

## Clinical And Diagnostic Studies Of Myocarditis Result From FMD In Lambs

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**Abstract:** The study was conducted on (125) local suckling lamb breeds (5-30) days old and from both sexes, reflecting different farms of Basrah, Iraq. Their dams have no history of vaccination against FMD and show classical foot and mouth disease signs (reared in Basrah Governorate). Suspected lambs show neither oral blisters belong to FMD lesions or rope salivation, nor lesions of interdigital space of a foot. However, all clinically examined lambs had heart problems, Reflected as murmur sounds, irregular heart rhythm and tachycardia on auscultation of the chest. Twenty five clinically healthy local lamb breeds were considered as controls. Completed clinical examinations have been carried out to all suspected and control animals. Diseased lambs show different clinical manifestations such as signs of depression and dullness, with inactivity, mouth breathing with panting. Animals are mostly recumbent and unable to suck their dams, However, some of diseased animals died soon within 24-72hrs, Since, macroscopic examinations of the autopsied dead lambs, exhibit necrotic myocarditis with enlargement of the heart and observing of different sizes of pale foci associated with a zone of hyperemia detected in different parts of heart tissue specially the papillar and ventricular cardiac muscles. Data concerning clinical examinations of diseased lambs shows a significant increase ( $p < 0.05$ ) in the body temperature, respiratory and heart rate of diseased animals than in controls. Furthermore, abnormal cardiac sounds (organic murmurs) were indicated on auscultation of the heart. RT-PCR results on gel electrophoresis, show that the FMD virus has 328 base-pair FMDV specific PCR amplicons were determined. Moreover, on histopathological examinations there were severe inflammatory cell infiltration in the interstitium of myocardial fibers with obvious areas of coagulation of myocardial fibers (coagulative necrosis of myocardial fibers) and marked area of hyalinization, Furthermore, High number of A large areas of vacuolated degenerative myocardial muscle cells and with several foci and A several vacuolated-degenerated myocardial muscle cells with possibility of interstitial cell edema will also detected . Results of hematological parameters indicated a significant increase ( $p < 0.05$ ) in ESR values of diseased lambs than controls. Moreover, the total leukocyte count was increased significantly with significant lymphocytosis. In addition that, The results also show a significant increase ( $p < 0.05$ ) in values of serum cardiac Troponin-I (cTnI), Homocystein (Hcy), Creatine kinase-myocardial band (CpK-MB), and Lactate dehydrogenase (LDH) in diseased lambs with FMD than in controls. The results of the acute phase response were indicating a significant increase ( $p < 0.05$ ) in both Haptoglobin values and Fibrinogen time in diseased lambs compared with controls. It has been concluded that, Myocarditis associated with FMD is a common unusual sequel always terminated by death and high mortalities of diseased animals.

**Key words:** FMD, Myocarditis, Lambs. Basrah, Iraq

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### I. Introduction

Foot-and-mouth disease (FMD) is a highly contagious disease of all cloven-footed animals (Domesticated and wild type of animals) caused by the foot-and-mouth disease virus (FMDV), A small, non-enveloped virus that belongs to the genus of *Aphthovirus*, family *Picornaviridae*, characterized by vesicles in the mouth and on the feet (Constable *et al.*, 2017). The causative agent of the foot-and-mouth disease (FMD), is classified within the *Aphthovirus* genus as a member of the *Picornaviridae* family with seven serotypes, including O, A, C, Asia-1, South African Territory SAT-1, SAT-2, and SAT-3 (Dukpa *et al.*, 2011). Foot and mouth disease is an economically subversive disease, causes significant production losses in infected domestic livestock (Alexandersen and Mowat, 2005., Aitken, 2008).

Unlike pigs and cattle, where the disease is usually characterized by overt clinical signs, FMD in sheep and goat is often mild and in apparent, Since, The clinical signs are generally milder in sheep than in cattle or pigs, However, Viremia may be present for up to 3 days before the appearance of vesicular lesions (Alexandersen *et al.*, 2003). During this time the sheep may be pyrexia and distressed with lameness spreading throughout the flock, Moreover, Agalactia may occur in lactating ewes, Furthermore, Vesicular lesions were

noted in the interdigital cleft along the coronary bands and on the bulb of the heels, Although, oral lesions are less common, but can detect on the dental pad, tongue and gums (Hughes *et al.*, 2002).

