Postmortem diagnosis of early myocardial infarction in adult albino rats

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Abstract

Introduction : Postmortem diagnosis of early myocardial infarction is still a puzzling problem in forensic pathology practice, especially in sudden cardiac death. Forensic autopsy data shows that sudden cardiac death victims usually die in less than 6 h after acute myocardial infarction occurs. **Aim of the study :** Postmortem diagnosis of early myocardial infarction within the first 6 hours based on biochemical and morphologic changes. **Materials and Methods :** Rats divided into control group and operated group which had a ligation of The left anterior descending coronary artery . The rats were killed at 5, 15, 30, 60, 120, 240 and 360 mines post ligation intervals. Serum and myocardial tissue samples was collected from two groups at the previous postmortem time intervals for measurement of Creatine kinase- MB, Troponin I (CTnI), myoglobin and histopathological examination by light and electron microscopy . The same work was repeated after induction early acute myocardial infarction by beta-adrenergic shock and by lethal hemorrhage. **Results :** Levels of CK-MB and CTnI had statistical significant rise with increasing postmortem intervals whatever method of myocardial infarction induction, but myoglobin level was less sensitive in different methods of induction. Ultrastructural changes were detected after 30 mines post-mortem in different methods of induction.
Conclusion: Diagnosis of early myocardial infarction can be performed within the first postmortem six hours depending on biochemical and morphological changes.

KEYWORDS: early myocardial infarction- postmortem diagnosis- CK-MB- CTnI- Myoglobin

Introduction

Myocardial infarction (MI) or acute myocardial infarction (AMI), commonly known as a heart attack, is the interruption of blood supply to part of the heart, causing heart cells to die. This is most commonly due to occlusion (blockage) of a coronary artery following the rupture of a vulnerable atherosclerotic plaque, which is an unstable collection of lipids (fatty acids) and white blood cells (especially macrophages) in the wall of an artery. The resulting ischemia (restriction in blood supply) and oxygen shortage, if left untreated for a sufficient period of time, can cause damage or death (infarction) of heart muscle tissue (myocardium) . Acute Myocardial Infarction as a cause of death is diagnosed in many cases of sudden death based on the indirect evidence of critical narrowing (75 percent) of one or more coronary arteries **(Thygesen et al., 2007) .**

Myocardial infarction is a common presentation of ischemic heart disease. The WHO estimated in 2002, that 12.6 percent of worldwide deaths were from ischemic heart disease with it the leading cause of death in developed countries and third to AIDS and lower respiratory infections in developing countries **(Robert , 2004)** .

At common law, a myocardial infarction is generally a disease but may sometimes be an injury. This has implications for no-fault insurance schemes such as workers' compensation . A heart attack is generally not covered; however, it may be a work-related injury if it results **(Kotabagi et al., 2000)**.

The diagnosis of myocardial infarction requires two components (E.C.G and cardiac enzymes). Cardiac enzymes or cardiac markers are proteins that leak out of injured myocardial cells through their damaged cell membranes into the bloodstream. Until the 1980s, the enzymes SGOT and LDH were used to assess cardiac injury. Now, the markers most widely used in detection of myocardial infarction are MB subtype of the enzyme creatine kinase, myoglobin and cardiac troponins T and I as they are more specific for myocardial injury **(Jeremias and Gibson, 2005)**.

The cardiac troponins T and I which are released within 4–6 hours of an attack of myocardial infarction and remain elevated for up to 2 weeks, have nearly complete tissue specificity and are now the preferred markers for assessing myocardial damage. Elevated troponins in the setting of chest pain may accurately predict a high likelihood of a myocardial infarction in the near future. Myoglobin is a specific marker for cardiac muscle injury but it becomes elevated later than troponin **(Moe and Wong , 2010)** .

Postmortem diagnosis of early myocardial infarction is still a puzzling problem in forensic pathology practice, especially in sudden cardiac death which remains one of the major challenges of contemporary cardiology and forensic pathology. Forensic autopsy data shows that sudden cardiac death victims usually die in less than 6 h, even within 1 h after acute myocardial infarction occurs. The current diagnostic approaches are not sensitive enough to make such an early diagnosis but immunehistochemistry may be partially useful in determination of early myocyte necrosis in sudden cardiac death. Myoglobin and troponin C may be better but its practical application in forensic pathology may be still limited **(Hu et al., 1996).**

Postmortem diagnosis of a "well developed" myocardial infarction is not difficult. In the initial stages of infarction, however, at the stage of gross examination, it is difficult to discriminate between the infarcted zone and the undamaged cardiac muscle. When a cardiogenic cause of death is suspected, the histopathological diagnosis of a "fresh" myocardial infarction employing a basic routine staining method (hematoxylin/eosin) is not sufficient either. This is because microscopic evidence of infarction is seen in H & E stained sections only if the person has survived for a minimum period of 6 hours after sustaining fatal ischemic attack. This makes it necessary to use additional special staining techniques to resolve diagnostic problems, thus verifying the precise diagnosis **(Khalifa et al., 2006)** .

