 showed a statistically significant decrease between groups with P value less than 0.05

Table (1): Comparison of postmortem oxidant (MDA), antioxidant (CAT), m TOR and LC3 expression in different groups depending on post mortem interval

	G0	G1	G2	G3	G4	G5	G6
MDA nmol/g	0.89±0.04	1.19±0.1 *	1.67±0.08 *#	2.15±0.11 *#$	2.45±0.11 *#$@	2.68±0.05 *#$@&	2.94±0.14 *#$@&%
CAT mmol/g	2.67±0.04	2.46±0.05 *	2.27±0.04 *#	1.9±0.03 *#$	1.47±0.03 *#$@	1.12±0.04 *#$@&	0.8±0.03 *#$@&%
m TOR ng/ml	0.39±0.02	0.56±0.06	0.77±0.04 *#	2.09±0.13 *#$	2.37±0.22 *#$@	2.26±0.05 *#$	2.41±0.03 *#$@
LC3	1.03±0.05	0.84±0.05 *	0.81±0.1 *	0.56±0.05 *#$	0.41±0.01 *#$@	0.34±0.05 *#$@	0.2±0.01 *#$@&%

Values are presented as mean ±SD

*: statistically significant compared to corresponding value in group 0 (P<0.05)

#: statistically significant compared to corresponding value in group 1(P<0.05)

$: statistically significant compared to corresponding value in group 2 (P<0.05)

@: statistically significant compared to corresponding value in group 3 (P<0.05)

&: statistically significant compared to corresponding value in group 4 (P<0.05)

%: statistically significant compared to corresponding value in group 5 (P<0.05)

The post hoc pairwise comparisons regarding MDA and CAT between each 2 groups, demonstrated that there was a statistically significant difference between all groups p value<0.001 as shown in table (2), figure (1, 2, respectively).

Table (2): Posthoc pairwise comparison (P value between each 2 groups)

		G0	G1	G2	G3	G4	G5	G6
MDA nmol/g	G0		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	G1	<0.001		<0.001	<0.001	<0.001	<0.001	<0.001
	G2	<0.001	<0.001		<0.001	<0.001	<0.001	<0.001
	G3	<0.001	<0.001	<0.001		<0.001	<0.001	<0.001
	G4	<0.001	<0.001	<0.001	<0.001		0.004	<0.001
	G5	<0.001	<0.001	<0.001	<0.001	0.004		<0.001
	G6	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
CAT mmol/g	G0		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	G1	<0.001		<0.001	<0.001	<0.001	<0.001	<0.001
	G2	<0.001	<0.001		<0.001	<0.001	<0.001	<0.001
	G3	<0.001	<0.001	<0.001		<0.001	<0.001	<0.001
	G4	<0.001	<0.001	<0.001	<0.001		<0.001	<0.001
	G5	<0.001	<0.001	<0.001	<0.001	<0.001		<0.001
	G6	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
m TOR ng/ml	G0		0.085	<0.001	<0.001	<0.001	<0.001	<0.001
	G1	0.085		0.021	<0.001	<0.001	<0.001	<0.001
	G2	<0.001	0.021		<0.001	<0.001	<0.001	<0.001
	G3	<0.001	<0.001	<0.001		0.001	0.085	<0.001
	G4	<0.001	<0.001	<0.001	0.001		0.570	0.986
	G5	<0.001	<0.001	<0.001	0.085	0.570		0.170
	G6	<0.001	<0.001	<0.001	<0.001	0.986	0.170	
LC3	G0		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	G1	<0.001		0.946	<0.001	<0.001	<0.001	<0.001
	G2	<0.001	0.946		<0.001	<0.001	<0.001	<0.001
	G3	<0.001	<0.001	<0.001		<0.001	<0.001	<0.001
	G4	<0.001	<0.001	<0.001	<0.001		0.408	<0.001
	G5	<0.001	<0.001	<0.001	<0.001	0.408		0.001
	G6	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	

The post hoc pairwise comparisons regarding m TOR between each 2 groups, demonstrated that there was a significant difference among all groups with p value less than 0.001 except groups (0 and 1), (3 and 5), (4 and 5), (4 and 6), (5 and 6) with p value > 0.05 as shown in table (2), figure (3).

The post hoc pairwise comparisons regarding LC3 between each 2 groups, demonstrate that every group showed a statistically significant difference (p value<0.001) except groups (1 and 2), (4 and 5), with p value > 0.05 as shown in table (2), figure (4).

PMI was positively correlated with MDA and m TOR with P-value less than 0.001 as shown in table (3), figure (5,7, respectively), while PMI was a negatively correlated with CAT and LC3 as shown in table (3), figure (6,8, respectively), (P-value less than 0.001)

Table (3): Correlation between PMI and measured parameters

		PMI (Day)
MDA nmol/g	**R**	0.984
	P value	<0.001
	N	42
CAT mmol/g	**R**	-0.992-
	P value	<0.001
	N	42
m TOR ng/ml	**R**	0.917
	P value	<0.001
	N	42
LC3	**R**	-0.974-
	P value	<0.001
	N	42

Table (4) showed that the linear regression analysis revealed that LC 3 was A potent independent PMI predictor as the 95% confidence interval (CI) in the form of upper as well as lower bounds were-6.329-, -7.345- and p less than 0.001, respectively. Furthermore, the point estimate between PMI and LC3 was -0.974-.