In young animals, The disease, characterized by necrosis of heart muscles and may result in death, before lesions developments in the most common and visible location such as the mouth or foot (Lubroth, 2002). The high mortality due to FMD in young animals, particularly in lambs is associated with acute myocarditis, As, The fatal form of FMD might occur without classic vesicular lesions, The myocarditis of young animals is acute, with hyaline degeneration and necrosis of muscle fibers and an intense infiltration of mainly lymphocytes (Alexandersen *et al.*, 2003., Glubahar *et al.*,2007., Tunca *et al.*,2008., Aslani *et al.*,2013). However, myocarditis may occur in young animals resulting in death (Mazengia *et al.*, 2010). The mortality rate is around 5% in mature animals, but can run as high as 50% in young animals because of myocardial damage (Grubman and Baxt, 2004). Foot and mouth disease was diagnosed with most parts of Iraq, as it seems to be an endemic disease causing a large animal health problems and high economic losses (Mahdi, 2010). Thereby the current study was aimed to, clinical evaluation of diseased animals with FMD via exploring the main clinical manifestations showed by diseased lambs, Moreover, Evaluation of hematological changes, cardiac enzymes and acute phase response in diseased lambs with Cardiac form of FMD and Post mortem examination of recently died and /or slaughtered lambs with laboratory histopathological evaluations, Furthermore, To, Confirm the clinical diagnosis of FMD using Biological methods, The PCR technique.

## **II. Materials and Methods**

**Animals and Study design:-** The study was conducted on (125) local suckling lamb breeds (5-30) days old and from both sexes, reflecting different farms of Basrah, Iraq, Their dams have no history of vaccination against FMD and show classical foot and mouth disease signs (reared in Basrah Governorate). Suspected lambs show neither oral blisters belong to FMD lesion or rope salivation, Nor lesions of interdigital space of a foot. However, all clinically examined lambs had heart problems, Reflected as murmur sounds, irregular heart rhythm and tachycardia on auscultation of the chest. Twenty five clinically healthy local lamb breeds were considered as controls. Completed clinical examinations have been carried out to all suspected and control animals.

**Hematology and collections of samples:-**Ten milliliters of blood (10 mL) were withdrawn from each lamb through puncture of the jugular vein and from these (2.5) milliliter of blood mixed with EDTA used to determine hemoglobin concentration (Hb), Total erythrocyte count (TRBc), packed cell volume (PCV), and Total leukocyte count (TLC), (Analysis done using, Hematology analyzer from Genex, USA). Furthermore, differential leukocyte count was done using Giemsa stain blood smear method according to (Weiss and Wardrop, 2010). Moreover, erythrocyte sedimentation rate, ESR was also estimated according to (Reagan *et al.*, 2008).

**Biochemical analysis and Evaluation of acute phase response:-** Serum was evaluated for Troponin I (cTnI) concentration measured by AFIAS-6 (AFIAS-automated fluorescent immunoassay system) from, boditech. Homocystein (Hcy) (Homocystein commercial kit from Axis<sup>®</sup> Homocystein EIA-UK), Creatine kinase-myocardial band (CpK-MB) and Lactate dehydrogenase (LDH) (spectrophotometer using commercial kits, Roche Diagnostics, Indianapolis, GMBH, Germany). Moreover estimation of acute phase response, including evaluation of Haptoglobin (Haptoglobin Elisa method) (Biotechnology co -china) and Fibrinogen time (using plasma) (Biolabo / France) were also done according to manufacture instructions of the producers.

**Gross post mortem examination and Histopathology :-** Recently died and /or slaughtered lambs were subjected to post mortem examinations and laboratory histopathological evaluations after owner's approval. Heart lesions were detected and investigated. Furthermore, The tissue samples were collected from different parts of the heart tissue, The interventricular, the right and left atria, and ventricular parts of the hearts, Collected tissue samples were fixed in 10% neutral buffered formalin solution for 72 hrs, It will trimmed to apposite and suitable sizes, washed, then dehydrated and cleared in xylol. Then, was embedded in paraffin wax, after that sectioned at 4-5  $\mu$  thickness, stained with hematoxyline and eosin, and examined under a light microscope (Maxie, 2015).

**PCR Assay:-** Another, Tow and half (2.5) milliliter of blood containing EDTA were used for viral PCR analysis. RNA extraction was performed on EDTA blood immediately following collection. Viral RNA was extracted from blood samples collected in EDTA-coated tubes according to the instructions given in the insert of the QIAamp<sup>®</sup> Viral RNA Mini Kit (QIAGEN, Germany).

### **Preparation of reagents:**

**Addition of carrier RNA to Buffer AVL:-**310  $\mu$ l Buffer AVE was added to the tube containing 310  $\mu$ g lyophilized carrier RNA to obtain a solution of 1  $\mu$ g/ $\mu$ l. The carrier RNA was dissolved thoroughly, divided it

into conveniently sized aliquots, and store it at  $-20^{\circ}\text{C}$ . The volume of Buffer AVL-carrier RNA mix needed per batch of samples was calculated using the following sample calculation:

$n \times 0.56 \text{ ml} = y \text{ ml.}$ ,  $y \text{ ml} \times 10 \mu\text{l/ml} = z \mu\text{l.}$ , where:  $n$  = number of samples to be processed simultaneously.,  $y$  = calculated volume of Buffer AVL, $mz$  = volume of carrier RNA-Buffer AVE to add to Buffer AVL., it was mixed by inverting the tube 10 times.

