Mansoura Journal of Forensic Medicine and Clinical Toxicology

Estimation of Postmortem Intervals by Some Biochemical Changes and DNA Degradation in Rat Brain and Skeletal Muscle Tissues

Amr R. Zaki, Tohamy, A.F, Nour El-houda, Y. H

KEYWORDS
Post-mortem intervals
Brain
Muscle
Oxidant and antioxidant
DNA fragmentation
Histopathological changes

ABSTRACT

Accurate estimation of postmortem interval (PMI) is one of the most important and difficult issues in forensic medicine. After death, the tissues undergo autolysis and bio-macromolecules degrade. The relationship of extent of DNA damage and certain components of the oxidant/antioxidant balance in brain and femoral muscle tissues of rats killed by cervical dislocation or drowning, with the post-mortem intervals was investigated and examined by making a relation with the histopathological finding in brain tissue. The results showed that the oxidant/antioxidant balance in brain and femoral muscle tissue was shift insignificant favour of the oxidants at 24 hrs postmortem and lasted tell 96 hours post-mortem toward the oxidant. The percentage of DNA fragmentation was detected increased parallel to significant increase of oxidant level from zero to 96 hrs post-mortem. The histopathological alterations in brain and muscle tissues of drowned or cervical dislocation were found to be in full accordance with biochemical finding and DNA fragmentation. Our results suggest that biochemical analysis, DNA fragmentation as well as histopathological examination of brain and muscle tissues provide an accurate estimation of post-mortem intervals.

Introduction

Postmortem interval (PMI) is the time elapsed between death of a person and the time of autopsy. Although the exact time of death can rarely be estimated on the basis of autopsy findings alone, an appropriate range of PMI can be deduced by intelligent interpretation of various changes that take place after death (Chandrakanth et al., 2013).

Following death, a complex series of biochemical and pathological processes are initiated resulting in considerable alterations of the structure and composition of the human body. Because many of these changes occur sequentially, it has been proposed that the evaluation of the types and degrees of changes may enable estimation of time since death (Ferreira and Cunha, 2013).

An estimation of the time since death is commonly inferred from the evaluation of physiological and physical postmortem changes, such as the distribution and amount of rigor mortis, death stains, changes in body temperature, changes in potassium concentration of the vitreous humor, the degree of decay in the body, proliferation of bugs on the corpse and their developmental stages

·*Amr R. Zaki,*
Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Beni-Suef University.
Tohamy, A.F,
Department of Toxicology and Forensic Medicine, Faculty of Veterinary Medicine, Cairo University.
Nour El-houda, Y. H,
Department of Toxicology and Forensic Medicine, Faculty of Veterinary Medicine, Beni-Suef University.

(Henssge and Madea, 2004; Knight et al., 2004). In fact, there are several internal factors: age of the deceased, gender, physical, pathological state and external factors as air temperature, humidity, bug and animal activity affect the postmortem process rendering the determination of time of death more complicated (Poloz and O'Day, 2009; Prieto et al., 2007).

In the field of forensic pathology one of the most important issues is the correct estimation of the PMI (Lendoiro et al., 2012). A precise estimation of the time of death enables the verification of witnesses' statements, limits the number of suspects and assesses their alibis (Kaliszan, 2012).

An accurate estimation of the PMI requires the evaluation of parameters that change constantly with time after death. This definition seems to fit well in post mortem degradation of nucleic acids (Liu et al., 2007). Indeed, with the advances of molecular biology, the analysis of time-dependent degradation of nucleic acids (both DNA and RNA) became a focus of attention in forensic science (Bauer et al., 2003). Forensic DNA analysis has advanced considerably over the past 20 years through the development of new techniques, such as the use of miniSTR analysis, which involves amplification of smaller DNA targets (Tate et al., 2012). DNA is one of the most stable components of cells, and its content is similar among different individuals and different cell types within the same species (Larkin, et al., 2010; Lin, et al., 2011), so DNA is often relied upon to identify missing persons and victims of mass fatality incidents (Marjanovic et al., 2007 & Parsons et al., 2007). DNA degradation is caused by intracellular enzymes and bacterial proliferation, then its estimation may lead to a diagnosis of the degree of postmortem changes, including the time after death (Swango et al., 2006).

Since DNA is known to be stable a long postmortem period, methods for quantification of DNA degradation level, such as flow cytometry or single cell electrophoresis, were described by several authors (Johnson and Ferris, 2002). Due to the rapid decomposition rate, tissues such as blood and kidney were found to be unsuitable for DNA fingerprinting after a period of one week. Brain, lymph nodes and skeletal muscles preserved high molecular DNA up to 3 weeks, whereas kidney, thyroid and spleen retained it for one week and liver lost all high molecular DNA after 2 days. Brain tissue seems to be one of the best sources of DNA for post-mortal studies followed by muscle and blood and then other internal organs, whereas liver is consistently a poor source of DNA (Parsons and Weedn, 1997).

Oxidative stress is defined as a marked imbalance between the reactive oxygen species (ROS) and its removal by antioxidant system. These free radicals associated with cellular death (Halliwell, 2001; Serafini and Del Rio, 2004). Brain tissue has multiple potential sources of ROS (Faraci, 2006) and a large oxidative capacity, but its ability to combat oxidative stress is limited (Mantha et al., 2006). Oxidants and antioxidants; malondialdehydes (MDA), reduced glutathione (GSH), and catalase (CAT), play important role in determining the early post-mortem intervals. Under physiological conditions, the oxidant/antioxidant defense system in human body is in a state of continuous equilibrium. Equilibrium in aerobic metabolism (in alive tissue) is characterized by the formation of free radicals and their removal by means of antioxidant systems (Clarkson and Thompson, 2000). Increase in oxidant levels and a decrease in antioxidant levels are observed in the damaged tissues of any live animal (Iraz et al., 2006; Aguilar et al., 2007).

The purpose of our study was to investigate and examine the correlation between the levels of oxidant/antioxidant

parameters, the extent of DNA damage, histopathological changes in brain and femoral muscle tissue of rats and post-mortem intervals.

Material and Methods

Animals

Forty adult male albino rats; each about (150-200 g) were used. They were obtained from the Egyptian holding company for biological products and vaccines, Cairo, Egypt. They were kept under good ventilation and standard hygienic conditions and allowed free access to balanced standard diet pellets and tap water.

Experimental design

Rats were classified into two groups, each group containing a total of 20 rats. The first group was subjected to death via drowning, while the 2nd group was anesthetized by diethylether inhalation, followed by cervical dislocation (at room temperature 20°C). Rats of each group were divided into 4 subgroups (n=5), the first group was dissected to obtain organs (brain and femoral muscle) immediately after death (0 time) while the 2nd, 3rd, 4th groups were dissected to get the same organs at 24, 48, 96 h postmortem, respectively.

The required biochemical, DNA fragmentation and histopathological investigations were performed on the specimens and evaluated them by direct comparison between groups.

Histopathological study:

Brain and femoral muscle tissues were taken from the dissected rats in each examined intervals. They washed using chilled saline solution then perfused with phosphate buffer saline (50 mM potassium phosphate, pH 7.4 containing 0.16 mg/ml heparin) to remove any red blood cells and clots. The tissues were homogenized in 5-10 ml cold buffer (i.e 50 mM potassium phosphate which composed of 9.4 ml of 1M monobasic solution and 40.6 ml of 1M dibasic solution and complete to 12 by distilled water, pH 5.1, 1mM EDTA) per gram tissue using tissue homogenizer and centrifuged at 4000 rpm/15 min at 4°C. The supernatant was washed and subjected to assay the activity of catalase (Aebi et al., 1984), reduced glutathione (Beutler et al., 1963) and lipid peroxidation (LPO) which were measured by estimation of thiobarbituric acid reactive substance (TBARS) method of Ohkawa et al. (1979).

1. Brain and femoral muscle tissues for DNA fragmentation
   Brain and muscle tissues were lysed in 1 ml buffer (10mM Tris-Hcl, pH 7.4, 10 mM EDTA, 0.5% TRITON X-100) by the method of Sellins and Cohen (1987).
2. Agarose gel electrophoresis of fragmented DNA
   For electrophoretic analysis of fragmented DNA, the total nuclear DNA was isolated from tissue according to the method of Kuo et al. (2005).
3. Brain and femoral muscle tissues for histopathological study:
   Autopsy samples were taken from the brain and muscle tissues of rats in different groups and fixed in 10% formal saline for 24 h. They were subjected to histopathological examination according to Banchroft et al. (1996).