PMI (Day)= 7.086-6.837* **LC3.**

As shown in table (5), the linear regression analysis revealed that MDA, CAT and m TOR were strong predictors of PMI as the 95% confidence interval (CI) in the form of upper and lower bounds of MDA(1.943, 0.967), CAT (-1.443-, -2.239-), respectively) with p < 0.001and m TOR were (-0.076-, -0.537-, respectively) with P value 0.011 and the Standard Error of Estimate of MDA and CAT were (0.241, 0.197, respectively) and m TOR was (0.114). Furthermore, the point estimate between PMI and MDA was 6.037, PMI with CAT was -9.369- and PMI with m TOR was -2.687-

PMI (Day)= 3.909+1.455* **MDA nmol/g**-1.841* **CAT mmol/g**-0.306* **m TOR ng/ml**

Table (4): Linear regression equation for PMI estimation with LC3

Model		Unstandardized Coefficients		Standardized Coefficients	t	P value	95.0% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
PMI (Day)	**(Constant)**	7.086	0.167		42.551	<0.001	6.749	7.423
	LC3	-6.837-	0.252	-0.974-	-27.182-	<0.001	-7.345-	-6.329-

Table (5): Linear regression equation for PMI estimation with all parameters (MDA, CAT and m TOR).

Model		Unstandardized Coefficients		Standardized Coefficients	t	P value	95.0% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
PMI (Day)	(Constant)	3.909	0.731		5.351	<0.001	2.430	5.388
	MDA nmol/g	1.455	0.241	0.523	6.037	<0.001	0.967	1.943
	CAT mmol/g	-1.841-	0.197	-0.603-	-9.369-	<0.001	-2.239-	-1.443-
	m TOR ng/ml	-0.306-	0.114	-0.132-	-2.687-	0.011	-0.537-	-0.076-

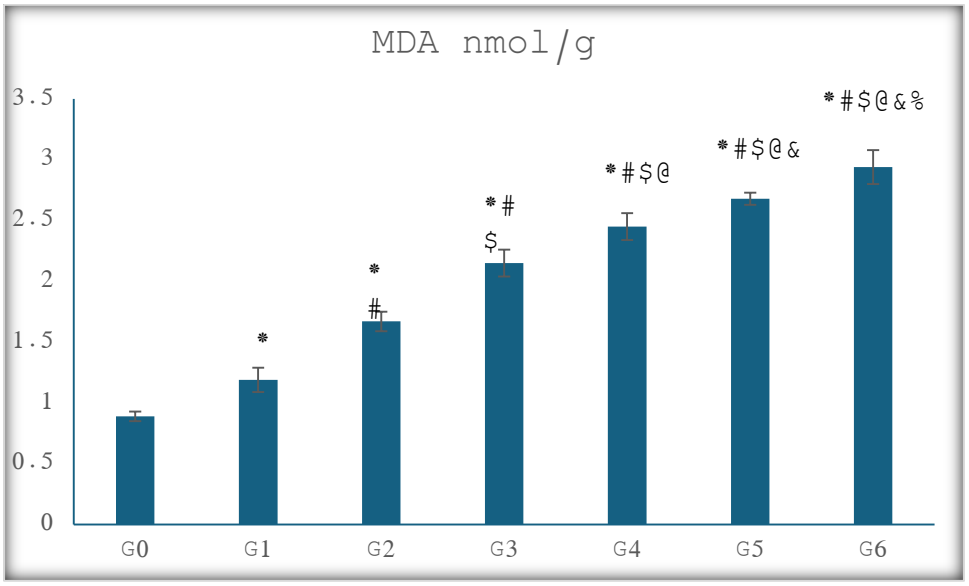


Figure (1): The post hoc pairwise comparisons regarding MDA between each 2 groups

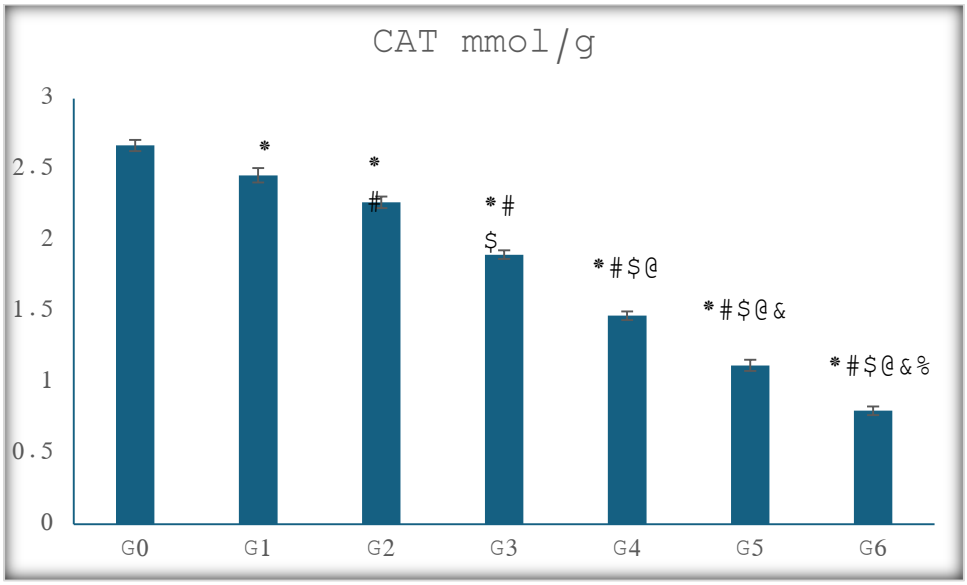


Figure (2): The post hoc pairwise comparisons regarding CAT between each 2 groups