**Preparation of Buffer AW1 and Buffer AW2:-**Absolute ethanol (25 mL) was added to a bottle containing 19 mL of buffer AW1 concentrate and 30 mL of absolute ethanol was added to a bottle containing 13 mL of buffer AW2 concentrate. The reconstituted buffers were stored at room temperature ( $15-25^{\circ}\text{C}$ ). The QIAamp® Viral RNA Mini Kit Procedure, It was done according to manufacture protocol. All the extracted viral nucleic acids were examined by Nano drop instrument in order to determine the concentration and purity of viral RNA.

**Oligonucleotide primers:-**

FMDV Universal primers: 1F 5'-GCCTGGTCTTTCCAGGTCT-3', 1R 5'-CCAGTCCCCTTCTCAGATC-3' (Jamal *et al.*, 2011) were used for the one-step RT-PCR method. These primers amplified an 328-bp dsDNA amplicon.

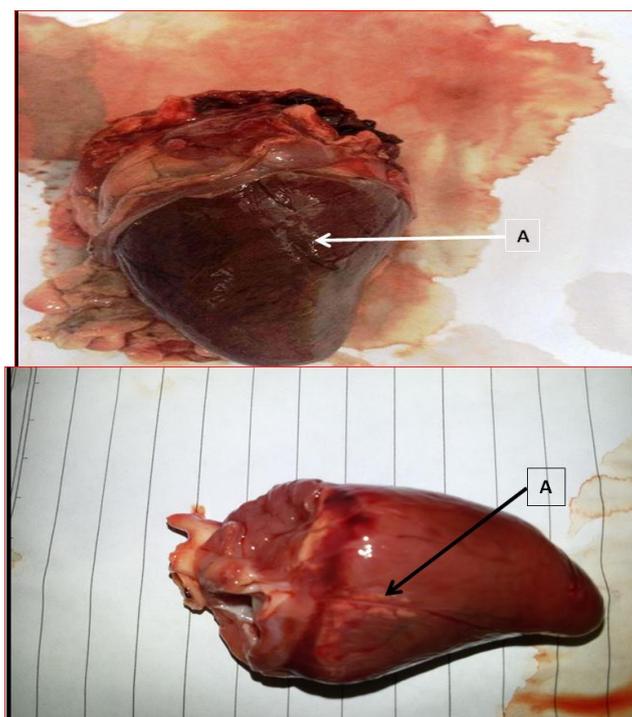
**Reverse transcription polymerase chain reaction (RT-PCR):-**

Published methods were used (Jamal *et al.*, 2011) for the detection of viral RNA of foot and mouth disease viruses by one step RT-PCR using AccuPower® RT-PCR PreMix Kit (Bioneer, South Korea).

**Statistical analysis:-** Analysis of statistics in the current study was applied according to (Leech *et al.*, 2007) and expressed as (mean $\pm$ SD). Since, the significance of variations between diseased and healthy lambs was evaluated via (SPSS) student *t*-test.

**III. Results**

Diseased lambs show different clinical manifestations such as signs of depression and dullness, with inactivity, mouth breathing with panting, animals are mostly recumbent and unable to suck their dams, However, some of diseased animals died soon within 24-72hrs, Since, macroscopic examinations of the autopsied dead lambs, exhibit necrotic myocarditis with enlargement of the heart and with different sizes of pale foci associated with a zone of hyperemia detected in different parts of heart tissue specially in the papillar and ventricular cardiac muscles.Fig 1.



**Fig 1: Necrotic myocarditis with enlargement of the heart and different sizes of pale foci associated with a zone of hyperemia detected in the papillar and ventricular cardiac muscles.**

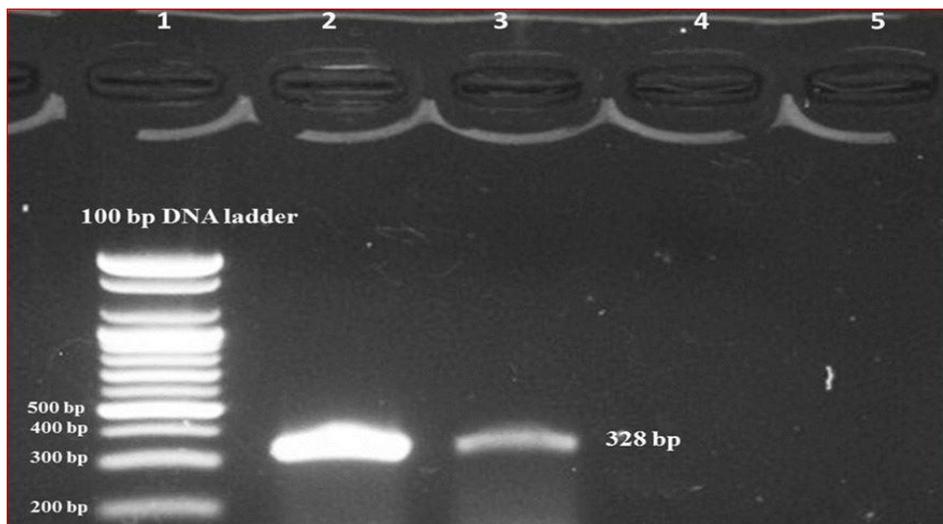
Data concerning clinical examinations of diseased lambs shows, A Significant increase ( $p < 0.05$ ) in the body temperature, respiratory and heart rate of diseased animals than in controls. Furthermore, abnormal cardiac sounds (organic murmurs) were indicated on auscultation of the heart (Table.1).