   Statistical Analysis

   The obtained data were analyzed using one way analysis of variances (ANOVA) followed by Duncan TEST using SPSS 11.0 statistical software (Spss, Inc, Chicago, IL, 2001). They were expressed as mean ± standard error (SE).

Results

As can been seen in tables (1, 2, 3 and 4) as well as in figures (1-6), that the difference between oxidant and antioxidant parameters at 0 to 96 hours post-mortem were found to be statistically significant ($p \leq 0.05$). These data were similar in case of brain and muscle samples in both cervical dislocation and drowning. Catalase and reduced glutathione were significantly decreased in brain and muscle tissue after 24 h of the post-mortem interval and continued in inhibition till 96 h PMI. There was a time dependent factor between the oxidant/antioxidant evaluated parameters. The concentration of lipid peroxidation was significantly increased in brain and muscle tissues of rats subjected to cervical dislocation or drowning after 24 h post-mortem interval and the elevation was continued in increase till 96 h.

DNA fragmentation percentage in brain and muscle tissues of rats that killed by cervical dislocation and drowning showed ascending increase from 0time till 96hrs post-mortem interval. Also, there were time dependent factors between the all tested interval and percent of DNA fragmentation, as seen in tables (5 and 6) and figures (7 and 9).

As demonstrated in figures (8 and 10), a quantitative analysis of DNA fragmentation related to post-mortem interval showed a strong correlation between increased fragmentation and increasing time since death. Brain DNA showed slower degradation than muscle DNA.

Table (1): The levels of analyzed oxidants and antioxidants in rat brain tissues after cervical dislocation (20 rats).

Time/Hours	Brain tissue		
	Catalase	LPO	GSH
0	0.268 ± 0.002^{bcd}	$8.431\pm0.0.28^{bcd}$	5.214 ± 0.06^{bcd}
24	0.234 ± 0.01^{acd}	9.089 ± 0.03^{acd}	4.857 ± 0.05^{acd}
48	0.210 ± 0.004^{abd}	10.450 ± 0.16^{abd}	4.259 ± 0.05^{abd}
96	0.171 ± 0.008^{abc}	13.025 ± 0.13^{abc}	3.848 ± 0.04^{abc}

([a]) Significantly different from control group (zero time) at $p\leq0.05$.
([b]) Significantly different from group of 24 h time intervals (after death) at $p\leq0.05$.
([c]) Significantly different from group of 48 hrs time intervals (after death) at $p\leq0.05$.
([d]) Significantly different from group of 96 hrs time intervals (after death) at $p\leq0.05$.

Table (2): The levels of analyzed oxidants and antioxidants in rat femoral muscle tissues after cervical dislocation (20 rats).

Time/Hours	Femoral Muscle tissue		
	Catalase	LPO	GSH
0	0.483 ± 0.007^{bcd}	10.823 ± 0.06^{bcd}	2.759 ± 0.04^{bcd}
24	0.304 ± 0.007^{acd}	11.871 ± 0.05^{acd}	2.369 ± 0.06^{acd}
48	0.231 ± 0.01^{abd}	12.805 ± 0.11^{abd}	1.757 ± 0.05^{abd}
96	0.137 ± 0.006^{abc}	13.751 ± 0.07^{abc}	1.117 ± 0.03^{abc}

([a]) Significantly different from control group (zero time) at $p\leq0.05$.
([b]) Significantly different from group of 24 h time intervals (after death) at $p\leq0.05$.
([c]) Significantly different from group of 48 h time intervals (after death) at $p\leq0.05$.
([d]) Significantly different from group of 96 h time intervals (after death) at $p\leq0.05$.

Table (3): The levels of analyzed oxidants and antioxidants in rat brain tissues after drowning (20 rats).

Time/Hours	Brain tissue		
	Catalase	LPO	GSH
0	0.285 ± 0.004^{bcd}	8.635 ± 0.24^{bcd}	3.812 ± 0.046^{bcd}
24	0.232 ± 0.01^{acd}	10.735 ± 0.13^{acd}	3.225 ± 0.07^{acd}
48	0.169 ± 0.002^{abd}	11.686 ± 0.12^{abd}	2.663 ± 0.048^{abd}
96	0.11 ± 0.002^{abc}	12.712 ± 0.12^{abc}	2.101 ± 0.046^{abc}

(^{a}) Significantly different from control group (zero time) at $p\leq0.05$.
(^{b}) Significantly different from group of 24 h time intervals (after death) at $p\leq0.05$.
(^{c}) Significantly different from group of 48 h time intervals (after death) at $p\leq0.05$.
(^{d}) Significantly different from group of 96 h time intervals (after death) at $p\leq0.05$.

Table (4): The levels of analyzed oxidants and antioxidants in rat femoral muscle tissues after drowning (20 rats).

Time/Hours	femoral Muscle tissue		
	Catalase	LPO	GSH
0	0.618 ± 0.006^{bcd}	8.313 ± 0.12^{bcd}	13.557 ± 0.04^{bcd}
24	0.384 ± 0.005^{acd}	11.214 ± 0.06^{acd}	7.1697 ± 0.13^{acd}
48	0.325 ± 0.004^{abd}	11.848 ± 0.04^{abd}	5.375 ± 0.06^{abd}
96	0.287 ± 0.01^{abc}	12.504 ± 0.13^{abc}	2.761 ± 0.05^{abc}

(^{a}) Significantly different from control group (zero time) at $p\leq0.05$.
(^{b}) Significantly different from group of 24 h time intervals (after death) at $p\leq0.05$.
(^{c}) Significantly different from group of 48 h time intervals (after death) at $p\leq0.05$.
(^{d}) Significantly different from group of 96 h time intervals (after death) at $p\leq0.05$.

Table (5): Percentage of DNA fragmentation in brain and muscle tissues after cervical dislocation (20 rats).

Time/hours	DNA % (Brain tissue)	DNA% (Muscle tissue)
0	18.8±0.75	41.39±0.42
24	32.3±0.69*	51.56±0.73*
48	36.23±0.73*	54.5±0.67*
96	37.4±0.7*	60.4±0.69*

(*) Significantly different from control group (zero time) at $p\leq0.05$.

Table (6): Percentage of DNA fragmentation in brain and muscle tissues after drowning (20 rats).

Time/hours	DNA % (Brain tissue)	DNA% (Muscle tissue)
0	31.39±0.75	36.36±0.76
24	35.36±0.69*	47.5±0.69*
48	47.6±0.81*	53.6±0.68*
96	56.52±0.92*	65.3±0.78*

(*) Significantly different from control group (zero time) at $p\leq0.05$.

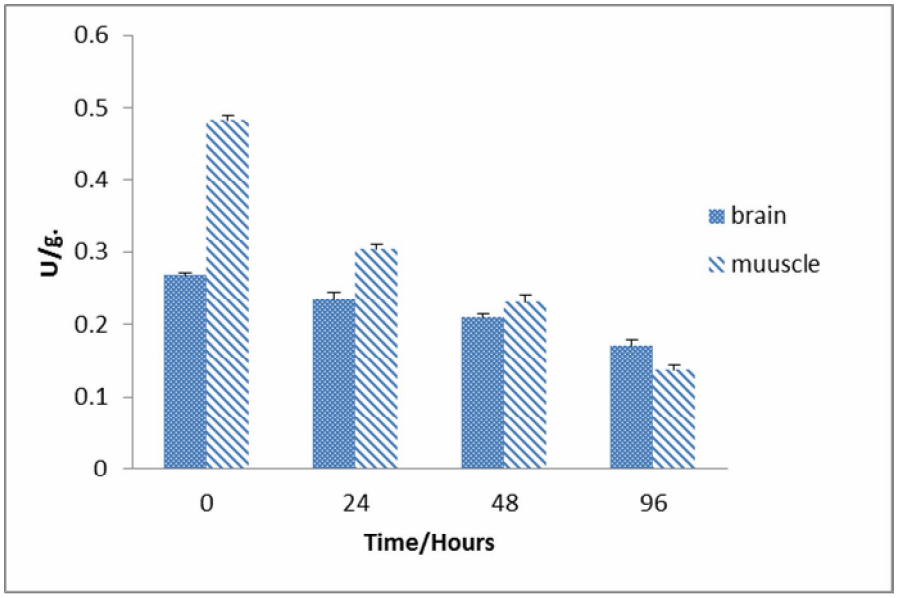


Fig. (1): The catalase enzyme activity (U/g tissue) in brain and muscle tissues of rats after cervical dislocation.