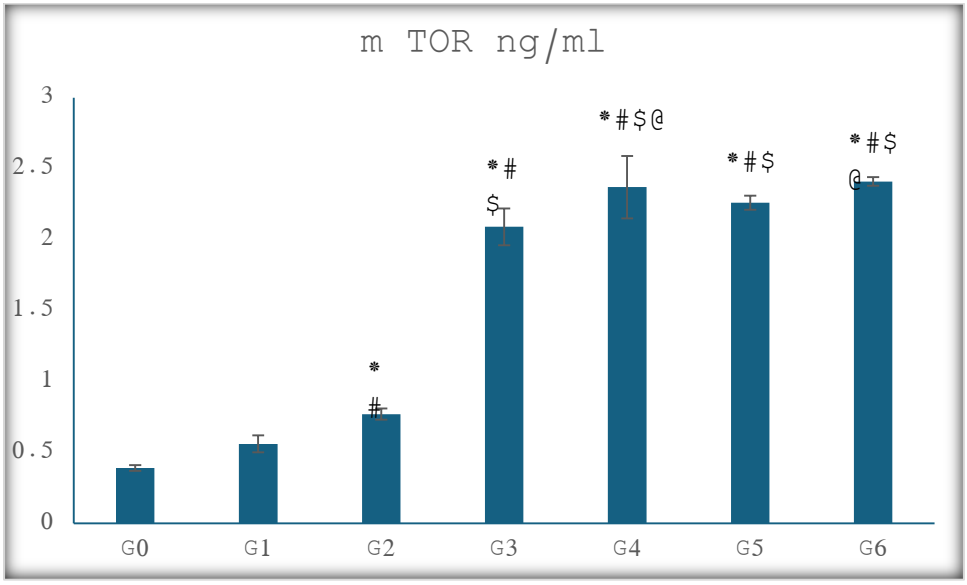


Figure (3): The post hoc pairwise comparisons regarding m TOR between each 2 groups

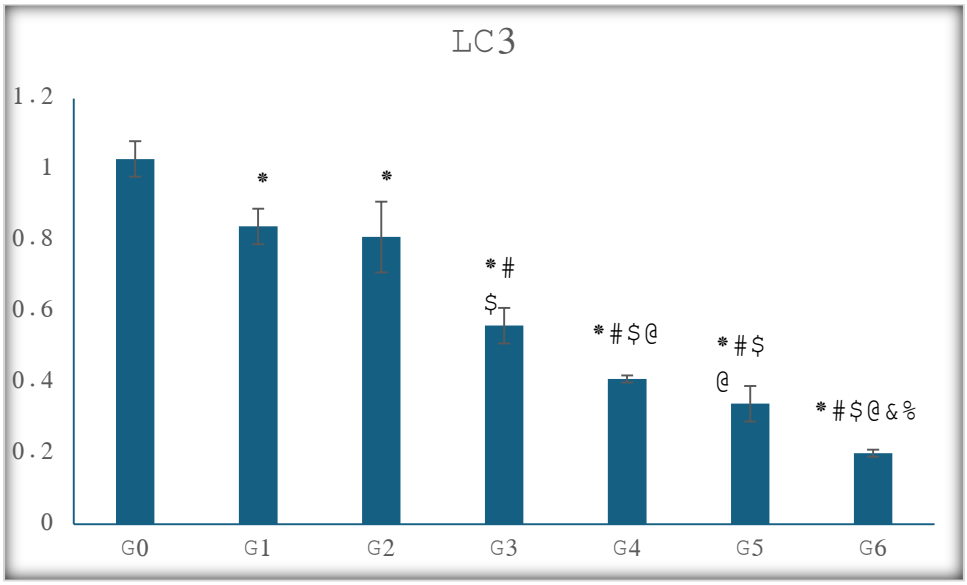


Figure (4): The post hoc pairwise comparisons regarding LC3 between each 2 groups