**Table.1: Body temperature, respiratory and heart rate of diseased lambs with FMD and controls.**

Parameters	Controls n=25	Diseased lambs n=100
Body temperature C°	38.7± 0.62	41.4± 0.63*
Respiratory rate/ mint	27.3± 3.58	63.6± 12.31*
Heart rate/ mint	103±4.78	156.5± 10.84*

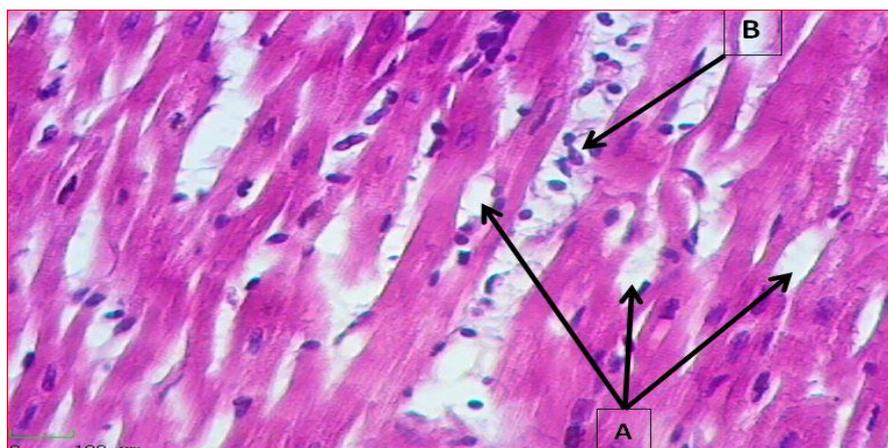
Values are mean ± standard error of mean. \* ( $P < 0.05$ ).

RT-PCR results on gel electrophoresis, show that the FMD virus has 328 base-pair FMDV specific PCR amplicons were detected (Fig.2).

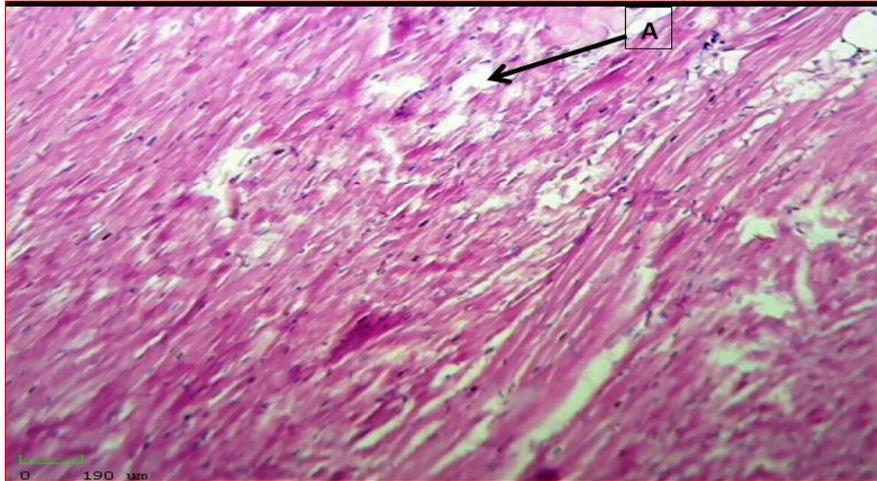


**Fig 2: RT-PCR results on gel electrophoresis, show that the FMD virus has 328 base-pair FMDV specific PCR amplicons were detected**

