Postmortem Interval Estimation Using Myoglobin Concentration in Different Glandular Tissues

Research Article

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Abstract

Postmortem interval (PMI) estimation is very important in a suspicious death investigation. Myoglobin is a hemoprotein presents in striated muscles of living body. After death, myoglobin is exuded from muscles to blood, tissues and organs. So, the aim of this study was to estimate PMI from myoglobin concentrations in glandular tissues. This study was done on rats to detect the Postmortem concentration of myoglobin in pituitary, parotid and adrenal glands at different time intervals. There was a significant correlation between myoglobin concentration in glandular tissues and PMI. By multiple stepwise linear regression analysis, the most predictable model was the combination between pituitary, adrenal and parotid myoglobin concentrations (R^2= 0.992).

The second predictable model was the combination between adrenal and pituitary myoglobin concentrations (R^2=0.99). The last predictable model was adrenal myoglobin concentration (R^2 = 0.980). It was confirmed that myoglobin concentration in glandular tissues is a helpful parameter in estimation of PMI.

Keywords: Forensic Science; Postmortem Interval; Myoglobin; Pituitary Gland; Parotid Gland; Adrenal Gland; Linear Regression.

Introduction

The most important medico-legal issue in any postmortem examination is to determine time passed since death. It's very important to know when the crime was occurred. Postmortem interval (PMI) is the time that has elapsed since a person has died. If the time in question is not known, a number of medical scientific techniques are used to determine it [1].

Estimation of postmortem interval is one of the most important tasks in forensic medicine. PMI estimation allows the police to deal more efficiently with the information available. Also, it enables them to exclude or include a suspect or accused of particular homicide. Accurate determination of PMI is very important in civil law in social and business matters [2].

Time passed since death is considered a major problem for the forensic pathologist and determination of PMI plays a vital issue in many medicolegal cases and the forensic experts are very often required to answer questions that are related to time passed since death in the courts of law [3].

Many methods are used to determine PMI such as calculations based on the measurement of body temperature or evaluating the changes that occur in the body after death [4]. Although calculation based on body temperature can sometimes give a reasonable accurate result in the first 12-24 hour after death, but this accuracy was done under controlled and stable environmental conditions, which rarely exist at the scene of crime [5].

There are many gross changes that occur after death e.g. loss of corneal reflex and changes in the eye, postmortem hypostasis, rig-

or mortis, decomposition and putrefactive changes. These gross changes will be quite unreliable at times. PMI could be estimated from the condition of food in stomach and intestine [6].

Studying the changes of electrolytes in blood, CSF, intraocular and synovial fluids after death play an important issue in determination of PMI [7]. Although the biochemical studies are very important in determination of PMI, but once decomposition occurs, these methods have been found to be of not much use [8]. Other studies found that histological and histochemical study of degenerative changes in various organs and tissues may be a good method for PMI estimation especially when the body is mutilated skeletonised or invaded by animals [9].

Myoglobin is an iron and oxygen binding protein that exists profusely during life in the striated muscles, cardiac muscles and only a little in the blood. Myoglobin is a cytoplasmic hemoprotein consisting of a single chain of 153 amino acids and its molecular mass is approximately 17.500 Dalton [10].

Myoglobin is similar to hemoglobin in that it is involved in the transportation of oxygen to cells. Myoglobin can only hold one oxygen and its affinity for that oxygen is very high compared to hemoglobin. During life, the plasma level of myoglobin is kept low because of its rapid excretion and its clearance by the kidney [11,12].

Wittenberg and Wittenberg [13], found that myoglobin is exuded into blood from the striated muscles and its concentration in blood started to increase extraordinarily after death. Owed to the fewer researches evaluating the P.M changes in myoglobin concentration in glandular tissues, the objective of this study was to estimate PMI from myoglobin concentration in different glandular tissues (pituitary, parotid and adrenal glands).

Materials and Methods

At this study 70 male Wistar albino rats of 12 weeks of age (weighing approximately 350-400gm) were used. All rats were obtained from Minia University laboratory animals growing center-Egypt. The animals were housed in plastic cages, fed a standard laboratory diet and water and maintained at a laboratory temperature of 23°C in an air-conditioned laboratory room.