The limitations of post mortem detection of early myocardial infarction by hematoxylin and eosin staining stimulated a search for the development of improved diagnostic methods based on biochemical and morphologic changes. These include electron microscopic examination, gross and microscopic histochemical stains (tetrazolium salts, phosphotungstic acid-hematoxylin, trichrome, periodic acid-Schiff, hematoxylin-basic fuchsin-picric acid), fluorescence and immunohistochemical techniques **(Vargas et al., 1999)**.

Animal model of myocardial infarction is very important for the development of new approaches of human myocardial infarction diagnosis. More easily applicable invasive and noninvasive methods of myocardial damage induction are used in small laboratory animals **(Reznik , 2010)** .

The aim of the present study is to establish postmortem diagnosis of early myocardial infarction via detection the morphological and biochemical changes of early acute myocardial infarction in various experimental models by different methods of induction.

Materials and methods

Eighty adult albino healthy rats of both sexes weighing (300–350 g) were subjected for the study. They were housed in air- conditioned, humidity-controlled cages. Rats had free access to water and food during the experimental period.

The rats were divided into two groups. Control group (N= 10) and operated group (N= 70) which was more subdivided into seven subgroups depending on the time of killing rats postoperatively. Animals will be narcotized with 350 mg/kg Chloral Hydrate. In the operated group animals, tracheostomy was performed and the rats were ventilated with room air from a positive pressure ventilator using compressed air at rate of 70 strokes/min and tidal volume of 10ml/kg to improve survival rate after ligation of left anterior descending coronary artery. A thoracotomy was performed at the fifth intercostals space and the pericardium was opened to expose the heart , the left anterior descending coronary artery was ligated 4 -5 mm from its origin with 5/0 silk suture by piercing the pericardial membrane. After the completion of the surgical procedure, the heart was returned to its normal position in the thorax. Ligation was confirmed by appearance of regional epicardial cyanosis.

The rats were sacrificed at 5 min, 15 min, 30 min, 60 min, 120 min, 240 min, and 360 min postoperative intervals. An incision of the right iliac artery was carried out to collect one milliliter (mL) of blood at the previous specific time intervals. The time interval after ligation

was used as a grouping index. The control group was treated similar to the operated animals except they did not receive LAD ligation to establish reference values of biochemical parameters and morphological cardiac muscle for comparative purpose .

Blood samples were collected from rats after they had received left anterior descending coronary artery ligation at 5, 15, 30, 60, 120, 240 and 360 min, respectively. These samples, along with blood samples from control animals, were stored at 4°C for 30 minutes, allowed to clot fully, centrifuged at 3000 rounds for 10 minutes and the serum stored at −80°C before batch analysis for measurement of Creatine kinase - MB (CK-MB), Troponin I (cTnI) and myoglobin.

Measurements of cTnI, and CK-MB mass were done using an Elecsys analyser by the troponin I STAT third generation, and CK-MB STAT methods (Roche Diagnostics, Tutzing, Germany). These assays are based on electrochemiluminescence immunoassay technology (ECLIA) using two mouse monoclonal antibodies in a sandwich format, two step assay. They were done on Elecsys 1010 and 2010 immunoassay analysers according to the manufacturer's instructions (Roche Diagnostics, Tutzing, Germany). Myoglobin concentrations were determined using the respective Stratus fluorometric enzymeimmunoassay (Dade Behring, Newark, Delaware, USA) **(Wu et al., 1999)**.

Following the serum collection, myocardial tissue specimens were collected from both control group and operated groups with interval time (5, 15, 30, 60, 120, 240 and 360 min, respectively) then fixed in 10% neutral buffered formalin. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol, cleared in xylene, embedded in paraffin, sectioned at 4-6 μm thickness and stained by haematoxylen and eosin according to **(Bancroft et al., 1996).**

Ultrastructural studies was done by Transmission Electron Microscope (TEM). Myocardial tissue specimens were cut into five pieces to analyze different myocardial areas. Fixation was done in (2% glutaraldyde and 0.6% paraformaldehyde). Post-fixation was continued with 2% osmium tetroxyde for 2 hrs. The specimens were then washed with phosphate buffer, dehydrated with graded acetone and then embedded in araldite CY212 to make tissue blocks. Semithin (1 μm) as well as ultrathin sections (70–80 nm) were cut by ultra microtome and toludine blue stain. The sections were stained with uranylacetate and lead acetate. Examination under a Transmission Electron Microscope was performed and evaluated by Philips 400T electron microscope with special focus on nucleus, mitochondria and myofibrils.