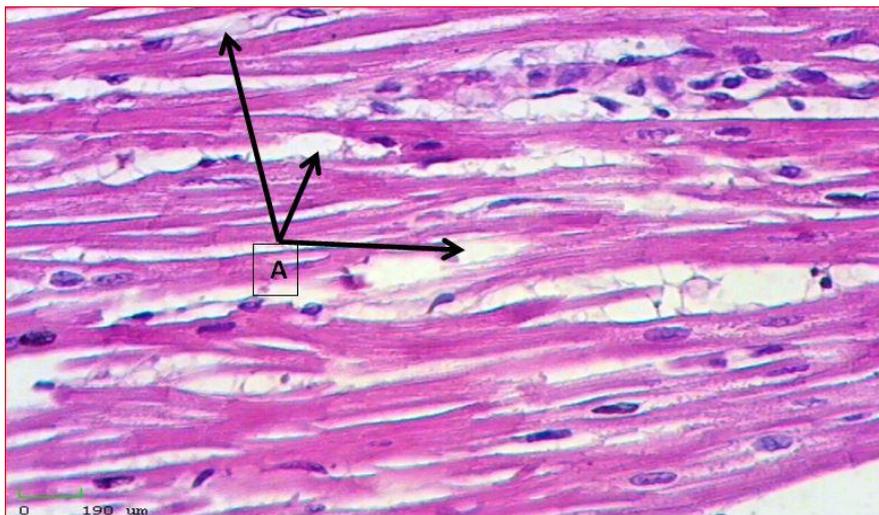
Moreover, on histopathological examinations there were severe inflammatory cells infiltration in the interstitium of myocardial fibers with obvious areas of coagulation of myocardial fibers (coagulative necrosis of myocardial fibers) and marked area of hyalinization, Furthermore, High number of vacuolated-degenerated myocardial muscle cells was found (Fig 3), Beside, A large areas of vacuolated degeneration myocardial muscle cells (Fig 4), and with a several foci of vacuolated-degenerated myocardial muscle cells had been detected (Fig 5), However, A several vacuolated-degenerated myocardial muscle cells with the possibility of interstitial cell edema was also detected (Fig 6).



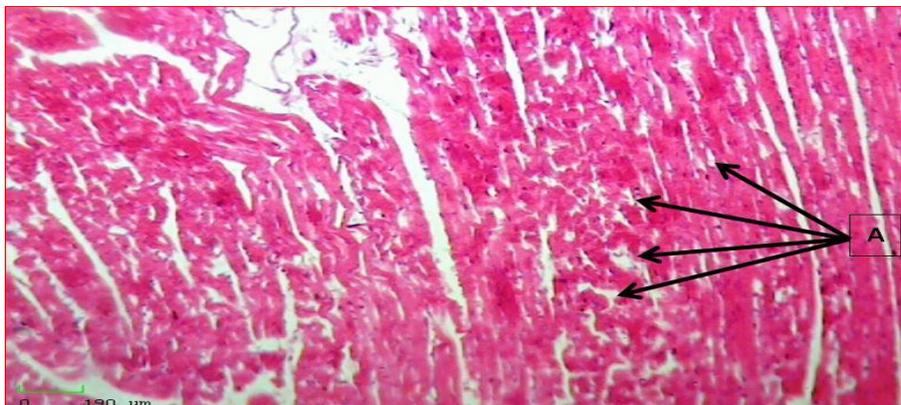
**Fig 3:High number of vacuolated-degenerated myocardial muscle cells (A) 40x Few mononuclear cells (B) 40x**



**Fig 4:**A large areas of vacuolated degeneration myocardial muscle cells (A) 10X



**Fig 5 :**Several foci Vacuolated-degenerated myocardial muscle cells(A) 40x



**Fig 6:**Several vacuolated-degenerated myocardial muscle cells with the possibility of interstitial cell edema (A) 10x

Results of hematological parameters indicated a significant increase ( $p < 0.05$ ) in ESR values of diseased lambs than in controls. Moreover, the total leukocyte count was increased significantly with significant lymphocytosis (Table. 2).

**Table 2: Hematological parameters of diseased calves with FMD and controls**

Parameters	Controls n=25	Diseased lambs n=100
RBC $\times 10^6$	7.91 $\pm$ 1.44	7.88 $\pm$ 1.63
Hb g/dl	13.22 $\pm$ 1.76	13.48 $\pm$ 2.65
PCV %	32.61 $\pm$ 4.76	33.12 $\pm$ 4.61
ESR mm/24h	11.14 $\pm$ 3.85	32.17 $\pm$ 6.88*
TLC $\times 10^3$	12.14 $\pm$ 1.53	14.56 $\pm$ 1.78*
Nutrophiles	5250 $\pm$ 421.15	5290 $\pm$ 363.21
Lymphocytes	5101 $\pm$ 362.63	8634 $\pm$ 111.54*
Monocytes	544 $\pm$ 375	539 $\pm$ 268
Eosinophiles	391 $\pm$ 26	392 $\pm$ 78
Basophiles	80 $\pm$ 65	79 $\pm$ 53

Values are mean  $\pm$  standard error of mean. \* ( $P < 0.05$ ).

Moreover, The results also show a significant increase ( $p < 0.05$ ) in values of serum cardiac Troponin-I (cTnI), Homocystein (Hcy), Creatine kinase-myocardial band (CpK-MB), and Lactate dehydrogenase (LDH) in diseased lambs with FMD than in controls (Table. 3) .