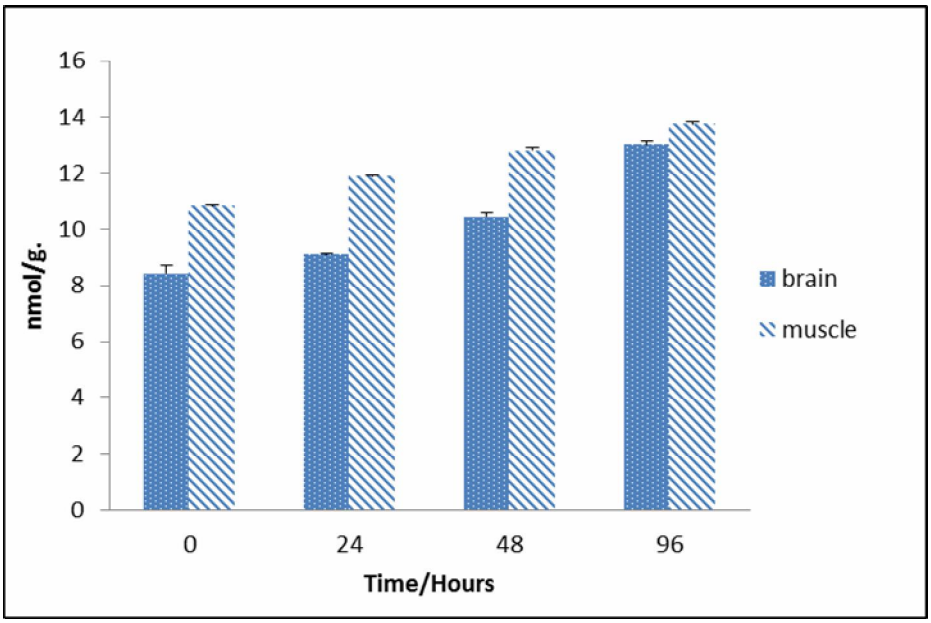


Fig. (2): The mean concentrations (Um/g tissue) of lipid peroxide in brain and muscle of rats after cervical dislocation.

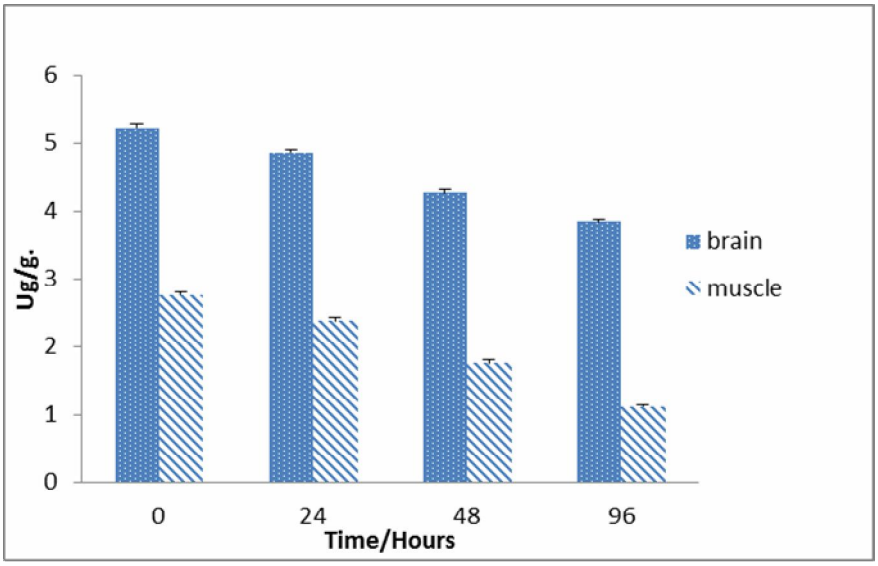


Fig. (3): The mean concentrations of reduced glutathion (ug/g) in brain and muscle tissues of rats after cervical dislocation**.**

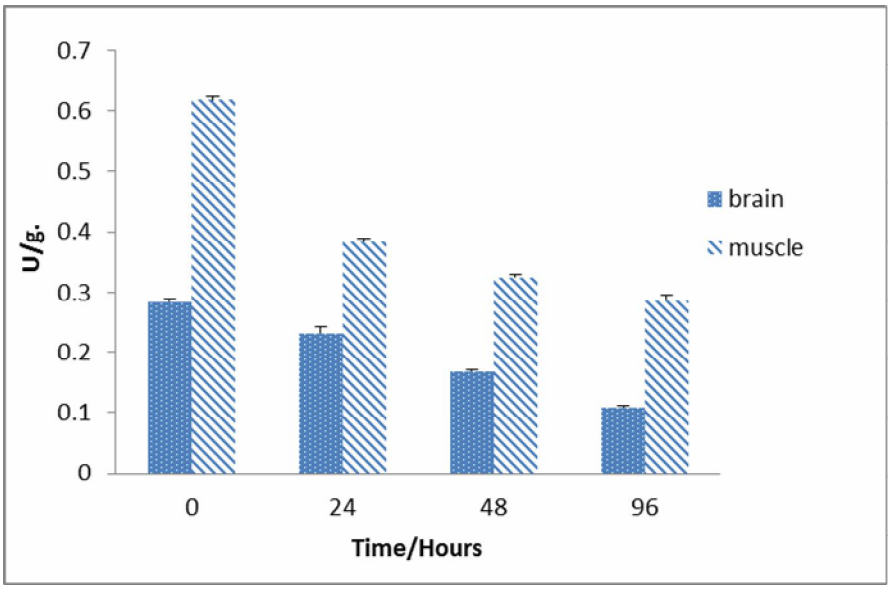


Fig. (4): The catalase enzyme activity (U/g tissue) in brain and muscle tissues of rats after drowning.

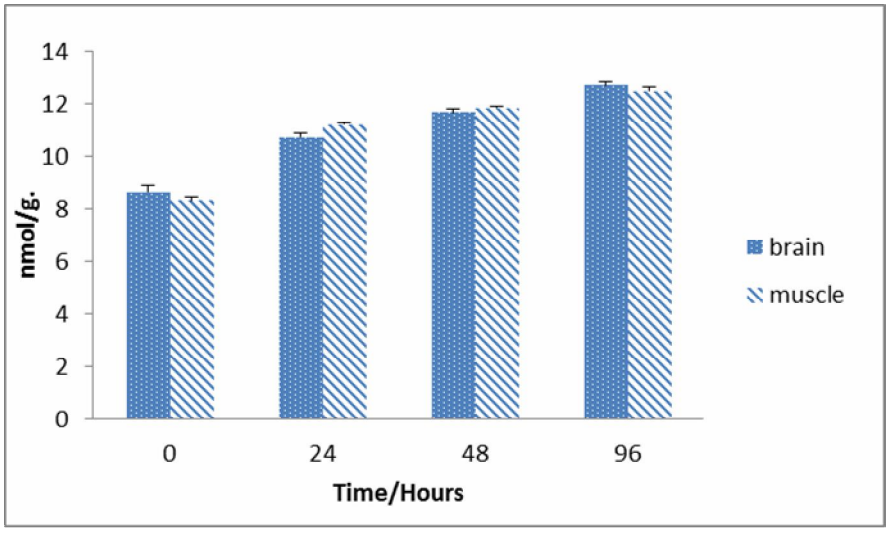


Fig. (5): The mean concentrations (Um/g tissue) of lipid peroxide in brain and muscle of rats after drowning.

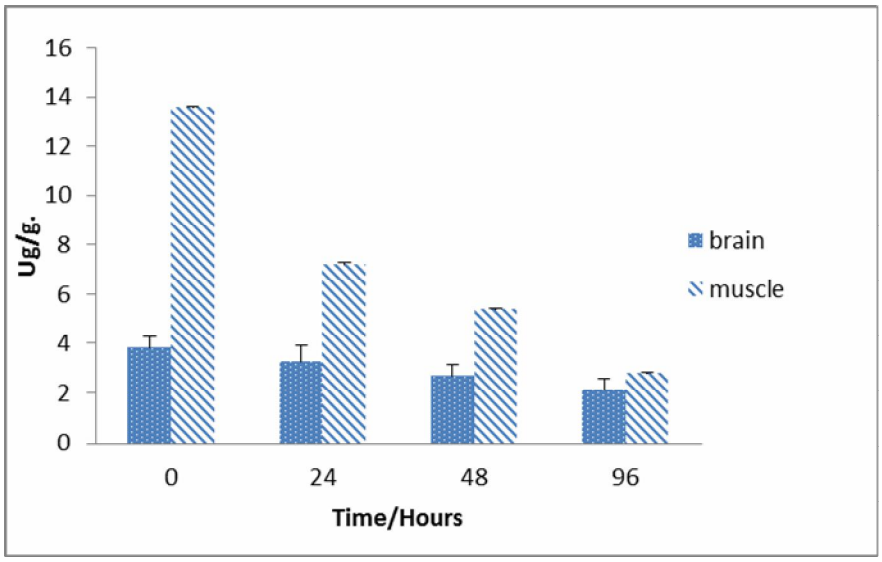


Fig. (6): The mean concentrations of reduced glutathion (ug/g) in brain and muscle tissues of rats after drowning.