The same work was repeated on eighty adult albino rats after induction early acute myocardial infarction by beta-adrenergic shock . The rats were divided into two groups. Control group (N= 10) and operated group (N= 70) which was more subdivided into seven subgroups depending on the time of killing rats postoperatively. Isoproterenol (ISO) is a synthetic catecholamine (beta-receptor agonists). It causes focal myocardial infarction through their hemodynamic effects by dose 100 mg / kg of rat body weight **(Hasic et al., 2011)**. The rats of operated group (N= 70) were treated with single subcutaneous dose of isoproterenol (100mg/kg body weight dissolved in 0.9% saline). Control group rats (N= 10) were treated with 0.9% of saline (NaCl) by the same route. The rats of operated and control groups were sacrificed at 5 min, 15 min, 30 min, 60 min, 120 min, 240 min, and 360 min after the injection of

Isoproterenol or saline solution . Serum and myocardial tissues samples were collected from rats with postmortem interval 5, 15, 30, 60, 120, 240 and 360 min, respectively. Isoproterenol hydrochloride was manufactured by Sigma Chemical company, USA.

The same work was repeated after induction early acute myocardial infarction by lethal hemorrhage. Carotid artery of the rats of operated group (N= 70) were cut. Operated group was more subdivided into seven subgroups depending on the time of killing rats postoperatively. The control group (N= 10) was treated similar to the operated animals except cutting of carotid artery. Blood and myocardial tissues samples were collected from rats with postmortem interval 5, 15, 30, 60, 120, 240 and 360 min, respectively.

A statistical analysis was performed using SPSS version 14. Nonparametric Kruskal-Wallis test was used to compare between biomarkers levels of myocardial infarction regarding to one method of induction of myocardial infarction **(Corder et al., 2009)**. ANOVA test was used to compare between variance of biomarker levels among three different induction methods of myocardial infarction.

Sample size

$$N = \frac{Z\alpha^2 * PQ}{D^2}$$

N = Number of sample size
Zα = Percentile of standard normal distribution determined by 95% confidence level .
Zα = 1.96
P = Prevalence of diagnosis of myocardial infarction within the first 6 hours.
Q = 1- P
D = Proportion of sampling error in a given situation.
D = 0.05. (**Leslie et al., 1991)**

Ethical considerations

The most appropriate animal species was chosen for this research. Promotion of a high standard of care and animal well-being at all times was done. Appropriate sample size was calculated by using the fewest number of animals to obtain statistically valid results. Surgical or other painful procedures were performed with appropriate sedation to avoid distress and pain. Our standards of animal care and administration met those required by applicable international laws and regulations.

Results

Table (1) (2) and (3) shows median values and ranges for each biomarker (Creatine kinase "CK-MB", Cardiac Troponin I "CTnI", myoglobin "MG") of control group and different study groups of myocardial infarction at different postmortem interval times regarding to method of myocardial infarction induction. Data of tables are presented along with the Kruskal – Wallis statistic and P- values.

Range of CK-MB (control) was 118-160 while 136.50 was represented as median value of control group. Range and median values of control group of CTnI was 0 (below the detection

limit of used immunoassay) . Range of myoglobin (control) was 88-130 while 105 was represented as median value of control group. Levels of CK-MB and CTnI were statistically significant rise with increasing postmortem intervals whatever method of myocardial infarction induction except myoglobin level which was statistically significant rise after 30 /m in case of myocardial infarction induced by ligation of coronary artery and after 60/m in cases of myocardial infarction induced by adrenergic shock and lethal hemorrhage **.**

The p value for all biomarkers among control and different groups was statistically significant because it was less than 0.01.

Table (1) Descriptive Statistics of Biomarkers Levels of Myocardial Infarction Induced In Coronary Artery Ligation Model Using Kruskal-Wallis Test

Marker		Control (N=10)	After 5 min (N=10)	After 15 min (N=10)	After 30 min (N=10)	After 60 min (N=10)	After 120 min (N=10)	After 240 min (N=10)	After 360 min (N=10)	*P* value
CK-MB (ng/ml)	**M***	136.50	149.50	183.50	228.00	714.50	1049.50	1267.00	1357.50	< 0.01
	R**	118- 160	138-165	165-195	213-289	650-741	986-1090	1176-1300	1326-1410	
CTnI (ng/ml)	**M***	0	0.015	.32	1.85	3.95	7.75	7.05	6.90	< 0.01
	R**	0 – 0	0-0	0-1	1-3	4-4	8-8	7-8	6-8	
MG (ng/ml)	**M***	105.00	86.50	92.00	109.00	110.50	220.00	372.50	505.00	< 0.01
	R**	88-130	79-117	75-123	78-130	80-153	180-245	365-383	480-544	

CKMB = Creatine kinase –MB CTnI = Cardiac Troponin I M G = Myoglobin
M* = Median R** = Range N = Number of samples per group
P < o.o5 = Statistical significant.