All aspects of animal care and handling were carried out according to the local guidelines of the Ethical Committee of Faculty of Medicine –Minia University, Egypt. Rats were sacrificed by cervical decapitation after light ether anesthesia. After scarification, pituitary, parotid and adrenal glands were dissected and removed as a whole organ at approximately 30min, and on 1,3,6,12,18,24 hour postmortem (10 rats for each time).

Sample Preparation & Measurement of Myoglobin Concentration in Glandular Tissues

For the quantitative analysis of myoglobin concentration in glandular tissues, 0.1g from each gland was removed and homogenized by ultrasonic homogenizer (Sonics- Vibracell, Sonics & materials inc., USA) in 0.5 ml of phosphate-buffered saline containing 1% triton x-100, 0.1% Sodium dodecylsulfate, 0.5% Sodium deoxicholate, 0.2% Sodium azide, and cocktail of protease inhibitors

(AmerSham, BioSciences-UK). Homogenization was carried out for 1 minute at 4°C. The homogenate was centrifuged at 4°C for 3 minutes at 10.000g [14].

After centrifugation, myoglobin concentrations in the supernatant were determined by Rat-tissue myoglobin ELISA kits at absorbance wave length 45nm by ELISA reader (Stat Fax-2100 microplate reader, Awareness Technology inc., Palm city) [15]. The kits were obtained from Bio-diagnostic Company-Egypt.

Results

In this study, there was a significant increase of myoglobin concentration in pituitary gland than the adrenal gland only at one hour PMI. Also, there was a significant increase in the previous parameter in parotid gland when compared with pituitary and adrenal gland at all PMIs (Table 1). There was a strong +ve significant correlation between myoglobin concentration & PMIs in all glandular tissues (Table 2) (Figure 1, 2, and 3).

By linear regression analysis, PMI can be estimated from this equation:
PMI= constant+ (slopping of unstandardized coefficient x gland myoglobin concentration).

By simple linear regression analysis, PMI for each gland can be estimated. In pituitary gland (R=0.974, R^2= 0.95), while in the parotid gland (R=0.987 & R^2= 0.974). Finally in adrenal gland, R=0.99 & R = 0.98 (Table 3).

By multiple linear regression analysis, PMI could be estimated by the use of combination of myoglobin concentration of the above three glands (R= 0.996 & R^2= 0.992) (Table 4). Finally, by multiple stepwise linear regression analysis three models for estimation of PMI appeared. The most predictable model in estimation of PMI was the combination between adrenal, pituitary and parotid glands myoglobin concentration (R=0.996 & R^2= 0.992). The second predictable model was the combination between adrenal & pituitary myoglobin concentration (R=0.995 & R^2= 0.99). The last model was adrenal myoglobin concentration (R=0.99 & R2= 0.980) (Table 5).

Internal validation test was done by recalculate the multiple stepwise linear regression models on randomly selected 70% of the sample size. It revealed that there were just very minimal differences in the R, R^2, coefficients and SEM. These changes were not exceeding 0.1 and no differences in the significance. So, multiple stepwise linear regression models were fit and internally valid (Table 6).

Discussion

A number of postmortem changes take place in the human body after death. Following irreversible cardiac arrest and death, cessation of cellular metabolic activity occur. Biochemical changes and cellular breakdown occur due to the conversion of the cellular metabolic pathways to autolytic activity and finally, structural changes in tissues & decomposition appear well [16]. There are 2 stages of death, somatic (immediate death) & cellular death. In somatic death, there is loss of movement, cessation of respiration and circulation [17].

Table 1 . Descriptive Statistics &One Way ANOVA Test Statistical Analysis of Myoglobin Concentration in Different Glandular Tissues at Different Pmis