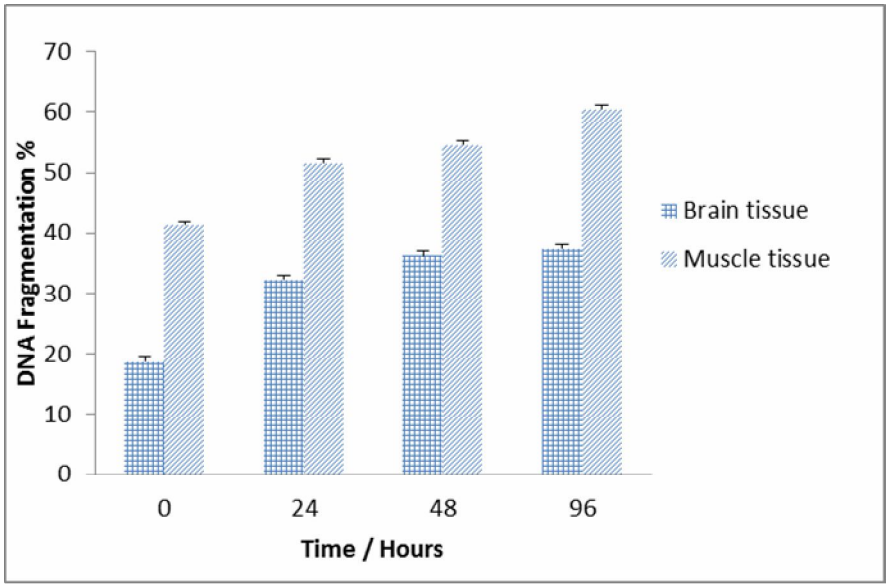


Fig. (7): Percentage of DNA fragmentation in brain and muscle tissues in cervical dislocation

M 50 bp DNA marker
1 brain 0 time
2 brain 24 hrs
3 brain 48 hrs
4 brain 96 hrs
5 muscle 0 time
6 muscle 24 hrs
7 muscle 48 hrs
8 muscle 96 hrs

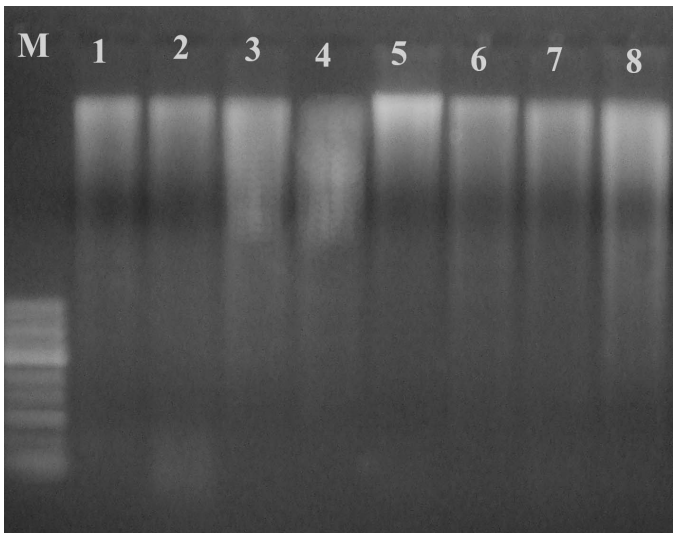


Fig. (8): Percentage of DNA fragmentation in brain and muscle tissues in cervical dislocation.

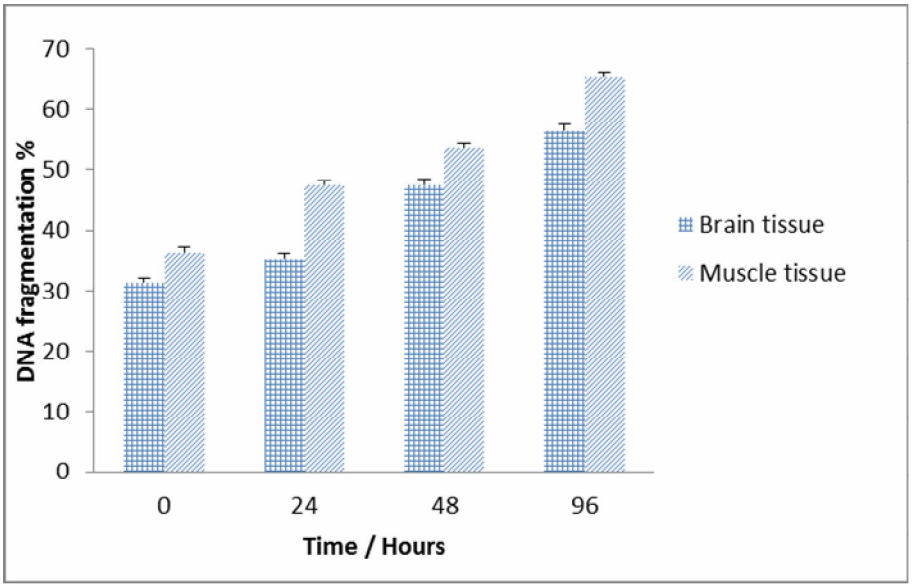
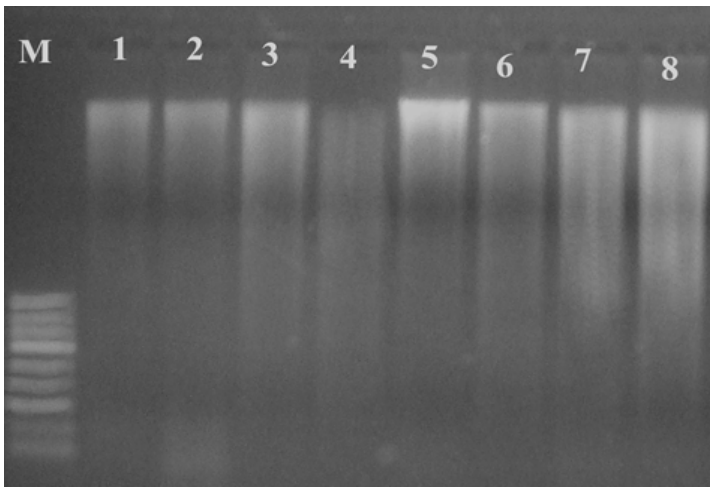


Fig. (9): Percentage of DNA fragmentation in brain and muscle tissues in drowning.



M 50 bp DNA marker
1 brain 0 time
2 brain 24 hrs
3 brain 48 hrs
4 brain 96 hrs
5 muscle 0 time
6 muscle 24 hrs
7 muscle 48 hrs
8 muscle 96 hrs

Fig. (10): Percentage of DNA fragmentation in brain and muscle tissues in drowning.