Table (2) Descriptive Statistics of Biomarkers Levels of Myocardial Infarction Induced In Adrenergic Shock Model Using Kruskal-Wallis Test

Marker	Control (N=10)	After 5 min (N=10)	After 15 min (N=10)	After 30 min (N=10)	After 60 min (N=10)	After 120 min (N=10)	After 240 min (N=10)	After 360 min (N=10)	*P* value
CK-MB (ng/ml) **M***	136.50	137.50	143.50	266.00	400.00	740.00	1155.00	1107.50	< 0.01
CK-MB (ng/ml) **R****	118- 160	120-150	136-153	225-285	380-422	686-831	1100-1210	1050-1220	
CTnI (ng/ml) **M***	0	0.045	.95	2.59	4.40	8.05	7.35	7.60	< 0.01
CTnI (ng/ml) **R****	0 - 0	0-0	1-1	2-3	4-5	8-9	7-8	7-8	
MG (ng/ml) **M***	105	86.50	87.50	93.00	111.50	263.00	405.00	520.00	< 0.01
MG (ng/ml) **R****	88-130	78-95	68-110	84-96	100-130	230-312	380-411	500-545	

CKMB = Creatine kinase –MB CTnI = Cardiac Troponin I M G = Myoglobin
M* = Median R** = Range N = Number of samples per group
P < o.o5 = Statistical significant.

Table (3) Descriptive Statistics of Biomarkers Levels of Myocardial Infarction Induced In Lethal Hemorrhage Model Using kruskal-Wallis Test

Marker	Control (N=10)	After 5 min (N=10)	After 15 min (N=10)	After 30 min (N=10)	After 60 min (N=10)	After 120 min (N=10)	After 240 min (N=10)	After 360 min (N=10)	*P* value
CK-MB (ng/ml) **M***	136.50	142.00	163.50	207.00	258.00	504.50	820.00	796.00	< 0.01
CK-MB (ng/ml) **R****	118- 160	118-162	139-180	195-214	240-302	470-515	780-895	780-854	
CTnI (ng/ml) **M****	0	0.065	.15	.70	1.67	3.01	6.04	6.06	< 0.01
CTnI (ng/ml) **R****	0 - 0	0-0	0-1	0-1	1-2	3-3	6-7	6-7	
MG (ng/ml) **M***	105	82.50	79.50	100.50	106.50	275.00	452.50	652.00	< 0.01
MG (ng/ml) **R****	88-130	79-87	77-93	91-103	98-115	250-281	382-495	605-711	

CKMB = Creatine kinase –MB CTnI = Cardiac Troponin I M G = Myoglobin
M* = Median R** = Range N = Number of samples per group
P < o.o5 = Statistical significant.

Table (4): there are statistical significant differences between levels of biomarkers in control and study groups at different times whatever method of induction of myocardial infarction. **There's** also 95% confidence interval for levels of biomarkers between control and study groups among three different induction methods of myocardial infarction.

Table (4) ANOVA-One Way Statistical Analysis of Three Biomarkers Levels In the Three Models of Myocardial Infarction Regarding to the Same Postmortem Interval.

Biomarker		95% Confidence interval for Mean		*P* Value
		Lower Bound	Upper Bound	
Control	CK-MB	130.89	144.31	
	CTnI	.00	.00	< 0.01
	MG	100.07	113.73	
After 5 min	CK-MB	140.03	152.47	
	CTnI	0.018	0.064	< 0.01
	MG	81.71	91.69	
After 15 min	CK-MB	164.39	178.71	
	CTnI	.19	.38	< 0.01
	MG	82.38	95.82	
After 30 min	CK-MB	209.95	232.75	
	CTnI	.98	1.64	< 0.01
	MG	94.28	109.42	
After 60 min	CK-MB	380.43	595.47	
	CTnI	2.29	3.38	< 0.01
	MG	99.45	119.05	
After 120 min	CK-MB	640.74	902.26	
	CTnI	4.20	6.50	< 0.01
	MG	226.27	258.53	
After 240 min	CK-MB	936.22	1143.58	
	CTnI	6.27	6.86	< 0.01
	MG	385.61	429.09	
After 360 min	CK-MB	952.37	1219.53	
	CTnI	6.27	6.75	< 0.01
	MG	541.47	614.13	

CKMB = Creatine kinase –MB CTnI = Cardiac Troponin I M G = Myoglobin
P < o.o5 = Statistical significant

Pathological findings

I- After induction early acute myocardial infarction by ligation of left anterior descending coronary artery:-