**Table 3: Serum cardiac troponin I (cTnI), Homocystein (Hcy) and enzyme activities of diseased lambs with FMD and controls**

Parameters	Controls n=25	Diseased lambs n=125
cTnI ng/ml	0.23 $\pm$ 0.07	25.83 $\pm$ 2.8*
Hcy $\mu$ mol/L	6.11 $\pm$ 0.47	18.22 $\pm$ 3.81*
CpK-MB U/l	69 $\pm$ 76	212 $\pm$ 53*
LDH U/l	132 $\pm$ 65	255 $\pm$ 67*

Values are mean  $\pm$  standard error of mean. \* ( $P < 0.05$ ).

The results of the acute phase response also indicated a significant increase ( $p < 0.05$ ) in both Haptoglobin values and Fibrinogen time in diseased lambs compare with controls (Table. 4).

**Table 4: Haptoglobin values and Fibrinogen time of diseased lambs with FMD and controls**

Parameters	Controls n=25	Diseased lambs n=100
Haptoglobin g/dl	0.018. $\pm$ 0.007	0.077 $\pm$ 0.019*
Fibrinogen / Sec	13.85 $\pm$ 10.47	42.45 $\pm$ 7.85*

Values are mean  $\pm$  standard error of mean. \* ( $P < 0.05$ ).

#### IV. Discussion

Foot and mouth disease is an important, highly contagious viral disease that affected domestic and wild ruminant, which lead to high mortality and high economic losses in young animals, Since, The classical clinical signs of FMD in sheep and goat are mostly mild and may go unnoticed, However, high mortalities due to the sudden death that occur in lambs due to myocarditis and cardiac degeneration or necrosis will indicated in most outbreaks (Glubahar *et al.*, 2007 .,Constable *et al.*,2017).

The cardiovascular system is a very important organ that plays a vital role in tissue functions of farm animals, the growth and high milk production depends on the function of this vivid system, As, The primary function of the cardiovascular system is to ensure an adequate circulation of blood so that nutrients are delivered and disbursing, waste products are eliminated, and a homeostatic environment is conserved at the organ and cellular level. An inadequate circulation interferes with nutrient delivery and waste product removal, and ultimately leads to circulatory failure, As, the primary concept in diseases of the cardiovascular system. (Andrews *et al* ,2008).

In the present study , diseased lambs show different clinical manifestations which are mentioned by others (Hammond , 2011., Constable *et al* ,2017., Pinto, 2017) such as signs of depression and dullness, with inactivity, mouth breathing with panting, collapse, fever, tachycardia, Moreover, animals are mostly recumbent and unable to suck their dams , In addition that, The characteristic lesions of FMD were not seen in any lambs that affected with myocarditis, However, myocarditis, were suspected by examining and indicated the organic murmurs through auscultation of the chest, Nevertheless, Diagnosis of FMD virus was confirmed by PCR analysis, Thereby, The final diagnosis of the myocarditis in suspected lambs due to FMD was done with the aid of histopathological changes, Biochemistry markers, acute phase response, respectively .

Myocarditis is an inflammation of myocardial muscle cells , However, there are a number of infectious and non-infectious agents that affected animals and result in myocarditis such as Viruses (FMD, canine parvovirus, encephalomyocarditis ), Bacterial (Clostridium spp , Mycobacterium spp), Protozoa (Toxoplasma gondii, Sarcocysti ssp., Neospora caninum), Deficiencies (vitamine E and selenium, copper and cobalt),

Rickettsia and spirochetes may also cause myocarditis by direct invasion or by their toxins (Zachary, 2017). In young lambs, FMD virus has a high affinity for myocardial muscle (cardiac tissue) and infection frequently cause death from acute myocardial damage and heart failure (Alexandersen *et al.*, 2003., Gulbahar *et al.*, 2007., Constable *et al.*, 2017)

Histopathological signs of dead or slaughtered lambs were indicated with several vacuolated-degenerated myocardial muscle cells with the possibility of interstitial cell edema and intense mononuclear cell infiltration, hyaline degeneration and necrosis, which consistent with the results of other studies, indicated by (Gunes *et al.*, 2005., Karapinar *et al.*, 2010).

A malignant form of the disease, without vesiculation, does occur in young animals and occasionally in adults. In these, death is common, as a result of myocarditis. Poorly defined pale foci of variable size are seen anywhere within the ventricular muscle. Although historically referred to as *tigerheart*, these gross lesions does not differ from those generated in any other syndrome of severe, acute myocardial damage. However, necrosis of fibers may be striking (Kostin *et al.*, 2003). Animals that survive the acute phase of FMD infection (or are not slaughtered during depopulation) can progress to develop a set of chronic lesions. Since, Chronic lesions include myocardial necrosis and scarring, heat intolerance, pancreatitis with acinar necrosis, and regeneration (Jamal and Belsham, 2013).

In the present work lymphocytosis has been indicated in diseased lambs, which was also mentioned by (Weiss and Wardrop, 2010). As, lymphocytosis might occur due to the stimulation of bone marrow responses and lymphoid system activity against any foreign parasite. However, lymphoid depletion and disorganization with massive lymphocytes could also expected. In addition that, Lymphocytosis in FMD were noted due to the formation of antibodies in response to FMD antigen (Knowles and Samuel, 2003).