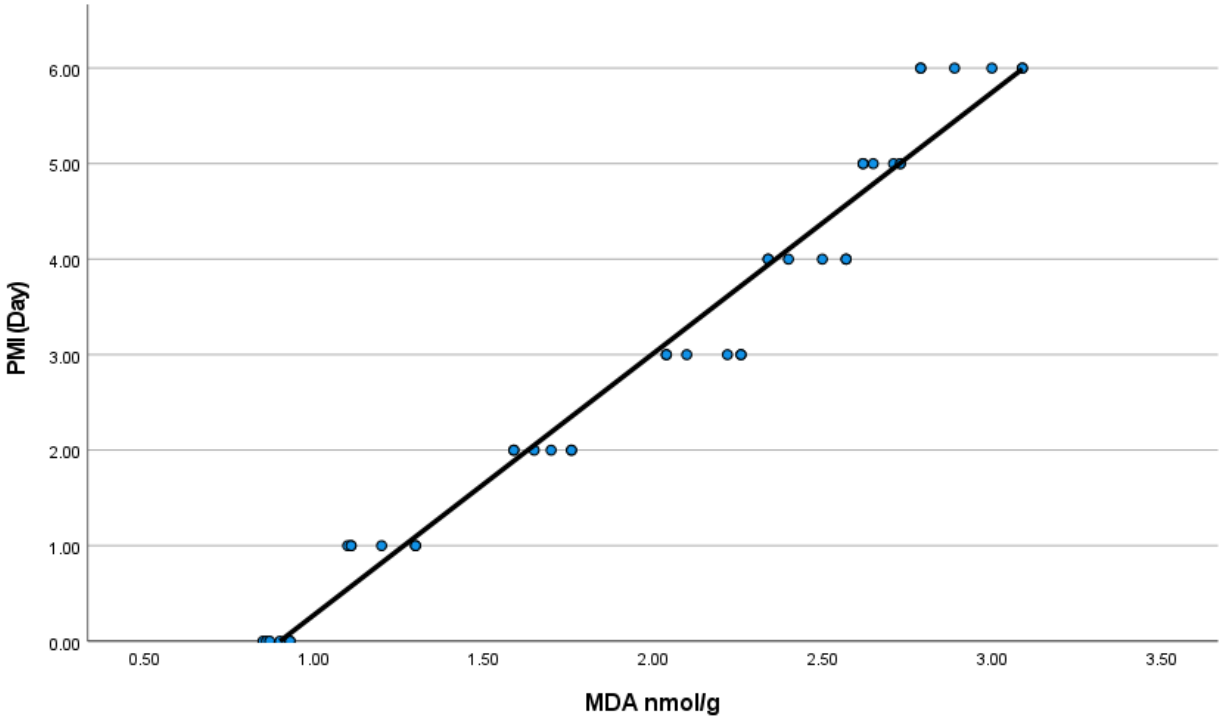


Figure (5): correlation of MDA with PMI in different groups

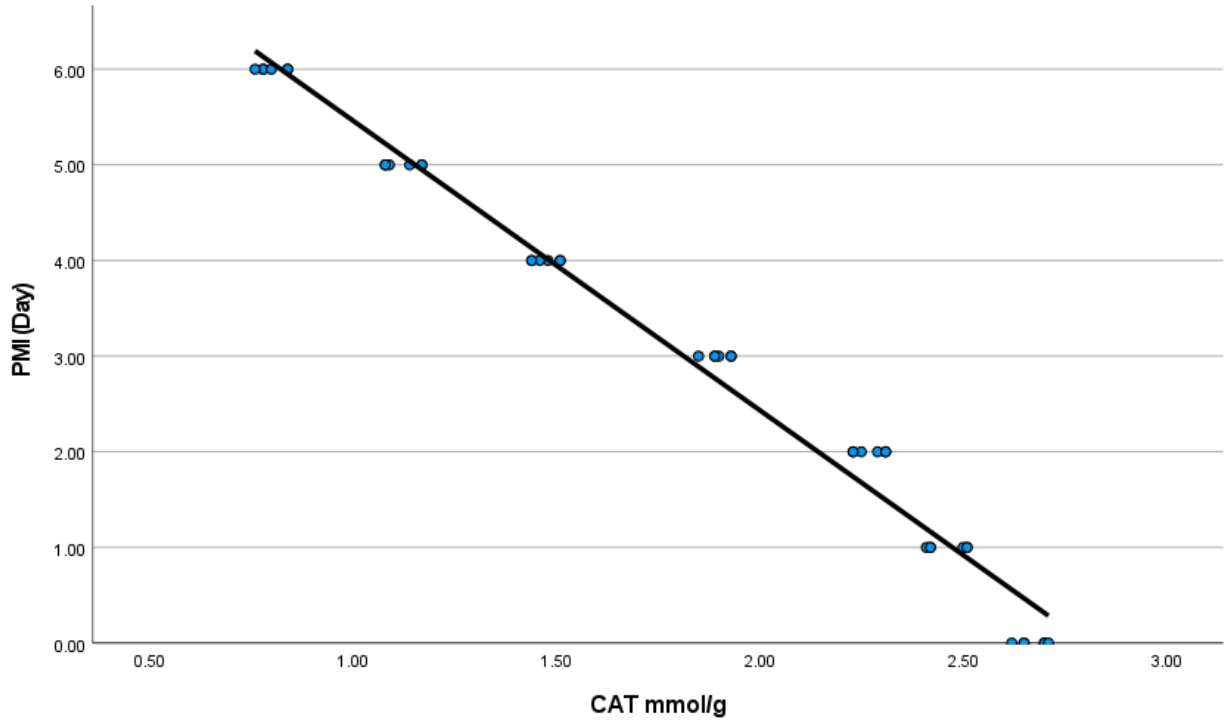


Figure (6): correlation of PMI with CAT in different groups

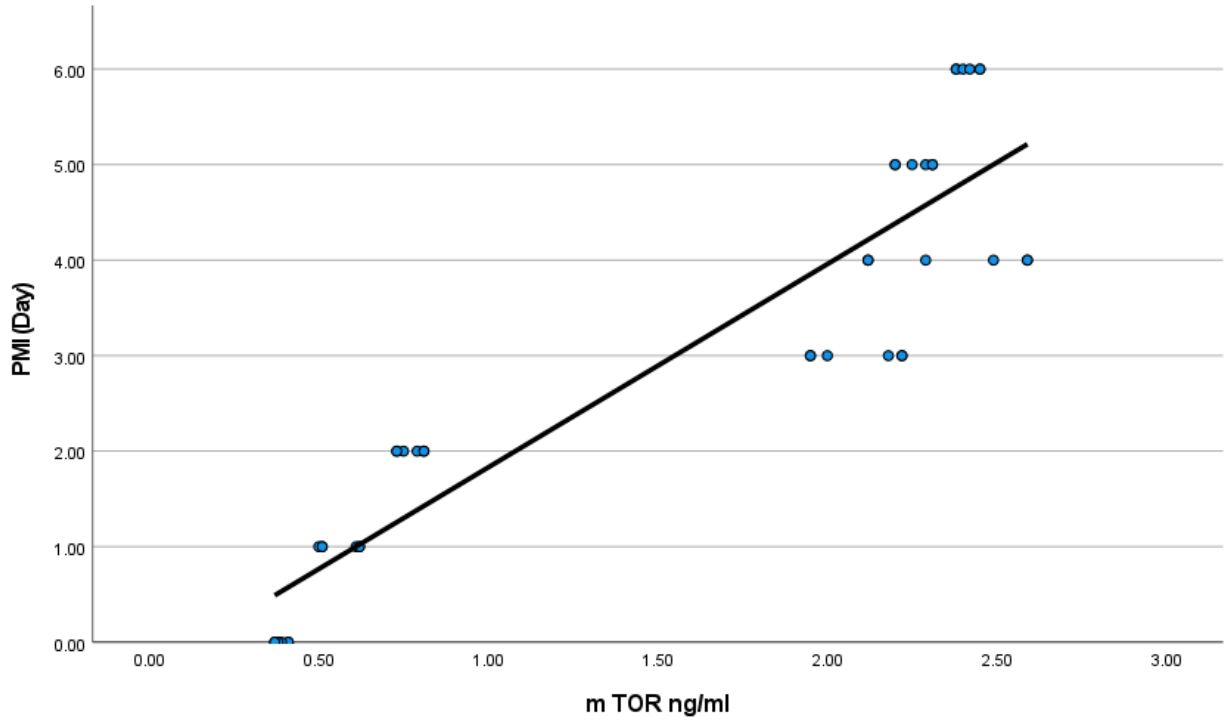