Ventricles of hearts of sacrificed rats of both control and study groups were taken for gross examination along the experimental period with interval time (5-15-30-60-120-240-360 minutes after the ligation of left anterior descending coronary artery). No definite gross changes were detected in infarcts areas of less than 360 minutes but the anterior wall of left ventricle and apex were appeared as pale, fairly well demarcated areas at 360 minutes. After 5, 15, 30 minutes of induction of infarction, histopathological findings revealed normal histological structure of myocardium that relatively similar to control animals. The muscle fibres appeared short branched join to each other and formed network. Cross striation and oval prominent central single nuclei was seen fig. (1) . Electron microscopy examination after 5-15 minutes of ligation showed ultrastructure similar to control animals. Nucleus showed several deep furrow-like

notches and a clear nucleus. The chromatin granule was homogenically spread and somewhat more densely accumulated along the nucleus membrane. Mitochondria were densely distributed around the nucleus fig.(2) . Alterations of histopathological findings of cardiac muscle fibres were seen after 360 minutes of ligation as slight swelling and separation. Few number of leukocytes mainly neutrophils and macrophages were seen between the muscle fibres. The muscle fibres were appeared swollen with faintly stained cytoplasm and central situated deeply basophilic nucleus fig. (3) . On the other side the ultrastructure changes were seen after 30 minutes of ligation. The nucleus showed margination chromatin granules as clots in the mid-nucleus. Many mitochondria showed destroyed cristae. Membrane like structured, fine filaments and glycogen granule were also seen in the cellular matrix fig. (4) . The ultrastructure changes were more pronounced after 60,120 and 240 minutes of ligation. The muscle cells showed peripheral condensation of nuclear chromatin and marked swelling of mitochondria. The cellular matrix contained circumscribes electron dense granules as lipid bodies. Dilation of the rough endoplasmic reticulum with detachment of ribosomes was also noticed fig. (5) . After 360 minutes, a strong clotting and a clear margination of the chromatin were observed. Mitochondria lied isolated around the nucleus in a niche. In the mitochondria lie several larger electron density granules, between them as round homogenous structure, which is enclosed by a membrane-like structure. This was identified as lipid bodies' fig. (6).

II- After induction early acute myocardial infarction by beta-adrenergic shock :-

Gross lesions were appeared only after 360 minutes of induction of myocardial infarction by Isoproterenol (ISO) injection as pale dilated flaccid ventricles. Histopathological findings were appeared after 60 minutes of Isoproterenol (ISO) injection, inform of mild swelling and derangement of myocardial fibres. The ultrastructural changes of ventricular wall especially at apex were noticed after 30 minutes of Isoproterenol (ISO) injection, the muscle fibres showed mild swelling of mitochondria and disappearance of perinuclear glycogen particles fig. (7) . After 60-120-240 minutes of Isoproterenol (ISO) injection, electron microscopy examination revealed marked swelling of mitochondria with dilatation of endoplasmic reticulum. The nucleus showed clumping of its chromatin. On the other hand the ultrastructural changes were prominent after 360 minutes of Isoproterenol (ISO) injection .The myocytes showed marked swelling and disappearance of mitochondrial cristae. The nucleus showed clear margination of nuclear chromatin. Coarse electron dense lipid bodies were seen in the cellular matrix fig. (8) .

III- After induction early acute myocardial infarction by lethal haemorrhage:-

Gross lesions were appeared only after 240& 360 minutes of cutting of carotid artery. The cardiac muscle appeared pale with dilatation of ventricles. Histopathology findings were seen after 360 minutes of cutting of carotid artery which revealed the cardiac muscle fibres showed swelling and some of them showed coagulative necrosis. Few number of leukocytes mainly neutrophils and macrophages were seen between the muscle fibres fig. (9) . Electron microscopy examination after 30 minutes of cutting of carotid artery revealed marked swelling of mitochondria with disappearance of its cristae. Complete absence of perinuclear glycogen particles was also seen. Cardiac fibres showed peripheral condensation of its nuclear chromatin and marked swelling of mitochondria. Electron dense granules were also seen. Several membrane bound electron dense granules were seen in the cytoplasmic matrix after 60-120-240 minutes of carotid cutting. After 360 minutes of carotid cutting, cardiac muscle fibres showed

clear margination of the nuclear chromatin. Mitochondria showed severe swelling with disappearance of its cristae and some of them showed complete destroyed fig. (10) .

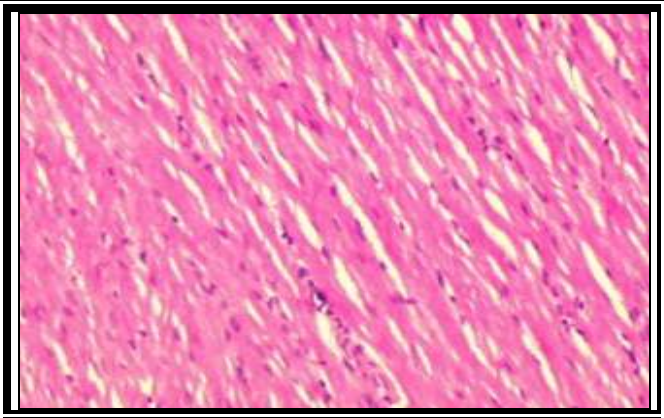

Fig.(1) showing normal histological structure of cardiac muscle after 5, 15, 30 minutes of infarction induction H&E (X200)