	Group I (pituitary) N=10	Group II (Adrenal) N=10	Group III (Parotid) N=10	P value			Eta2
At 30 min				< 0.001*			0.819
Range	(0.61-0.94)	(0.53-0.84)	(1.26-2.66)	I vs II	I vs III	II vs III	
Mean ± SD	0.73 ± 0.11	0.68 ± 0.10	1.83 ± 0.42	0.89	< 0.001*	< 0.001*	
At 1st h				< 0.001*			0.873
Range	(1.56-2.87)	(0.92-1.43)	(3.19-5.38)	I vs II	I vs III	II vs III	
Mean ± SD	2.21 ± 0.44	1.22 ± 0.19	4.22 ± 0.71	< 0.001*	< 0.001*	< 0.001*	
At 3rd h				< 0.001*			0.827
Range	(3.01-4.73)	(2.59-4.15)	(5.2-7.17)	I vs II	I vs III	II vs III	
Mean ± SD	3.91 ± 0.54	3.43 ± 0.48	6.12 ± 0.64	0.156	< 0.001*	< 0.001*	
At 6th h				< 0.001*			0.780
Range	(4.89-8.09)	(5.01-7.36)	(8.18-10.35)	I vs II	I vs III	II vs III	
Mean ± SD	6.43 ± 0.99	5.93 ± 0.71	9.23 ± 0.69	0.362	< 0.001*	< 0.001*	
At 12th h				< 0.001*			0.592
Range	(7.23-11.79)	(9.12-12.96)	(12.05-15.96)	I vs II	I vs III	II vs III	
Mean ± SD	10.18 ± 1.38	10.06 ± 1.2	13.19 ± 1.21	0.972	< 0.001*	< 0.001*	
At 18th h				< 0.001*			0.634
Range	(10.33-16.07)	(13.34-17.26)	(16.89-19.33)	I vs II	I vs III	II vs III	
Mean ± SD	14.71 ± 1.66	15.17 ± 1.35	18.39 ± 0.73	0.713	< 0.001*	< 0.001*	
At 24th h				< 0.001*			0.880
Range	(17.08-19.53)	(17.16-20.9)	(22.96-28.23)	I vs II	I vs III	II vs III	
Mean ± SD	18.28 ± 1	18.77 ± 1.17	25.77 ± 1.72	0.691	< 0.001*	< 0.001*	

Effect size (Eta2): around 0.2 = small effect, around 0.5 = moderate effect, around 0.8 = large effect
*P value is significant if < 0.05. All measurements in microgram/gram

Table 2 . Correlation between Myoglobin Concentration and Postmortem Interval

Myoglobin level in	Postmortem interval (day)	
	r	p
Pituitary gland	0.984	< 0.001*
Adrenal gland	0. 990	< 0.001*
Parotid gland	0.987	< 0.001*

r: correlation coefficient "weak (r = 0-0.24), fair (r = 0.25-0.49) , moderate (r = 0.5-0.74), strong (r = 0.75-1)
*: significant difference at p value < 0.05

Table 3 . Simple Linear Regression Analysis of Postmortem Interval

Model	B	SEM	R	R²	P value	Regression equation
Pituitary myoglobin	1.34	0.03	0.974	0.95	<0.001*	PMI= -1.6 + (1.34 x Pituitary myoglobin)
Constant	-1.6	0.3			<0.001*	
Adrenal myoglobin	1.27	0.02	0.99	0.98	<0.001*	PMI= -0.82 + (1.27 x Adrenal myoglobin)
Constant	-0.82	0.23			0.001*	
Parotid myoglobin	1.05	0.02	0.987	0.974	<0.001*	PMI= -2.57 + (1.05 x Parotid myoglobin)
Constant	-2.57	0.29			<0.001*	

PMI= Post-Mortem Interval, B= unstandardized coefficient, R=correlation coefficient, R² =effect size,
SEM= standard error of estimate *: significant difference at p value < 0.05

Shereen A Abdelaleem, Rehab H.A. Younis, Rasha F. Ahmed (2016) Postmortem Interval Estimation Using Myoglobin Concentration in Different Glandular Tissues. *Int J Forensic Sci Pathol.* 4(8), 259-265.