Figure (7): correlation of PMI with m TOR in different groups

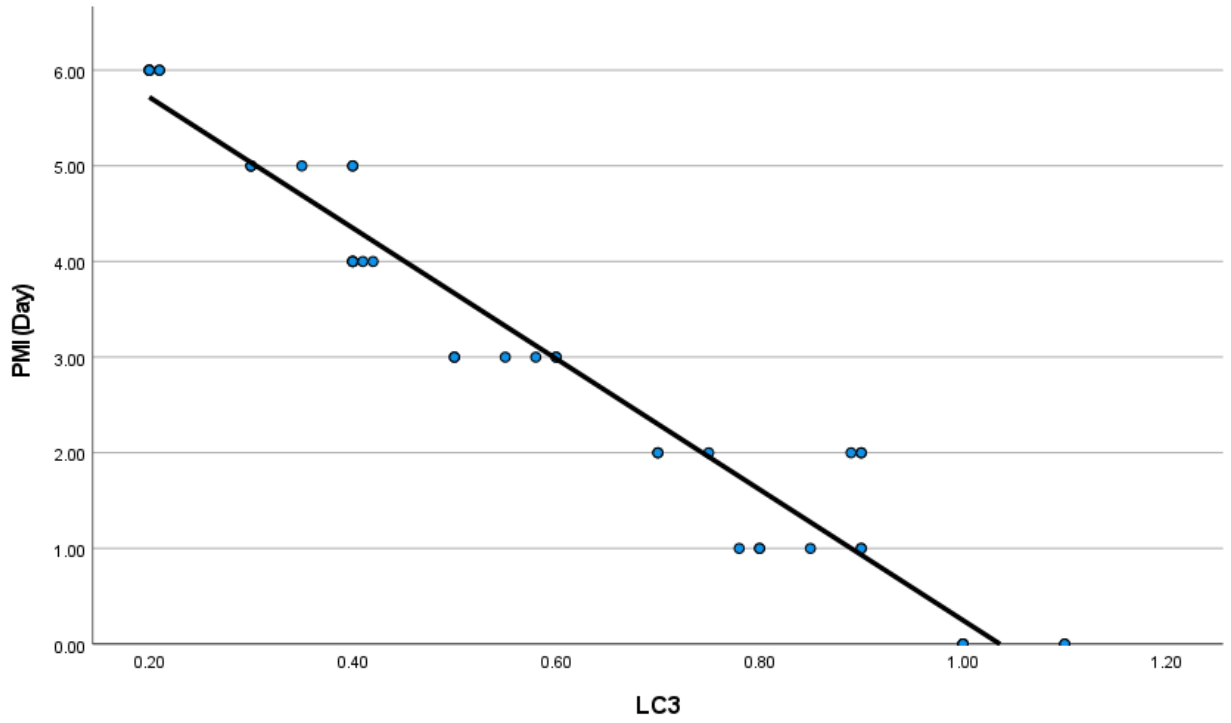
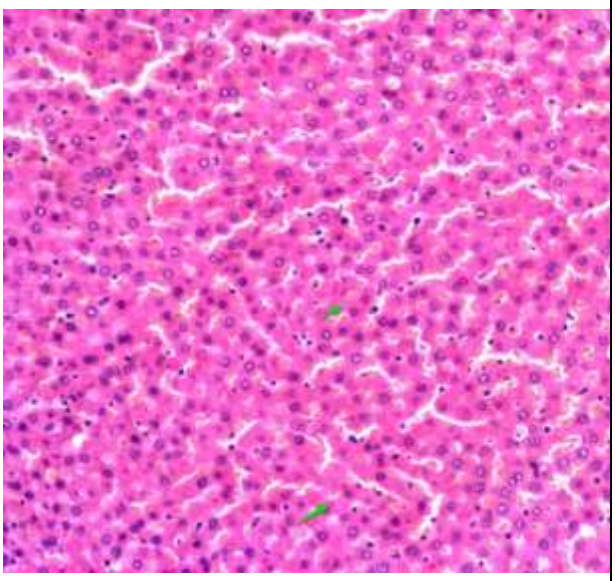


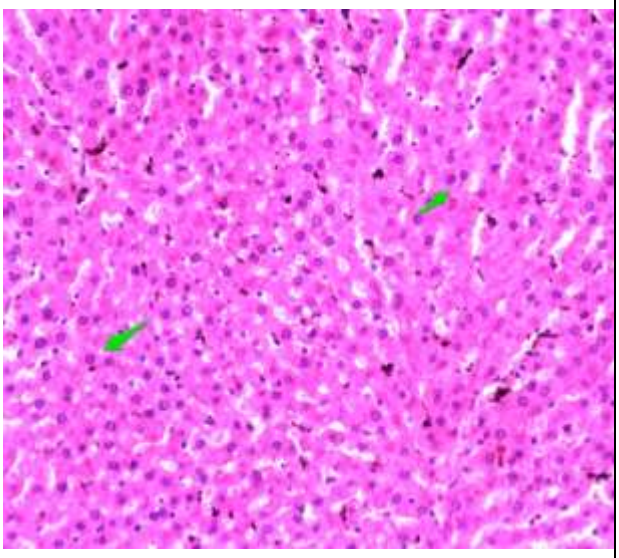
Figure (8): correlation of PMI with LC3 in different groups