Fig.(2) showing normal ultrastructure of myofibrils of cardiac muscle after 5, 15 minutes of ligation of coronary artery (10.000 X)

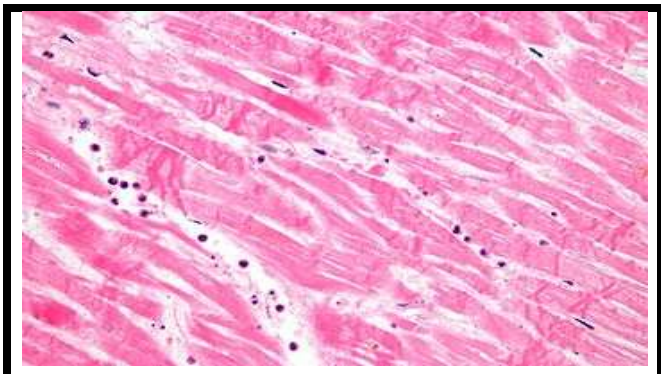

Fig. (3) showing marked swelling of cardiac muscle fibers with few leucocytic infiltration after 360 minutes of ligation of coronary artery H&E (X200)

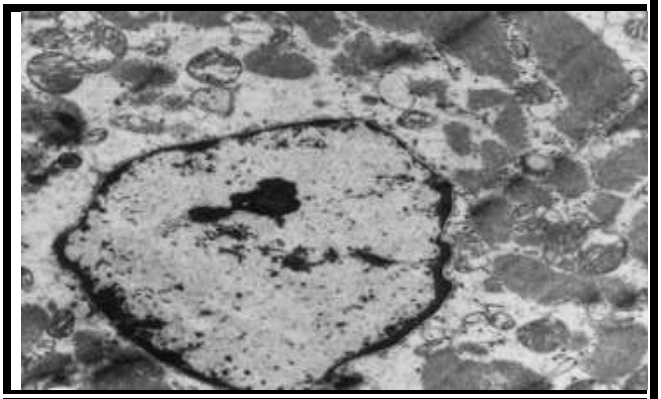

Fig. (4) showing margination of nuclear chromatin and destruction of mitochondrial cristae after 30 minutes of ligation of coronary artery (10.000 X)

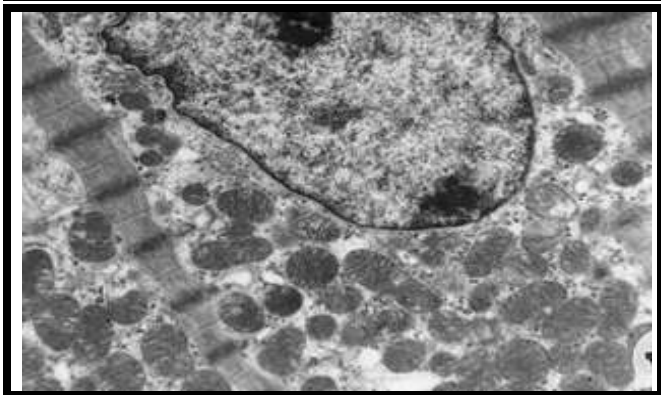

Fig. (5) showing marked swelling of mitochondria and electron dense granules as lipid bodies after 60,120 and 240 minutes of ligation of coronary artery (10.000 X)

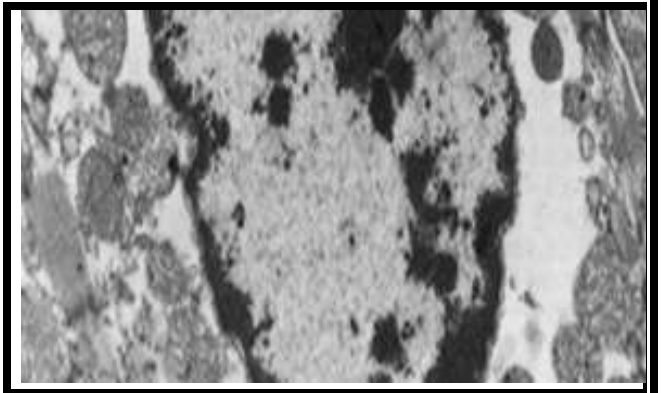

Fig.(6) showing clumping of nuclear chromatin and marked swelling of mitochondria after 360 minutes of ligation of coronary artery (10.000 X)

Fig.(7) showing mild swelling of mitochondria and disappearance of perinuclear glycogen particles after 30 minutes of Isoproterenol (ISO) injection (10.000x)