Figure 1. Correlation between Pituitary Gland Myoglobin Concentration & Different PMIs
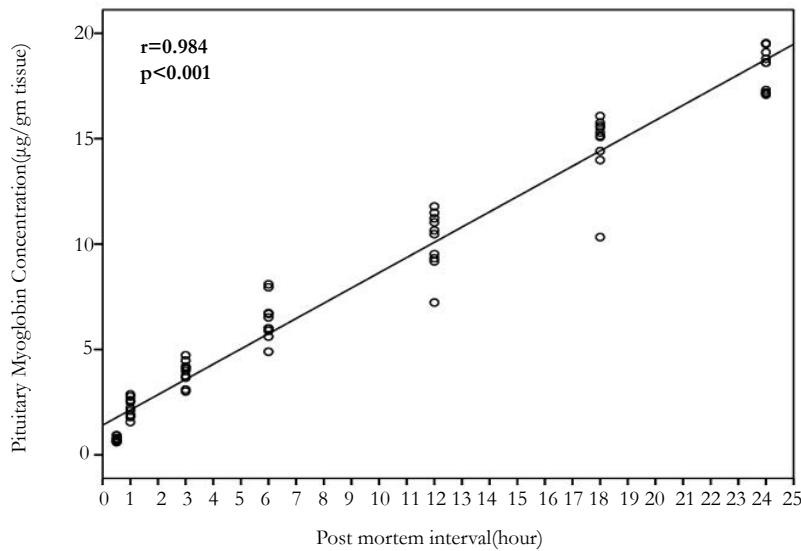

Figure 2. Correlation between Adrenal Gland Myoglobin Concentration & Different PMIs

Figure 3. Correlation between Parotid Gland Myoglobin Concentration & Different PMIs
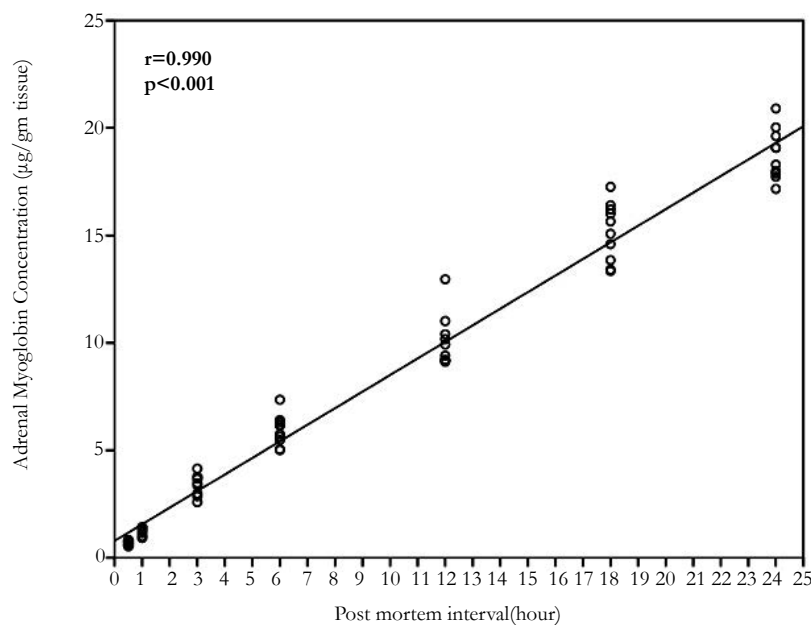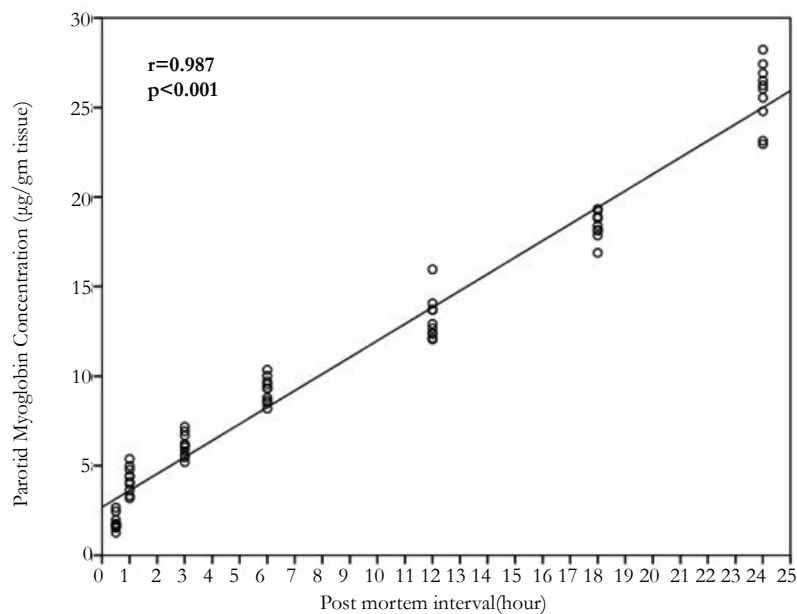
Shereen A Abdelaleem, Rehab H.A. Younis, Rasha F. Ahmed (2016) Postmortem Interval Estimation Using Myoglobin Concentration in Different Glandular Tissues. *Int J Forensic Sci Pathol.* 4(8), 259-265.