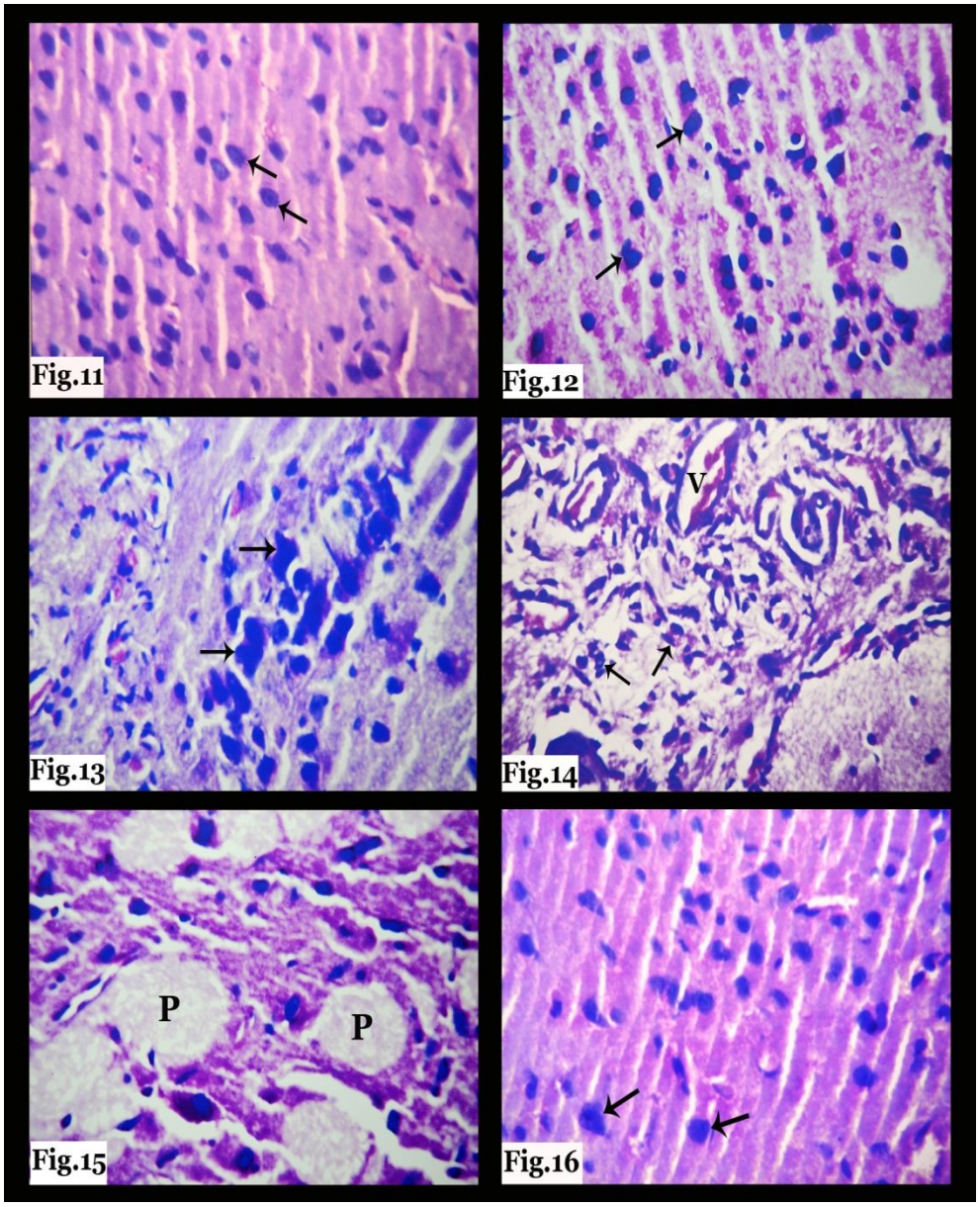


Fig. (11): Brain of cervical dislocated rats at 0 time showed blue color of neuron (arrows). H&E stain, X 80. **Fig. (12):** Brain of cervical dislocated rats at 24 hrs post-mortem showed deep blue color staining with shrinkage size of neuron in the cerebrum (arrows)) (H & E stain X 80). **Fig. (13 and 14)**: Brain of cervical dislocated rats at 48 hrs post-mortem showed clumping of the neuronal cells (arrows) in cerebrum with lyses in the wall of dilated blood vessels (V). H&E stain, X 80. **Fig. (15):** Brain of cervical dislocated rats at 96 hrs post-mortem showed homogenous eosinophilic circumscribed round plagues (P) were replaced the cerebral matrix) (H & E stain X 80). **Fig. (16):** Brain of drowned rats at 0 time showed irregular distribution of the neurons with ill-defined nucleus (arrows) in the cerebrum) (H & E stain X 80).

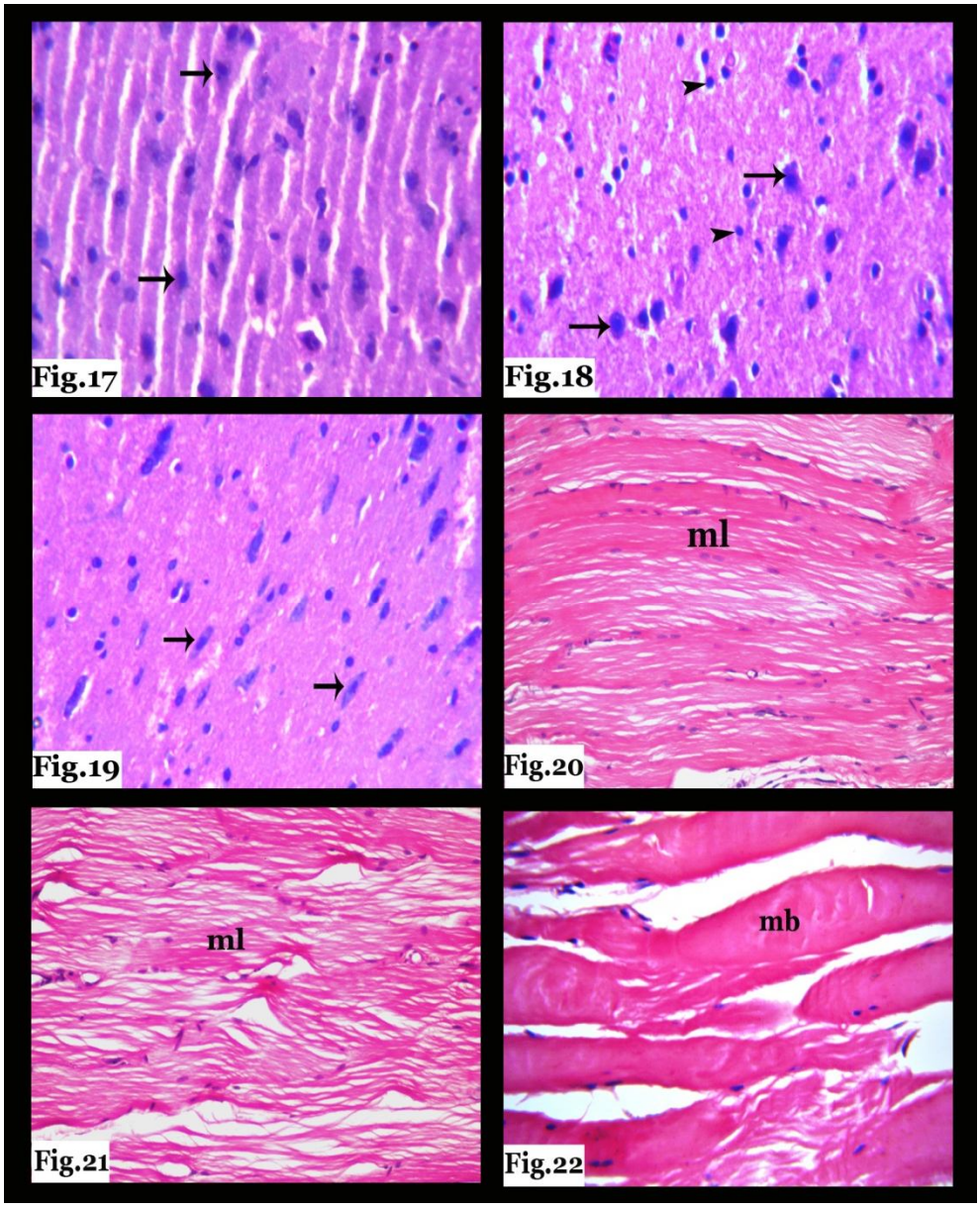


Fig. (17): Brain of drowned rats at 24 hrs post-mortem showed neuronal shrinkage with deep blue colour staining (arrows) (H & E stain X 80). **Fig. (18):** Brain of drowned rats at 48 hrs post-mortem showed glia cell (arrowheads) were the predominant one while the neuronal cells showed deep blue color (arrows) (H & E stain X 80). **Fig. (19):** Brain of drowned rats at 96 hrs post-mortem showed flattening in the neuronal shape (arrows) with wide distance in between (H & E stain X 80). **Fig. (20):** There was no histopathological alteration and the normal histological structure of the striated bundles (ml) (H & E stain). **Fig (21)**: Necrosis, hyalinization and lose of striation were detected in focal manner. **Fig (22):** Skeletal muscle bundles (mb) of rat showing massive number of inflammatory cell infilteration with congested blood vessels in between hyalinned muscle bundles (mb) (H & E stain)**.**

Discussion

Determination of the time of death has always been one of the primary goals of the forensic medicine. Traditional methods for estimating PMI were based on post mortem changes of cadaveric phenomenology or biochemical arrays of cadavers. Unfortunately, these methods were strongly influenced by many unpredictable internal and external factors. Biochemical markers that help to evaluate the time since death has been investigated. They include protein fractions, urea, creatinine, glucose, iron, potassium, calcium, enzyme and the immunohistochemical detection of insulin in pancreatic B cell (Wehner et al., 1999; Thaik et al., 2002).