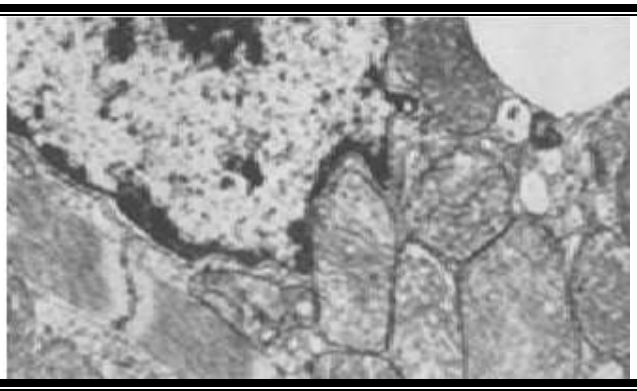

Fig. (8) showing marked swelling and disappearance of mitochondrial cristae after 360 minutes of Isoproterenol (ISO) injection (10.000X)

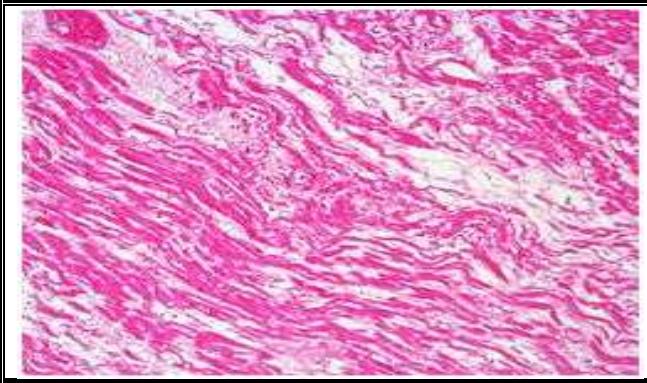

Fig. (9) showing cardiac muscle fibres showing swelling and some of them showed coagulative necrosis after 360 minutes of cutting of carotid artery H&E (X100)

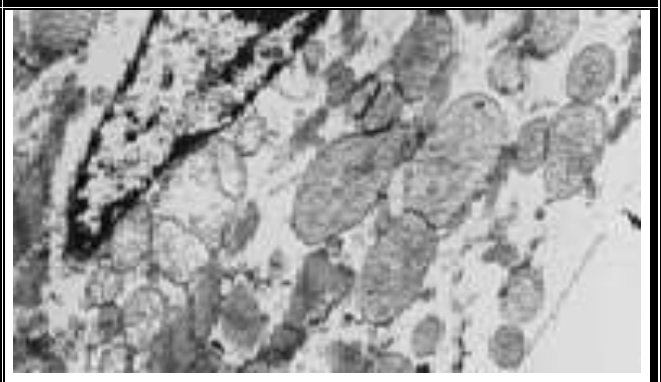

Fig. (10) showing clear margination of the nuclear chromatin. Mitochondria revealed severe swelling and some of them complete destroyed after 360 minutes of cutting of carotid artery (10.000 X)

Table (5) . All histopathological examination results using haematoxylen and eosin show no minor/or major cellular changes related to myocardial infarction before 60/mines of postmortem interval in three methods of induction of myocardial infarction, however all transmission electron microscope examination results show cellular changes related to myocardial infarction after 30/mines of postmortem interval in three methods of induction of myocardial infarction.
P values of histopathological examination using haematoxylen and eosin were statistically significant positive after 120/mines of postmortem interval in beta- adrenergic shock and lethal hemorrhage methods while *P* values were statistically significant positive after 240 mines of postmortem interval in ligation of coronary artery and lethal hemorrhage methods.

Table (5) *Chi-Square Statistical* Analysis of The Histopathological findings In Three Models of Myocardial Infarction Regarding to Postmortem Interval

Pathological Exam / Type of Induction	H.E After 60/min	H.E After 120/min	H.E After 240/min	T.E.M After 15/min	T.E.M After 30/min
Ligation	0.77	0.17	<0.01*	0.46	0.92
Shock	0.89	0.01*	0.92	1.00	1.00
Hemorrhage	0.78	<0.01*	<0.01*	0.46	0.92

* P < o.o5 = Statistical significant.
T.E.M = Transmission Electron Microscope.
H.E = Histopathological Examination by haematoxylen and eosin.

Discussion

Cardiac deaths comprise a significant volume of a forensic pathologist' s caseload .The cardiac markers have not shown the predictive value in the autopsy although there are many cases of assumed acute myocardial infarction with little or no gross histological evidence of myocardial injury because of the utility of these markers in the autopsy setting has not been fully established with it is well documented and widely use by clinicians (**Jaffe et al., 2006)**.

Recently, there is a rapid development of electron microscopy technology and description of histological ultrastructure to assess significance of early changes which is no detected by usual histological exam. So we need to investigate the role of electron microscopy and cardiac markers in postmortem diagnosis of early myocardial infarction by studying biochemical and morphological changes related postmortem time interval less than 6 hours.