262

Table 4 . Multiple Linear Regression Analysis of Postmortem Interval

	B	SEM	R	R^2	P value	Regression equation
Pituitary myoglobin	0.4	0.07			<0.001*	PMI= -1.7 + (0.4 x Pituitary myo-
Adrenal myoglobin	0.57	0.07	0.996	0.992	<0.001*	globin) + (0.57 x Adrenal myoglo-
Parotid myoglobin	0.28	0.06			<0.001*	bin) + 0.28 x Parotid myoglobin)
Constant	-1.7	0.18			<0.001*	

PMI= Post-Mortem Interval, B= unstandardized coefficient, R=correlation coefficient, R^2 =effect size,
SEM= standard error of estimate *: significant difference at p value < 0.05

Table 5 . Multiple Stepwise Linear Regression Analysis of Postmortem Interval

Model		B	SEM	R	R^2	P value	Regression equation
1	Adrenal myoglobin	1.27	0.02	0.99	0.98	<0.001*	PMI= -0.82 + (1.27 x
	Constant	-0.82	0.23			0.001	Adrenal myoglobin)
2	Adrenal myoglobin	0.76	0.06			<0.001*	PMI= -1.3 + (0.76 x Ad-
	Pituitary myoglobin	0.56	0.07	0.995	0.99	<0.001*	renal myoglobin) + 0.56
	Constant	-1.3	0.17			<0.001*	x pituitary myoglobin)
3	Adrenal myoglobin	0.57	0.07			<0.001*	PMI= -1.7 + (0.57 x Ad-
	Pituitary myoglobin	0.4	0.07			<0.001*	renal myoglobin) + (0.4
	Parotid myoglobin	0.28	0.06	0.996	0.992	<0.001*	x Pituitary myoglobin) +
	Constant	-1.7	0.18			<0.001*	(0.56 x parotid myoglo- bin)

PMI= Post-Mortem Interval, B= unstandardized coefficient, R=correlation coefficient, R2 =effect size,
SEM= standard error of estimate *: significant difference at p value < 0.05

Table 6 . Internal Validation Test of Estimation Of Postmortem Interval From Myoglobin Concentration In Glandular Tissues

Model		B	SEM	R	R^2	P value
1	Adrenal myoglobin	1.27	0.03	0.99	0.98	<0.001*
	Constant	-0.74	0.27			0.001*
2	Adrenal myoglobin	0.76	0.06			<0.001*
	Pituitary myoglobin	0.56	0.07	0.996	0.991	<0.001*
	Constant	-1.23	0.19			<0.001*
3	Adrenal myoglobin	0.56	0.07			<0.001*
	Pituitary myoglobin	0.41	0.07			<0.001*
	Parotid myoglobin	0.29	0.07	0.997	0.993	<0.001*
	Constant	-1.6	0.19			<0.001*

B= unstandardized coefficient, R=correlation coefficient, R2 =effect size, SEM= standard error of estimate *: significant difference at p value < 0.05
There are minimal differences in B, R, R2, SEM & no differences in the significance from multiple stepwise linear models.

Shereen A Abdelaleem, Rehab H.A. Younis, Rasha F. Ahmed (2016) Postmortem Interval Estimation Using Myoglobin Concentration in Different Glandular Tissues. *Int J Forensic Sci Pathol*. 4(8), 259-265.

The stage of cellular death includes eye changes in the form of loss of corneal reflex, shrunked eye and pale retina. Algor mortis (body cooling), hypostasis, rigor mortis are early postmortem changes that occur in cellular death. Late postmortem changes include putrefaction. The process of postmortem decomposition divided into five stages: Fresh (autolysis), putrefaction, black putrefaction, butyric fermentation and dry decay [18].