Evidently, determination of the PMI requires a parameter change constantly and linearity from the time of death (Liu et al., 2007). Indeed with the advances of molecular biology the analysis of time dependant on the degradation of nucleic acids (both DNA and RNA), which became a focus of attention in forensic science (Bauer et al., 2003). In the present study, brain and femoral muscle tissue of rats that killed by cervical dislocation and drowning were examined in an attempt to disclose whether or not a significant correlation was present among the levels of oxidant /antioxidant parameters, the extent of DNA damage and hitopathological alterations post mortem interval (PMI).

The results of our experiment documented that both the level and the amount of oxidant /antioxidant parameters as well as the percentage of DNA fragmentation in brain and femoral muscle tissues extracted after different periods of decapitation (0-96 h post mortem) were significantly different. The oxidant represented by LPO concentration displayed a significant increase from 0 time till 96hrs post mortem, in contrast to the antioxidants which showed a significant decrease. Under physiological conditions, the oxidant / antioxidant defense system in human body is in a continuous equilibrium state which is characterized by formation of free radicals and the removal by means of antioxidant system (Clarkson and Thompson, 2000). The oxidant / antioxidant equilibrium in injured tissue models is different. Increase in oxidant levels and a decrease in antioxidant levels are observed in the damage tissue of any live animals (Aguilar et al., 2007, Iraz et al., 2006). This event is a reaction induced against damage in a certain area of body (Cheeseman and Slater, 1993).

Differences between biochemical parameters were observed, no matter how much viability the cells possessed in the early hours of death. Differences in post mortem period of oxidant and antioxidant parameters must be assessed as biochemical disturbances , as it cannot be proposed that the oxidant /antioxidant equilibrium be controlled sufficiently by the body thus no matter how much viability the cells show in the early hours of death. The activity of catalase and concentration of GSH were significantly decreased from the 0 time till 96hrs PMI. This decline was parallel with the time since death, their peak of inhibition were recorded at 96 h. This excessive decrease in CAT and GSH in our experiment propels one to consider its independency from the increase in LPO, since the CAT activity in a damaged tissue decreases under normal conditions to such a level that is half that of after wise healthy subjects (Dengiz et al., 2007; Kisaoglu et al., 2011; Kurt et al., 2011; Polal et al., 2011). Reduced glutathione (GSH) has proved an important antioxidant compound, protecting the cells from oxidative damage (Yilmoz et al., 2012). Reduced glutathione (GSH) deficiency has been known to induce oxidative stress,

hence emerging as the underlying cause of numerous pathological events (Ross, 1998).

It can simply be inferred from the literature that the average increase in LPO in an experimental damaged tissue has not been that vastly different from that observed during further hours after death. LPO occurs through oxidation of the fatty acids containing three or more double bounds. LPO directly affects ion transfer through cell membrane, causing the membrane compounds to be cross linked (Niki et al., 2005).

It was reported that the amount of LPO increased in parallel to the increase in the extent of damage (Celik et al., 2004).

Ozturk et al. (2013) also revealed that oxidant /antioxidant balance in the striated muscle tissue of rat was documented to remain constant at 0 hour post mortem to shift mildly in favour of the oxidants at 2 hours post mortem to shift moderately in favour of the oxidants at 3 hours post mortem and to shift severely in favour of the oxidants, against the antioxidants at 4 and 5 hours post mortem. Also, Khalaf et al. (2010) mentioned that lipid perioxidation, expressed as MDA level, recorded significant elevations in the muscles of all post mortem groups which subjected to a different manner of asphyxia (anaesthesia over dose, drowning and electrocution) which were more pronounced at 120 min PMI which the antioxidant capacity represented by SOD, CAT, GSH and total antioxidants were greatly decreased at 120 min PMI.

Upon the death of an organism, internal nucleus contained within the cells should cause degradation of chromosomal DNA into increasing smaller fragment overtime (Gomaa et al., 2013). This post mortem degradation of nucleic acids has been suggested as an elegant alternative to classical methods for PMI estimation (Hao et al., 2007). So, this study aimed to profile postmortem degradation of DNA in relation to PMI. DNA was extracted from the brain and femoral muscles of rats at different PMI (0, 24, 48 and 96hrs postmortem). The results of the current study revealed a significant increase in percentage of DNA fragmentation in both brain and femoral muscle tissues from the zero time till 96 hrs post mortem. The increase in DNA fragmentation percentage was in parallel with increasing post mortem interval and the process of degradation was gradual, progressive and regular. These findings was in correlation with those of Gomaa et al. (2013) who reported that within 24hrs post mortem the brain and liver cells of rats showed increased DNA degradation parameters. Also Luo et al. (2006) revealed gradual decrease of bone marrow DNA with prolongation of PMI and Zhen et al. (2006) recorded that an evident comet tailing was observed in DNA of myocardium cells after electrophoresis and their changes related with the extension of PMI, which indicates that DNA degradation rate has a close correlation with post mortem interval in the period from 0 to 72 hours in rats.

Post mortem DNA degradation subjects to degradation by endogenous nucleases released by host cells or exogenous nucleases released by microorganisms and environmental invertebrates. Moreover, spontaneous degradation hydrolysis and oxidation will farther modify DNA structure at much slower speed (lindahl, 1993; Hofreiter et al., 2001). This DNA fragmentation can be quantified and appears to be a time dependant process which has the potential as a predictor of PMI in the field of forensic pathology.

Ferreir and Cunha (2013) reported that PMI estimation is complicated by numerous factors, some endogenous such as cause of death, body build, drug use , some exogenous causes which include temperature, pH and oxygen concentration as well as other soil characteristics. Environmental conditions have more influence on DNA degradation compared to time elapsed since deposition of the tissue.

Neutral or slightly alkaline pH in the sample or in the soil favors DNA preservation (Lindahal, 1993). Due to the rapid decomposition rate tissue such as blood and kidney were found to be unsuitable for DNA finger printing after a period of 1 week (Giannakis et al., 1991). Brain, lymph nodes and skeletal muscles preserved high molecular DNA up to 3 weeks, whereas thyroid and spleen retained it for 1 week and liver lost all high molecular DNA after 2 days. Brain tissue seems to be one after best sources of DNA for post mortem studies followed by muscle and blood and then other internal organs whereas liver is consistently a poor source of DNA (Gavrieli et al., 1992; Alaeddini et al., 2010).

The results of the current study revealed that brain DNA showed slower degradation than skeletal muscles DNA, such variability appears to be related to the antemortem ribonuclease activity of the tissue with relatively ribonucleases poor tissue such as brain and retina exhibiting greater nucleic acid stability (Johnsen et al., 1986; Malik et al., 2003) when compared to ribonuclease rich tissue as liver, stomach and pancreas (Finger et al., 1987). Mild histopathological findings become apparent at the 24 h after death, when the amount of oxidants significantly increased accompanied by a significant decrease in the antioxidant amounts and increased percentage of DNA fragmentation.

The histopathological findings in brain of cervical or drowning rats indicate the brain tissue deterioration in conjunction with an increase in oxidant level and DNA fragmentation and a decrease in antioxidant levels. Such more prominent and severe histopathological alterations as neuronal shrinkage, with deep blue color were the predominant findings. There was flattening in the neuronal shape with wide distance in between in cervical dislocation. The brain histological alterations at 48 h and 96 h PM were in the form of clumping of the neuronal cells in the cerebrum with lyses in the wall of the dilated blood vessels and homogenous eosinopholic circumscribed round plaques replacing cerebral matrix. It is now established that generation reactive oxygen species (ROS) through lipid perioxidation (LPO) can cause cell death either by apoptosis or necrosis (Higuchi and Yoshimoto, 2002).

No available literature was found about the histopathological alterations in brain and evaluation time of death, but all studies were localized to other organs as kidney, muscles, liver, heart and pancreas. The present results revealed histopathological alterations in muscle tissue of rats subjected to cervical dislocation and (or) drowning. The lesions were more prominent in drowned rats than in cervical dislocations. They were in the form of inflammatory cell infiltration with congested blood vessel in between the hyalinized muscle bundles at 24 and 48 hours PMI. While in case of cervical dislocation, the lesion was in moderate picture, and at 96 h, the histopathological lesions were in the form of necrosis, hyalinization and loss of striation of the muscle bundles.