A significant increase in the sedimentation rate of erythrocytes was also indicated in lambs suffering from FMD. Since, the erythrocyte sedimentation rate were indicated the generalized inflammation as the rate will increase due to any reason for inflammation. Therefore, when an inflammatory process is present, Fibrinogen enters the blood in high amounts and causes red cells to stick to each other, which raises the ESR (Reagan, *et al.*, 2008).

It had been documented that, the use of specific biomarkers for the evaluation of heart damage is important in the diagnosis of myocarditis. Therefore, In this study, plasma cardiac troponin concentration, Homocystain concentration, CpK-MB concentration, LDH concentration were measured. Serum cardiac troponins may be detected through myocardial damage cells in human and various animals, including dogs, cats, horse, and sheep as indicator of myocardial injury (Leonardi *et al.*, 2008). In addition to many diseases such as (idiopathic pericarditis, traumatic reticuloperitonitis and foot and mouth disease in young lambs) that cause increased concentration of circulating troponin (Gunes *et al.*, 2005). In the present study, serum cardiac troponin concentration, CpK-MB concentration, Homocystain concentration, LDH concentration in the diseased lambs with myocarditis were higher significantly than the values obtained from the non-myocarditis and healthy lambs. Cardiac troponin is a highly sensitive and specific for myocardial damage in different animals. However, It has been found that Cardiac troponin I levels in lambs with myocarditis due to FMD is higher than those healthy lambs. The same results were also documented in this study, which confirmed by (Leonardi *et al.*, 2008., Tunca *et al.*, 2008).

Troponins are protein molecules that are a part of cardiac and skeletal muscle. However, Smooth muscle cells do not contain troponins (Gunes *et al.*, 2005). Troponins are generally less detectable in healthy, normal animals, although this may eventually change as more sensitive assays become available. The absolute abnormal value varies depending on the clinical status in which the animal is evaluated and the test used. Troponins are released in response to myocardial harm, regardless of cause. Ischemia is the most common cause of cardiac muscle damage, and the initial tests were evolved as a marker to detect the presence of myocardial ischemia. However, the higher levels of troponin can occur in a lot of conditions other than ischemic damage (Leonardi *et al.*, 2008). It is proposed that there is a small cytosolic unit and a bigger muscular unit of troponins. During cardiac damage and depending on the intensity, troponins are released from both unit the small and the larger one. An initial small elevation occurs when troponins are released from the cytosolic unit, when troponin molecules in the cytosol of

Heart muscle diffuse towards the sarcolemma into the surrounding lymphatic system and blood vessels, will be detected in the blood. If the injury persists and necrosis progresses, further troponins are released from the muscular unit (Lim *et al.*, 2006). The troponin level may also elevate in the setting of a demand for an increased cardiac output and relatively inadequate myocardial blood flow. This can occur in specific conditions such as systemic inflammatory response, septic, hypotensive, and hypovolemic shock, as well as in cardiac arrhythmias, atrial fibrillation with rapid ventricular rate, and In animals with acute congestive heart failure due to etiologies even. Moreover, troponin levels may be rising because of the inability of the weak heart to maintain the proper coronary perfusion (Ammann *et al.*, 2001). Furthermore, Tachycardia from any etiology increases the cardiac oxygen demand. Therefore, it must be owing to reduced diastolic filling time, reduces

coronary perfusion and this relative supply, demand mismatch can cause myocardial injury to some extent, increasing the level of troponins (Tunca *et al.*, 2008).

Another type of biomarkers is the Homocysteine (Hcy) which is a thiol-containing amino acid produced by the intracellular demethylation of methionine and released into plasma where it circulates mostly in its oxidized forms bound to plasma proteins, Furthermore, It is known that blood homocysteine levels increase in heart and coronary diseases, myocardial infarction, peripheral vascular diseases, and intravascular thrombosis, Moreover, epidemiological studies have investigated the relationship between (Hcy) levels in blood and cardiovascular disease (Brattstrom and Wilcken, 2000). It had been postulated that the association between serum Homocystein and myocarditis still not well defined (Baszczuk and Kopczyński, 2014), On the contrary it had been proven that the determination of serum (Hcy) concentration in calves and sheep with FMD might useful as a guide to quantify cardiac associated heart damage (Kaya *et al.*, 2013), Where, homocystein is an intermediate amino acid in the methionine metabolism which is not present in proteins as hyperhomocysteinemia is an independent risk factor for cardiovascular disease and had a role in generation oxidative damage (Baszczuk and Kopczyński, 2014). Significant increase of Hcy was detected in diseased lambs in the present study, Moreover, it had been reported that plasma and heart tissue homocystein were higher in the acute nutritional muscular dystrophy caused by Vit.E / Selenium deficiency affecting the heart (Razaei and Dalir-Neghadeh, 2009).