The current study showed that histopathological changes also showed time-dependent variations. There was normal liver structure at time if death. All of the liver cells and cell nuclei were clearly visible 1-day postmortem, the most prominent changes appearing affected the shape and size of hepatocytes, minor changes 2-day postmortem, the nuclei were observed to shrink, and partial degradation was seen in the hepatocytes 3-day postmortem, there were pyknosis and swelling of the hepatocytes 4- day postmortem, the nuclei completely disappeared; cell walls appeared to become less distinct at 5- day postmortem and there were lysis of RBCs and loss of cell outline 6-day postmortem as shown in figure (9).

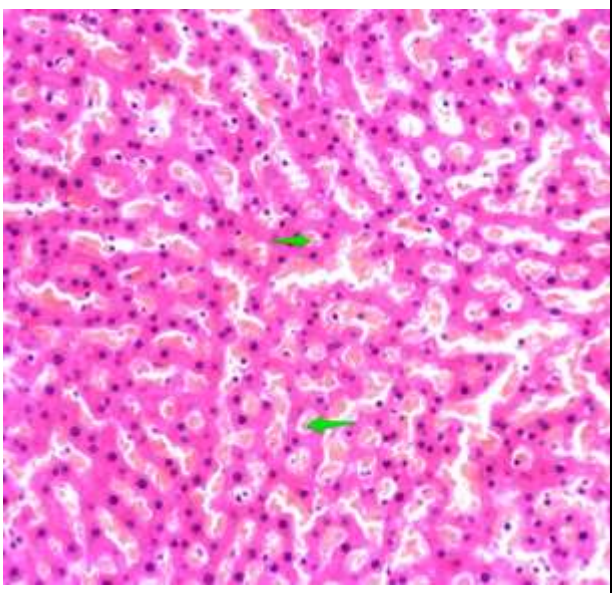
A)



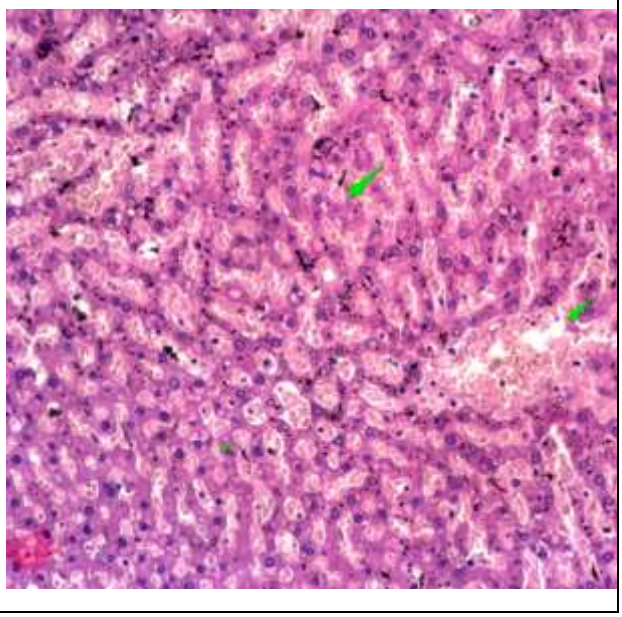
B)



C)



D)