The present findings were closely correlated to study adopted by Ozturk et al. (2013) who demonstrated prominent histopathological findings as striated muscles sarcoplasmic fragmentations and myofibrillary necrosis were visualized at 4 and 5 hours post-mortem when the amount of the DNA damage displayed a significant increase. Scarpelli (1990) mentioned that the onset of autolysis was rapid in tissue with a high content of hydrolytic, such as the pancreas and gastric mucosa, where it was intermediate in the heart, liver and kidney and slow in brain and fibroblasts which have relatively few lysosomal and a low level of hydrolytic enzymes.

In the current study, there was obvious correlation between the degree of degeneration or autolysis changes and time of PMI and also

with oxidant /antioxidant levels and DNA fragmentation. The result of current study revealed that antioxidant- oxidant and DNA alterations slower in degradation in brain than in muscles. Also the previous studied parameters are more affected in case of drowning than in cervical dislocation. Hence the depletion of the endogenous antioxidant and the inhibition of antioxidant enzymes and DNA damage were mostly more remarkable in drowning death than in case of cervical dislocation. In conclusion the results of our study showed that there was a good correlation between increase in oxidant, DNA fragmentation and decreased antioxidant levels in brain and muscle tissue and PMI within 96 hours after death. Furthermore, the biochemical findings were found to be consistent with the histopathological findings.

Collectively, data generated from the analysis indicated that biochemical genetic profile and histopathological findings generated by this approach can provide useful information for estimating the post mortem interval during the first 96 h after death.

References

Aebi, H. (1984): "Catalase in vitro". Methods Enzymol., 105:121-126.

Aguilar, A.; Alvarez-Vijande, R.; Capdevila, S.; et al. (2007): "Antioxidant patterns (superoxide dismutase, glutathione reductase, and glutathione peroxidase) in kidneys from non-heart-beating-donors: experimental study". Transplant Proc., 39: 249–252.

Alaeddini, R.; Simon, J.; Abbas, A. (2010): "A Forensic implications of genetic analyses from degraded DNA-A review". Forensic Sci. Inter. Genetics, 4: 148–157.

Bonchroft, J.D.; Steven, A.; Turner, D.R. (1996): Theory and practice of histological techniques. 4th Ed. Churchil Livingstone, New York, London, San Francisco, Tokyo.

Bauer, M.; Gramlich, I.; Polzin, S.; et al. (2003): "Quantification of mRNA degradation as possible indicator of postmortem interval–a pilot study". Legal Medicine (Tokyo), 5: 220–227.

Beutler, E.; Duron, O.; Kelly, B.M. (1963): "Improved method for the determination of blood glutathione". J. Lab. Clin. Med., 61:882-888.

Celik, O.; Turkoz, Y.; Hascalik, S.; et al. (2004): "The protective effect of caffeic acid phenethyl ester on ischemia-reperfusion injury in rat ovary". Eur. J. Obstet. Gynecol. Reprod. Biol., 117(2):183-188.

Chandrakanth, H.V.; Kanchan, T.; Balaraj, B.M.; et al. (2013): "Postmortem vitreous chemistry – An evaluation of sodium, potassium and chloride levels in estimation of time since death (during the first 36 h after death)". J. Forensic Legal Med., 20(4): 211-216.

Cheeseman, K.H.; Slater, T.F. (1993): "An introduction to free radical biochemistry". Br. Med. Bull., 49: 481–493.

Clarkson, P.M.; Thompson, H.S. (2000): "Antioxidants: what role do they play in physical activity and health". Am. J. Clin. Nutr., 72:637–646.

Dengiz, G.O.; Odabasoglu, F.; Halici, Z.; et al. (2007): "Gastroprotective and antioxidant effects of montelukast on indomethacin-induced gastric ulcer in rats". J. Pharmacol. Sci., 105(1):94-102.

Faraci, F.M. (2006): "Reactive oxygen species: Influence on cerebral vascular tone". J. Appl. Physiol., 100:739–743.

Ferreira, M.T.; Cunha, E. (2013): "Can we infer post mortem interval on the basis of decomposition rate? A case from a Portuguese cemetery". Forensic Sci. Inter., 226(1–3)10:298.

Finger, J.M.; Mercer, J.F.; Cotton, R.G.; et al. (1987): "Stability of protein and mRNA in human postmortem liver analysis by two dimensional gel electrophoresis". Clin. Chim. Acta, 170:209-218.

Gavrieli, Y.; Sherman, Y.; Ben-Sasson, S.A. (1992): "Identification of programmed cell deathin situ via specific labeling of nuclear DNA fragmentation". J. Cell. Biol., 119(3):493–501.

Giannakis, C.; Forbes, I.J.; Zalewski, P.D. (1991): "Ca^{+2}/Mg^{+2} - dependent nuclease: tissue distribution, relationship to inter-nucleosomal DNA fragmentation and inhibition by Zn^{+2}". Biochem. Biophys. Res. Commun., 181(2):915–920.

Mie, G.; Mohamad, A.; Mohamad, M. (2013): "The relationship between the postmortem interval and the DNA degradation in brain and liver of adult albino rats". J. American Sci., 9(5):535.

Halliwell, B. (2001): "Role of free radicals in the neurodegenerative diseases: Therapeutic implications for antioxidant treatment". Drugs Aging, 18:685–716.

Hao, L.G.; Deng, S.X.; Zhao, X.C. (2007): "Recent advancement in relationship between DNA degradation and postmortem interval". Fa. Yi. Xue. Za. Zhi, 23(2):145–147.

Henssge, C.; Madea, B. (2004): "Estimation of the time since death in the early post-mortem period". Forensic Sci. Int., 144:167–175.

Higuchi, Y.; Yoshimoto, T. (2002): "Arachidonic acid converts the glutathione depletion-induced apoptosis to necrosis by promoting lipid peroxidation and reducing caspase-3 activity in rat glioma cells". Arch. Biochem. Biophy., 400(1):133-140.

Hofreiter, M.; Jaenicke, V.; Serre, D.; et al. (2001): "DNA sequences from multiple amplifications reveal artifacts induced by cytosine deamination in ancient DNA". Nucleic Acids Res., 29(23):4793–4799.

Iraz, M.; Ozerol, E.; Gulec, M. (2006): "Protective effect of caffeic acid phenethyl ester (CAPE) administration on cisplatin-induced oxidative damage to liver in rat". Cell Biochem. Funct., 24:357–361.

Janssen, W. (1984): "Forensic histopathology". Berlin, 15–47.

Johnson, L.A.; Ferris, J.A. (2002): "Analysis of postmortem DNA degradation by single-cell gel electrophoresis". Forensic Sci. Inter., 126:43–47.

Johnson, S.A.; Morgan, D.G.; Finch, C.E. (1986): "Extensive postmortem stability of RNA from rat and human brain". J. Neurosci. Res., 16:267-280.

Kaliszan, M.K. (2012): "First practical applications of eye temperature measurements for estimation of the time of death in casework. Report of three cases'. Forensic Sci. Inter., 219(1-3):13-15.

Khalaf, A.A.; Mekawy, M.M.; Elsayd, M. (2010): "Post mortem changes of some biochemical parameters in rat skeletal muscles related to different types of death". Egypt. J. Forensic Sci., 10(1):1-18.

Kisaoglu, A.; Ozogul, B.; Cetyn, N.; et al., (2011): "The role of alpha-2 adrenergic receptors in the anti-ulcerative activity of famotidine and omeprazole in rats and its relationship with oxidant-antioxidant parameters". Int. J. Pharmacol., 7(6):682-689.

Knight, B.; Knight, B.F.P; Saukko, P.J. (2004): Knight's Forensic Pathology. Arnold, London. 3rd Ed., P.P.76–97.

Kuo, M.; Lou, S.; Postlethwait, J.; Chung, B. (2005): "ChungoBc. chromosomal organization, evolutionary relationship and expression of zebra fish Gn RH

family members". J. Biomed. Sci., 12(4):629-639.

Kurt, A.; Isaoglu, U.; Yilmaz, M.; et al. (2011): "Biochemical and histological investigation of famotidine effect on post ischemic reperfusion injury in the rat ovary". J. Pediatr. Surg., 46(9):1817-1823.

Larkin, B.; Iaschi, S.; Dadour, I. (2010): "Using accumulated degree-days to estimate postmortem interval from the DNA yield of porcine skeletalmuscle". Forensic Sci Med. Pathol., 6:83-92.