Autolysis of normal tissues in a dead body must be distinguished from autolysis in the living body by the fact that the former is diffuse rather than focal and doesn't invoke an inflammatory cell. Autolysis is a non bacterial postmortem self destruction of tissue by its own enzymes. It is quick in tissues that have a high concentration of autolytic enzymes e.g gastric mucosa & pancreas. It is moderate in liver, kidney & heart & slow in fibroblastic tissues which poor in lysosomes and hydrolytic enzymes [9].

PMI is the interval between death & time of examination. PMI can be estimated by traditional methods of appearance of postmortem changes. There are many methods of estimation of PMI such as biochemical study of changes of electrolytes in vitreous humor, CSF, pericardial & synovial fluids. Muscle action potential and forensic entomology are useful in determination of elapsed time since death [19, 20]. Deepti et al., [21] found that there were a lot of postmortem histological & ultrastructural changes in kidney, pancreas, liver, heart & adrenal gland that can be used for estimation of PMI.

There are more advanced methods in estimation of PMI such as DNA quantification, Infrared spectroscopy and for buried individuals. PMI can be estimated from changes in the level of methane, phosphate, nitrates and volatile organic compounds in the soil [22].

Myoglobin is a cytoplasmic hemoprotein found in the cardiac myocytes and oxidative skeletal muscle fibers. Myoglobin was so named because of its functional and structural similarity to hemoglobin. Myoglobin was found in type I muscle, type IIA and type IIB and not found in smooth muscle. Myoglobin reversibly binds O_2 & facilitates its transport from RBCs to mitochondria during the periods of increased metabolic activity [23].

The normal range of human blood myoglobin during life is 0-85ng/ml. When muscle is damaged, myoglobin in muscle cells is released into the blood stream & started to increase. An increased level of myoglobin may be due to rhabdomyolysis, myositis, heart attack, muscular dystrophy and skeletal muscle trauma [24, 25].

Suzuki et al., [26] revealed that postmortem myoglobin permeation into the blood occurs within 108h after death. Their study demonstrated that myoglobin appeared early in heart blood of cadavers than in femoral blood within one day after death. The appearance of myoglobin in blood seemed to be related to the high length between death and blood taking.

The study of Zhu et al., [27] demonstrated that postmortem urinary myoglobin level increased in fire victims, heat stroke victims, and also in some cases of acute and subacute death from polytrauma, asphyxia drowning, electricity and spontaneous cerebral bleeding.

Owed to the few numbers of researches evaluating the estimation

of PMI from the postmortem changes of myoglobin concentrations in glandular tissues, the objective of this study was to analyze the ability of myoglobin concentrations in glandular tissues (pituitary, adrenal and parotid glands) to estimate PMI.

In this study, there was a significant increase of myoglobin concentration in pituitary gland than the adrenal gland only at one hour PMI. Also, there was a significant increase in the previous parameter in parotid gland when compared with pituitary and adrenal gland at all PMIs. There was a strong +ve significant correlation between myoglobin concentration & PMIs in all glandular tissues.

Miura et al., [28] studied the post mortem changes in myoglobin content in organs (liver, kidney, heart) and thyroid gland at 30min, and on 1,3,5,7 & 14 days postmortem. Their postmortem intervals were different from those in this study. Evaluation of myoglobin concentrations in the present study was done in glandular tissues at 30min, and on 1,3,6,12,18 & 24 hour postmortem.

By multiple stepwise linear regression analysis three models for estimation of PMI appeared. The most predictable model in estimation of PMI was the combination between adrenal, pituitary and parotid glands myoglobin concentration .The second predictable model was the combination between adrenal & pituitary myoglobin concentration. The last model was adrenal myoglobin concentration.

Miura et al., [28] obtained a different result. Their study revealed that myoglobin concentration in the thyroid gland & lung were being useful in estimation of PMI. Myoglobin concentration in thyroid gland increased markedly by day 1 postmortem & still maintained by day 7 postmortem. From day 7- 14, no increase was observed.