Our results showed that level of CK-MB and CTnI increased with increasing postmortem intervals whatever method of myocardial infarction induction and this consistent with **(Puleo et al., 1994; Goldmann et al., 2004)** who decided that measurement of creatine kinase (CK) and its isoenzyme MB (CK-MB) has become the gold standard for detection of early acute myocardial infarction although it is not specific for myocardial damage. They explained that these markers can originate from both skeletal and cardiac muscles, yet they are sensitive.

Jeremias and Gibson (2005) suggested that cardiac troponin I (CTnI) is highly specific for the myocardium and is present only in cardiomyocytes, the contractile apparatus .

World Health Organization (1997) suggested that elevation of troponin level should replace elevation of other enzymes for definition of acute myocardial infarction. The present study confirm that there was no significant difference in performance between CTnI assay and CK-MB measurement for the same induction method of myocardial infarction or in other different methods of induction of myocardial infarction and this consistent with **(Eisenman, 2006).**

In contrast with **(Winter et al ., 1995)** who referred that troponin appears in the blood within 4-8 hours after early myocardial infarction and remains abnormal for four days and then it may be insufficient for diagnosis of early myocardial infarction .

According to **(Wu et al., 1999)**, combining multiple sampling of CK-MB and troponin testing may provide a reliable and effective testing for diagnosis of early acute myocardial infarction and this consistent with results of the present study.

O' Brien (2008) confirmed that cardiac troponin I is considered to be the standard for the non-invasive diagnosis of myocardial injury in people and small animals but its concentrations can be altered in samples collected from deeply sedated animals as a result of generalized hypoxia.

According to **(Hessel et al., 2008)** intracellular CTnI degradation and subsequent release of its degradation products in blood occur during myocardial necrosis development.

Results of this study showed that myoglobin appears after 30/minutes in myocardial infarction induced by ligation and after 60/minutes in myocardial infarction induced by adrenergic shock or by lethal hemorrhage and this contrast with **(Fesmire et al., 2004)** who suggested that myoglobin appears earlier than other cardiac markers.

Pathological findings of present study showed that electron microscopy examination has an important role for detection ultrastructural changes after 30/mines whatever method of myocardial infarction induction in contrast the usual histopathological examination by light microscopy which did not detect any definite gross changes less than 360/ mines regarding to induction of myocardial infarction by ligation of coronary artery or by adrenergic shock but gross changes appeared after 240/mines in induction by the lethal hemorrhage .

The current study is consistent with **(Adegboyega et al., 1997)** who indicated that gross findings can be detected in the infracted tissue only if the infarct is six hours old and it could not be differentiated from normal myocardium by light microscopy.

Kotabagi et al., (2000) explained that changes include microscopic and metabolic changes occurring in the early part of the process. They showed that after the death of myocardial cells, electrolytes like K^{+}, Mg^{++} , phosphates , etc which were kept inside the cell by active energy pumps, are lost to the interstitial space. Similarly, enzymes and low molecular weight co-factors diffuse out of the dead cell and enter the blood stream. They concluded that the detection of enzymes specific to the myocardial cell, in increased quantities in the peripheral blood thus indicates irreversible myocardial cell death. Other intracellular substances like the energy binding phosphates and glycogen are degraded inside the cell. Even at this stage the morphology of these dead cells remains normal to routine technique of light microscopy.

McVie (1990) agree with our results and mentioned that electron microscopic studies must be used for diagnosis of the early changes in infarct tissues and it will remain only an experimental tool applicable to study artificial induced infarcts in animal models. Our results showed correlation between early significant rise of CK-MB and CTnI levels with increasing postmortem intervals and progression of changes in the ultrastructure of myofibers in the infarcts which were readily demonstrated by electron microscopy.

Hougen et al., (1992) showed that these early ultrastructural changes were related to hyperosmolarity of mitochondria and sarcoplasmic reticulum especially after cell death and became progressively severe with time as a result of the increasing of ischemic duration.

Conclusion

Forensic pathologist can perform accurate diagnosis of early myocardial infarction within the first postmortem six hours depending on biochemical and morphological changes.
The assay of CK- MB and CTnI proved to be valuable for diagnosis of early acute myocardial infarction whatever the method of induction. Electron microscopic studies must be used for diagnosis of the early changes in infarct tissues and it will remain only an experimental tool applicable to study artificial induced infarcts in animal models.

Recommendations

This study recommends using of improved diagnostic methods based on biochemical and morphologic changes to resolve diagnostic problems of early acute myocardial infarction during the first postmortem six hours. Assay of cardiac markers especially CK- MB and CTnI is one of these methods. We suggest that electron microscopic examination to replace the usual histopathological examination by light microscopy for post mortem detection of early myocardial infarction.

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