Lendoiro, E.; Cordeiro, C.; Rodríguez-Calvo, M.S.; et al. (2012): "Applications of tandem mass spectrometry (LC–MSMS) in estimating the post mortem interval using the biochemistry of the vitreous humour". Forensic Sci. Inter., 223:(1–3)30:160-164.

Lin, X.; Yin, Y.S.; Ji, Q. (2011): "Progress on DNA quantification in estimation of postmortem interval". Fayixue Zazhi., 27:47-49.

Lindahl, T. (1993): "Instability and decay of the primary structure of DNA". Nature, 362(6422):709–715.

Liu, L.; Shu, X.; Ren, L.; et al. (2007): "Determination of the early time of death by computerized image analysis of DNA degradation: which is the best quantitative indicator of DNA degradation"? J. Huazhong Univ. Sci. Technolog. Med. Sci., 27:362–366.

Luo, G.H.; Chen, Y.C.; Cheng, J.D.; et al. (2006): "Relationship between DNA degeneration and postmortem interval of corrupt corpse". Fa. Yi. Xue. Za. Zhi., 22(1):7 – 9.

Malik, K.J.; Chen, C.D.; Olsen, T.W. (2003): "Stability of RNA from the retina and retinal pigment epithelium in a porcine model simulating human eye bank conditions. Invest". Ophthalmol. Vis. Sci., 44:2730-2735.

Mantha, A.K.; Moorthy, K.; Cowsik, S.M. (2006): "Neuroprotective role of neurokinin B (NKB) on betaamyloid (25–35) induced toxicity in aging rat brain synaptosomes: Involvement in oxidative stress and excitotoxicity". Biogerontology, 7:1–17.

Marjanovic, D.; Durmic-Pasic, A.; Bakal, N.; et al. (2007): "DNA identification of skeletal remains from World War II mass graves uncovered in Slovenia, Croat". Med. J., 48:513–519.

Niki, E.; Yoshida, Y.; Saito, Y.; et al. (2005): "Lipid peroxidation: mechanisms, inhibition, and biological effects". Biochem. Biophys. Res. Commun., 338(1):668-676.

Ohkawa, H.; Ohishi, N.; Yagi, K. (1997): "Assay for lipid perioxidation in animal tissues by thiobarbituric acid reaction". Anal. Biochem., 95:351-358.

Ozturk, C.; Sener, M.; Sener, E.; et al. (2013): "The investigation of damage in the muscle tissue with the oxidant/antioxidant balance and the extent of postmortem DNA damage in rats". Life Sci. J., 10(3):13-15.

Parsons, T.J.; Huel, R.; Davoren, J.; et al. (2007): "Application of novel mini-ampliconstr multi-plexes to high volume casework on degraded skeletal remains". Forensic Sci. Int. Genet., 1(2):175–179.

Parsons, T.J.; Weedn, V.W. (1997): Chemical and ultra-structural aspects of decomposition. In: Forensic Taphonomy: The Post-mortem Fate of Human Remains, W.D. Haglund, M.H. Sorg (Eds.). Stern, CRC Press, Inc. P.P.93–108.

Polal, B.; Albayrak, Y.; Suleyman, B.; et al. (2011): "Antiulcerative effect of dexmedetomidine on indomethacin-induced gastric ulcer in rats". Pharmacol. Rep., 63(2):518-526.

Poloz, Y.O.; O'Day, D.H. (2009): "Determining time of death: temperature-dependent postmortem changes in calcineurin A, MARCKS, CaMKII, and protein phosphatase 2A in mouse". Int. J. Legal Med., 123:305–314.

Prieto-Castello, M.J.; Hernandez, D.; Rincon, J.P.; et al. (2007): "Perez-Sirvent C: Application of biochemical and X-ray diffraction analyses to establish the postmortem interval". Forensic Sci. Int., 172: 112–118.

Ross, D. (1988): "Glutathione, free radicals and chemotherapeutic agents. Mechanisms of free-radical induced toxicity and glutathione-dependent protection". Pharmacol. Ther., 37(2):231-249.

Scarpelli, D.G.; Iannaccone, P.M. (1990): Cell death, autolysis and necrosis. In: Anderson's pathology. Kissane JM, editor, 9th Ed. St Louis. MO: Mosby, P.13.

Sellens , A.; Cohen, S. (1987): "A flow – cytometric method to study DNA fragmentation in lymphocyte". J. Immunol. Method., 152:171-176.

Serafini, M.; Delrio, D. (2004): "Understanding the association between dietary antioxidants, redox status and disease: is the total antioxidant capacity the right tool"? Redox Rep., 9(3):145-152.

SPSS software, Inc. Chicago, IL. (2001).

Swango, K.L.; Timken, M.D.; Chong, M.D.; et al. (2006): "A quantitative PCR assay for the assessment of DNA degradation in forensic samples". Forensic Sci. Inter., 158:14–26.

Tate, C.M.; Nuñez, A.N.; Goldstein, C.A.; et al. (2012): "Evaluation of circular DNA substrates for whole genome amplification prior to forensic analysis". Forensic Sci. Inter. Genetics. (6)2:185-190.

Thaik-Oo, M.; Tanaka, E.; Tsuchiya, T. (2002): "Estimation of postmortem interval from hypoxic inducible levels of vascular endothelial growth factor". J. Forensic Sci., 47:186-189.

Wehner, F.; Wehner, H.D.; Schieffer, M.C.; et al. (1999): "Delimitation of the time of death by immunohistochemical detection of insulin in pancreatic beta-cells". Forensic Sci. Inter., 105:161-169.

Yilmaz, M.; Ozgeris, F.B.; Isaoglu, U.; et al. (2012): "Effects of adrenalin on ovarian injury formed by ischemia reperfusion in rats". Latin American J. Pharmacy, 31(7):1032-1037.

Zhen, J.L.; Zhang, X.D.; Niu, Q.S. (2006): "Relationship between the postmortem interval and nuclear DNA changes of heart muscular cells in mice". Fa. Yi. Xue. Za.Zhi., 22:173-176.

تقدير فترات ما بعد الوفاة من خلال بعض التغيرات البيوكيميائيه و تدهور الحمض النووي في أنسجة مخ وعضلات جرذان التجارب

عمرو رضا زكي و عادل تهامي* و نور الهدي ياسين**
قسم الطب الشرعي والسموم الاكلينيكيه كليه الطب البشري–جامعه بني سويف
قسم الطب الشرعي والسموم الاكلينيكيه كليه الطب البيطري – جامعه القاهره *
قسم الطب الشرعي والسموم الاكلينيكيه كليه الطب البيطري– جامعه بني سويف **

التقدير الدقيق للفترة التالية للموت (PMI) هي واحدة من أكثر القضايا أهمية وصعوبة في الطب الشرعي. بعد الوفاه يحدث تحلل ذاتي في الأنسجة و الجزيئات الحيوية. لذلك كان الهدف من الدراسة هو دراسه العلاقة بين مدى تلف الحمض النووي و التوازن بين مكونات الاكسدة و مضادات للأكسدة في المخ والعضلات في أنسجة الفئران التي تعرضت للقتل بالخنق أو الغرق، وعلاقتها بالتغيرات الهستوباثولوجيه في أنسجة المخ. وقد اجريت هذه الدراسه علي ٤٠ جرذ من جرذان التجارب البيضاء، وقسمت الي مجوعتين علي النحو التالي :

المجموعه الاولي: وهي مجموعه تعرضت للوفاه عن طريق الغرق.

المجموعه الثانيه: وهي مجموعه تعرضت للوفاه عن طريق الخنق.

وتم تشريح الفئران في المجموعه الاولي والثانيه والثالثه والرابعه للحصول علي انسجه المخ والعضلات في الاوقات الاتيه: بعد الوفاه مباشره ، بعد مرور ٢٤ساعه من الوفاه ، بعد مرور ٤٨ ساعه من الوفاه وبعد مرور ٩٦ ساعه من الوفاه علي التوالي.

و أظهرت نتائج هذه الدراسة أن هناك علاقة جيدة بين الزيادة في أكسدة، وتفتيت الحمض النووي وانخفاض مستويات مضادات الأكسدة في أنسجة المخ والعضلات خلال ٩٦ ساعة بعد الموت. وعلاوة على ذلك، وجدت ان النتائج البيوكيميائية متسقة مع التغيرات الهستوباثولوجيه. وأشارت البيانات التي تم إنشاؤها من التحليل أن الصورة الجينية و البيوكيميائيه والتغيرات الهستوباثولوجيه الناتجة عن هذا النهج يمكن أن توفر معلومات مفيدة لتقدير فترة ما بعد الوفاة خلال أول ٩٦ ساعة بعد الموت.