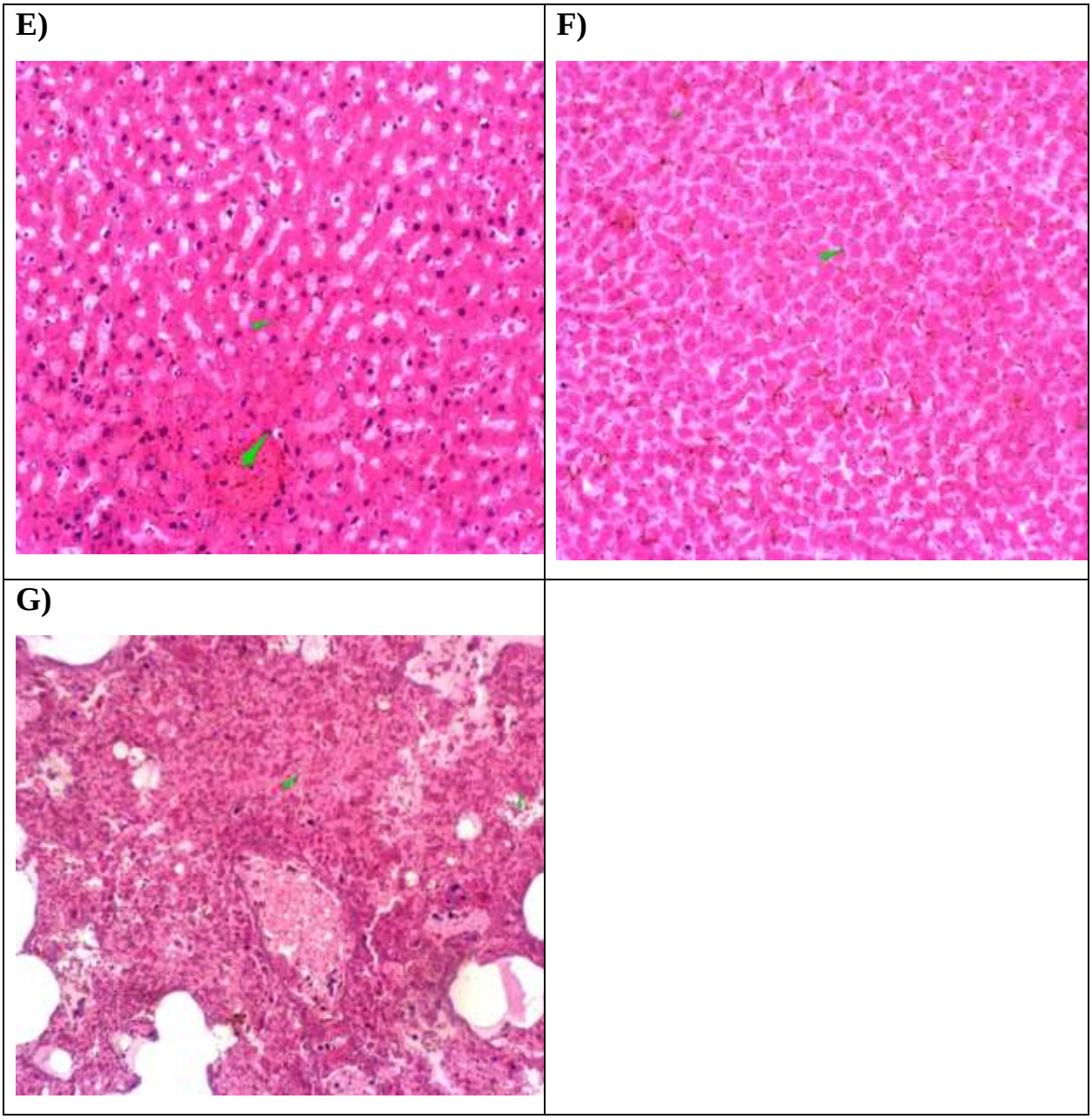


Figure (9): Photomicrograph of liver at different postmortem intervals. **A** normal liver structure at time of death (green arrows). **B** All of the liver cells and cell nuclei were clearly visible (green arrows) 1-day postmortem. **C** The most prominent changes appearing affected the shape and size of hepatocytes, minor changes (green arrows) 2-day postmortem. **D** The nuclei were observed to shrink, and partial degradation was seen in the hepatocytes (green arrows) 3-day postmortem. **E** Pyknosis and swelling of the hepatocytes (green arrows) 4- day postmortem. **F** the nuclei completely disappeared; cell walls appeared to become less distinct (green arrows) at 5- day

postmortem. **G** There were lysis of RBCs and loss of cell outline (green arrows) 6- day postmortem. (H&EX200)

4. Discussion:

A crucial task in every death investigation is to estimate PMI. Physical factors (algor mortis, postmortem hypostasis), physicochemical (postmortem regidity), biochemical (electrolyte level and enzyme activity), microbiological (decomposition), entomological, as well as botanical elements are all utilized to determine the PMI [14].

According to the science of "thanatochemistry," current trends in calculating the PMI aim to determine it quantitatively based on chemical changes occuring on and in the corpse. Even though PMI estimations have improved thanks to these methods, more study has been done to increase precision and accuracy of the calculation. The fields of "thanatobiology" and "thanatomicrobiome" have developed as a result. The foundation of thanatobiology is the estimation of PMI from protein analysis, cell death signaling pathways as well as DNA/RNA degradation [15].

The autolysis process has been utilized in recent developments in PMI estimate, specifically through the analysis of apoptosis as well as autophagy gene expression. As a result of cell death or as a regulatory mechanism, oxidative stress plays a role in this signaling [16].

Aluminum phosphide (AlP) is a chemical compound that can cause death in some countries. AlP inhibits the functioning of cytochrome C oxidase in the mitochondria, leading to toxicity. The poisoned patient's AlP-related toxicity is caused by oxidative stress, ROS production, as well as inflammatory signaling [17]**.** AlP poisoning results in multi-organ failure, especially in the liver, kidneys, and heart—the three most important organs that depend on oxidative phosphorylation [18]**.**

Therefore, we aim to evaluate of PMI in aluminum phosphide toxicity using oxidative stress markers as well as the expression of autophagy gene and apoptosis genes.

The results of the current research demonstrated that MDA was upregulated with difference that is statistically significant between groups (P value <0.05), while CAT showed a significant decrease (P value <0.05) depending on time passed since death. The post hoc pairwise comparisons regarding MDA and CAT between each 2 groups, demonstrate that there was a significant

difference between all groups with p value<0.001.

Similarly, ***Sener et al.*** [19] observed that the rat liver's MDA levels significantly increased after death, though at different PMIs, and that the rat liver's catalase levels significantly decreased within one to two hours after death.

Shaaban et al. [20] investigated how oxidant in addition to antioxidant factors affected the estimation of PMI and discovered a notable increase in MDA levels, but there was no significant decrease of CAT was observed during the period of experiment (8 hours) and PMI was positively correlated with MDA and negatively correlated with CAT with P-value less than 0.05.

Similarly, ***El-Noor et al.*** [21] examined the changes in oxidative stress indicators in the heart as well as kidney after death and found that M DA significantly increased and at one to two and six to seven hours after death, the levels of catalase in the kidney and heart, respectively were significantly lower. ***Welson et al.*** [22] discovered a strong correlation between the PMI and oxidative stress parameters in the kidney and heart.