There are many mechanisms that can explain the increase of myoglobin concentrations in glandular tissues postmortem. The 1st mechanism is the postmortem distribution of myoglobin through the blood, in which the myoglobin concentrations raised to a level approximately 6.600 times that of antemortem blood in one day after death[11,29].

Significant increase in myoglobin concentrations in glandular tissues postmortem can be explained from the direct diffusion of myoglobin from the surrounding striated skeletal muscles, the closer the gland to the muscle , the higher the diffusion and concentration of myoglobin in such glands [30]. This mechanism explained such increase in parotid gland which lies directly on masseter muscle & pterygoid muscles. Also, this gland related anterolaterally to mastoid process and its relations to sternomastoid muscle. Also, adrenal gland is closely related to the diaphragm and so direct diffusion of myoglobin from these muscles to the glands occurred after death [31].

It is difficult to explain such an increase in the myoglobin concentration in pituitary gland by different diffusion from the striated muscles as the cranial cavity was not surrounded by skeletal muscles. Such increase can be explained by the difference in myoglobin concentrations between the sites of the blood (the central & peripheral blood) [32].

Shereen A Abdelaleem, Rehab H.A. Younis, Rasha F. Ahmed (2016) Postmortem Interval Estimation Using Myoglobin Concentration in Different Glandular Tissues. *Int J Forensic Sci Pathol.* 4(8), 259-265.

264

Conclusion & Recommendations

In conclusion, it was confirmed that myoglobin concentration in glandular tissues increased after death. There was a strong correlation between myoglobin concentrations in glandular tissues with PMI. By multiple stepwise linear regression analysis, the most predictable model in estimation of PMI is the combination between adrenal, pituitary, and parotid gland myoglobin concentrations ($R=0.996$ & $R^2 = 0.992$).

It is recommended to use the equation derived from this study in estimation of early PMI from myoglobin concentrations in glandular tissues. Finally, it is recommended to upgrade such study to the human level to estimate PMI from the concentration of myoglobin in other organs and tissues.

References

[1]. Lin X, Yin YS, Ji Q (2011) Progress on DNA quantification in estimation of postmortem interval. Fa yi xue za zhi. 27(1): 47-9.

[2]. Singh D, Prashad R, Sharma SK, Pandey AN (2006) Estimation of postmortem interval from human pericardial fluid electrolytes concentrations in Chandigarh zone of India: Log transformed linear regression model. Leg Med. 8(5): 279-287.

[3]. Kaliszan M, Hauser R, Kernbach G (2009) Estimation of time of death based on assessment of postmortem process with emphasis on body cooling. Leg Med. 11(3): 111-117.

[4]. Vas A (2012) Odor mortis. Forensic Sci Int. 222: 234-241.

[5]. Henssage C, Madea B (2004) Estimation of the time since death in the early postmortem period. Forensic Sci Int. 144: 167-175.

[6]. Goff ML (2009) Early postmortem changes and stages of decomposition in exposed cadavers. Exp Appl A carol. 49: 21-36.

[7]. Kawamoto O, Michiue T, Ishikawa T, et al., (2013) Comprehensive evaluation of pericardial biochemical markers in death investigations. Forensic Sci Int. 22 : 73-79.

[8]. Nidhi S, Yashoda R, Ritu S, Autal M (2011) Estimation of postmortem interval from the changes in vitreous biochemistry. J Indian Acad Forensic Med. 33: 171-174.

[9]. Tomita Y, Nihira M, Ohno Y, Sato S (2004) Ultrastructural changes during in situ early postmortem autolysis in kidney, pancreas, liver, heart and skeletal muscle of rats. Leg Med. 6 : 25-31.

[10]. Collman JP, Boulatov R, Sunderland CJ, FU L (2004) Functional analogues of cytochrome C oxidase, myoglobin, and hemoglobin. Chem Rev. 104(2): 561-88.

[11]. Puschel K, Lockemann U, Bartel J (1995) Postmortem investigation of serum myoglobin levels with special reference to electrical fatalities. Forensic Sci Int. 72(3): 171-177.

[12]. Reeder BJ, Svistunenko DA, Cooper CE, Wilson MT (2004) The radical and redox chemistry of myoglobin and hemoglobin: from in vitro studies to human pathology. Antioxid Redox Signal. 6(6): 954-66.