Oxygen deprivation after death results in oxidative stress and cell damage [23]**.** A notable imbalance between ROS and the antioxidant system's ability to eliminate them is known as oxidative stress. This can be the result of a decline in antioxidant defenses or an increase in ROS production. Tissue injury and repair are closely related to the oxidant/antioxidant parameters ***[24].***

The current study showed that m TOR were up regulated with statistically significant difference among groups (P value less than 0.05). The post hoc pairwise comparisons regarding m TOR between each 2 groups, demonstrate that there was a statistically significant difference among all groups (p value less than 0.001) except groups (0 and 1), (3 and 5), (4 and 5), (4 and 6), (5 and 6) with p value > 0.05. There was positive correlation between PMI with m TOR (P-value <0.001).

To the best of our knowledge, no prior research has used mTOR to predict PMI; however, ***C. Zapico et al.*** [25] examined early PMI (zero to eight hours) in the rat gastrocnemius muscle. The apoptosis regulator phosphatase and tensin homologue deleted on chromosome ten (PTEN) was examined for mRNA expression. The results showed the PMI and the mRNA expression of this protein have a positive linear connection, as well as a time-dependent rise in PTEN protein mRNA expression levels up

until six hours after death and a decrease in PTEN expression eight hours later. Increased oxidative stress brought on by the cell death process or RNA degradation may be the cause of the drop.

PTEN's detrimental role in the PI3K/AKT/mTOR pathway is well known, and mTOR has been demonstrated to be involved in a number of biological processes, like food metabolism, progression of cell cycle, as well as autophagy. AKT is rendered inactive by PTEN loss. AKT activating mutation (AKT1 E17K) and PTEN deficiency both cause mTOR to be sequentially activated via negatively regulating Rheb [(26)].

The present study found that LC3 showed a significant decrease between groups (P value <0.05). The post hoc pairwise comparisons regarding LC3 between each 2 groups, demonstrate that there was a significant difference between all groups (p value less than 0.001) except groups (1 and 2), (4 and 5), with p value > 0.05. There was a negative correlation between PMI with LC3

The expression of autophagy genes in the rat gastrocnemius muscle during the first eight hours of PMI was examined by [(16)]. LC3, which functions in the initial phases, rose for two hours before declining.

This rise is then explained by the fact that autophagy signaling seems to initially increase shortly after death in an effort to lessen stress and save the cell. However, because autophagy and apoptosis have been shown to interact [(27)]**,** cells may react by triggering apoptosis if the stress continues and a survival strategy can no longer be supported by autophagy [(15)]**.**

In the current study, the linear regression equation analysis for PMI estimation with LC3 revealed that LC 3 was a substantial independent indicator of PMI as the 95% confidence interval (CI) in the form of upper as well as lower bounds were-6.329-, -7.345- and p < 0.001, respectively. Furthermore, the point estimate between PMI and LC3 was -0.974-. PMI (Day)= 7.086-6.837* **LC3** and the linear regression equation analysis for PMI estimation with all parameters (MDA, CAT and m TOR) revealed that MDA, CAT and m TOR were strong predictors of PMI with Standard Error of Estimate of MDA and CAT were (0.241, 0.197, respectively) with p < 0.001and m TOR was (0.114) with P value 0.011. Furthermore, the point estimate between PMI and MDA was 6.037, PMI with CAT was -9.369- and PMI with m TOR was -2.687-. PMI (Day)= 3.909+1.455* **MDA nmol/g**-1.841* **CAT mmol/g**-0.306* **m TOR ng/ml.**

To the best of our knowledge, no prior study utilized LC3 as in the equation to predict PMI.

Similarly, ***Shaaban et al.*** [20] found that in the liver, MDA was a strong independent predictor of PMI, PMI= (0.124 MDA) + 2.367 with Standard Error of Estimate = 0.363 and p value = 0.011 and found that CAT in the brain was a strong independent predictor of PMI, PMI= (-0.008 CAT) + 7.522 with Standard Error of Estimate = 0.001 and p value <0.001.

To the best of our knowledge, no prior study utilized the m TOR equation to predict PMI.

The current study showed that histopathological changes also showed time-dependent variations. There was normal liver structure at time if death. All of the liver cells and cell nuclei were clearly visible 1-day postmortem, the most prominent changes appearing affected the shape and size of hepatocytes, minor changes 2-day postmortem, the nuclei were observed to shrink, and partial degradation was seen in the hepatocytes 3-day postmortem, there were pyknosis and swelling of the hepatocytes 4- day postmortem, the nuclei completely disappeared; cell walls appeared to become less distinct at 5- day postmortem and there were lysis of RBCs and loss of cell outline 6- day postmortem.

According to ***Yahia et al.*** [10], liver samples taken from dogs showed normal architecture at the time of death. Four hours after death, the hepatocytes showed some minor changes, including pyknosis in a few nuclei. Twelve and twenty-four hours after death, there was an increase in cytoplasmic expansion and nuclear pyrknosis. Cell outlines disappeared 24 and 72 hours after death, while hepatocytes and RBCs in the sinusoids were totally lysed.

Histological testing is a dependable technique for measuring PMI, as evidenced by the histological alterations found in numerous studies. ***Welson et al.*** [22] shown that PMI in rats can be ascertained by using histological abnormalities in the kidneys, testis, as well as heart. They found that until 120 hours after death, postmortem anoxia resulted in oxidative stress in the different tissues, gradual autolysis, as well as loss of cellular structure, form, and organization. ***Mostafa et al.*** [28] found a strong correlation between PMI and histological alterations in muscle tissue.

The release of lysosome autolytic enzymes may be the source of these changes, which lead to the breakdown of cellular

constituents and the loss of tissue architecture [(22)].

5. Conclusion:

According to the current study's findings, the level of LC3 and mTOR expression in liver tissue can be utilized to calculate the PMI. LC3 was strong independent indicator for PMI estimation.

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