[13]. Wittenberg JB, Wittenberg BA (2003) Myoglobin function reassessed. J.Exp. Biol. 206 : 2011-2020.

[14]. Waller P et al., (1984) EMBO J . 3 : 439-446.

[15]. Akaboshi E (1985) Gene. 33: 241-249.

[16]. Arroyo A, Rosel P, Marron T (2005) Cerebrospinal fluid postmortem biochemical study. J Clin Forensic Med. 12(3): 153-156.

[17]. Grassberger M, Reiter C (2002) Effect of temperature on development of liopygia (=Sacrophaga) argyrostoma (Diptera: Sarcophagidae and its forensic implications. J Forensic Sci. 47(6): 1332-1336.

[18]. Forbes SL, Stuart BH, Dadour IR st al., (2004) A preliminary investigation of the stages of adipocere formation. J Forensic Sci . 49(3): 566-574.

[19]. Chandrakanth HV, Kanchan T , Balaraj BM (2013) Postmortem vitreous chemistry-An evaluation of sodium, potassium and chloride levels in estimation of time since death during first 36h after death. J Forensic Leg Med. 20(4): 211-216.

[20]. Carvalho LML, Thyssen PJ, Goff ML, Linhares AX (2004) Observations on the succession patterns of necrophagous insects on a pig carcass in an Urban area of south eastern Brazil. Journal of Forensic Medicine and Toxicology. 13 : 5-8.

[21]. Deepti G, Meena G, Bichitrananda R (2015) Sequential postmortem histological changes in human adrenal gland up to thirteen hour& thirty minutes postmortem interval . International Journal of Advanced Research. 3(7): 1138-1155.

[22]. Pringle JK, Cassella JP, Jervis JR, Williams A, Cross P et al., (2015) Soilwater conductivity analysis to date and locate clandestine graves of homicide victims. J Forensic Sci. 60(4): 1052-1061.

[23]. Mirceta S , Signore AV, Burns JM, Cossins AR, Campbell KL st al., (2013) Evolution of mammalian diving capacity traced by myoglobin net surface charge. Science. 340: 1234192.

[24]. Weber M , Rau M, Madlene Kr, Elsaesser A, Bankovic D et al., (2005) Diagnostic utility of new immunoassays for the cardiac markers cTnI, myoglobin and CK-MB mass. Clin Biochem. 38(11): 1027-30.

[25]. Selcen D (2016) Muscle diseases. Goldman,s Cecil Medicine, Elsevier Saunders: Philadelphia.

[26]. Suzuki T , Kashimura S, Umetsu K (1983) Postmortem permeation of myoglobin into the blood. Z Rechtsmed . 90(4): 297-301.

[27]. Zhu BL , Ishida K, Quan L, Taniguchi M, Oritani S et al., (2001) Postmortem urinary myoglobin levels with reference to the cause of death. Forensic Sci Int. 115(3): 183-8.

[28]. Miura M , Naka T, Miyaishi S (2011) Postmortem changes in myoglobin content in organs. Acta Med Okayama. 65(4): 225-230.

[29]. Miyaishi S , Moriya F, Yamamoto Y, Kitao T, Ishizu H (1994) Discrimination between postmortem and antemortem blood by a dot-ELISA for human myoglobin. Nihon Hoiqaku Zasshi. 48(6): 433-438.

[30]. Miyaishi S (1990) An enzyme immunoassay for human myoglobin and its application to forensic medicine. J Leg Med. 45(1): 6-25.

[31]. George A, Daniel J (2004) Myoglobin: an essential hemoprotein in striated muscles. J Exp Bio. 207: 3441-3446.

[32]. Kitao K, Miyaishi S , Ishizu H (1995) Identification of human skeletal muscle from a tissue fragment by detection of human myoglobin using a double-sandwich ELISA. Forensic Sci Int. 71(3): 205-214.

Shereen A Abdelaleem, Rehab H.A. Younis, Rasha F. Ahmed (2016) Postmortem Interval Estimation Using Myoglobin Concentration in Different Glandular Tissues. *Int J Forensic Sci Pathol.* 4(8), 259-265.

265