It was reported that CpK-MB levels, as a marker of myocardial cell damage, have often increased in its range during the very early stage of myocarditis (Vishal *et al.*, 2012). Therefore, this result was indicated in the current study it could suggest that the stage of the disease is very early.

It was shown that Creatine phosphokinase-MB (CpK-MB) is the most specific indicator available for the diagnosis of an heart problems especially, acute myocardial infarction. The degree and the duration of Creatine phosphokinase -MB elevation in serum approximates the range of an acute myocardial infarction, although a different factors may affect the accuracy of such an index (Karapinar *et al.*, 2010). Differences in the analysis methods for the creatine phosphokinase isoenzymes have produced a clashing documentation as to the presence of CpK-MB in tissues other than myocardium and the release of CpK-MB under conditions other than an acute myocardial infarction. The embryological development of the CpK-MB isoenzymes, as well as the different conditions involving increase level of CpK-MB in serum might also deserve more attention, Moreover, The CK and isoenzymes found in the skeletal and heart muscles, and the brain are the an organ specific enzymes used in mostly in the clinical studies (Fredericks *et al.*, 2001). In the present study it was observed that the CpK-MB levels show a significant increase when compared with healthy controls, This finding corroborates the results reported for myocarditis in other studies, Since, It was considered that this fact was due to the effects of an ongoing increase CK activity as a result of muscular pathology that develops in myocarditis and other

Heart problems (Jafari *et al.*, 2014).

In the current study, it has been found that there is a significant increase in LDH level in the diseased lambs than in controls, It can be concluded that the increase of cardiac dysrhythmias in the myocardial diseased animals may be resulted from metabolic and electrolyte disorders, Since, LDH enzyme is found in most tissue such as heart, liver, erythrocyte, leukocytes, and kidney, Furthermore, it has been mentioned that, A single LDH molecule is a tetramer built of two subunits, subunit H which belong to the heart and subunit M which belong to the muscle, As, The fraction LDH1 consists of subunits H only, whereas the fraction LDH5 contains only subunits M. (Sobiech *et al.*, 2002). LDH catalyzes a reversible reaction of pyruvate conversion into lactate in the presence of NADH. Differences in the tissue activity of particular isoenzymes result from the fact that cathodic fractions (LDH4 and LDH5) catalyze this reaction when considerable amounts of lactate are accumulated (especially when the anaerobic metabolism dominates), Whereas anodic isoenzymes (LDH1, LDH2 and LDH3) can be found in tissues with aerobic pyruvate conversion in the tricarboxylic acid cycle (Valvona *et al.*, 2016).

The results of the present work indicated a significant increase in Haptoglobin and Fibrinogen reflected the increase of acute phase response. The acute phase response, is the aggregate of the systemic and metabolic alteration occurred by release of acute phase proteins in response to inflammatory stimuli, Moreover, Acute phase proteins are plasma proteins which become higher in its concentration following the infection, inflammation, or Trauma, Furthermore, they are a class of proteins whose plasma concentrations increase or decrease according to inflammatory responses. This response is called the *acute-phase reaction* or *response* (Guzelbektes *et al.*, 2010). The acute phase response is a complex reaction, involving local and systemic effects, As, One of these effects corresponds to changes in the concentration of some plasma proteins, basically synthesized in the liver, which are called acute phase proteins (APP). The APR is induced by protein hormones called cytokines acting as messengers between the local site of injury and the hepatocytes responsible for synthesizing the APPs, In addition, The Major Functions of acute phase response are, Opsonisation and trapping of microorganisms and their products, Inactivating complement system, binding cellular remnants, neutralizing enzymes, Scavenging free hemoglobin and radicals and Modulating the host's immune response (Cary *et al.*, 2009). Haptoglobin the glycoprotein acute phase reactant that binds to free hemoglobin and forms a stoichiometric

metrically stable complex (Jain *et al*,2011). The major biologic function of haptoglobin is to bind hemoglobin in an equimolar ratio with very high affinity to prevent hemoglobin-mediated renal parenchymal injury and loss of iron following intravascular hemolysis. In addition, Haptoglobin can also inhibit prostaglandin synthesis and is believed to have anti-inflammatory and antioxidant properties in the body (Cary *et al*, 2009). It has been also documented that, the Haptoglobin binds to free hemoglobin, with extremely high affinity probably the highest nature. However, Hemoglobin, is the richest source of iron in the body, The major hazard posed by iron and iron containing compounds is the formation of reactive oxygen species, As, Free hemoglobin also enhances the peroxidation of purified arachidonic acid and other polyunsaturated fatty acids within neuronal cell membranes, Furthermore, Iron released from heme proteins can catalyze oxidative injury to neuronal cell membranes and might have a role in post traumatic central nervous system (CNS) damage. Also, oxidation of low density lipoprotein is catalyzed by heme and leads to vascular endothelial cell damage and atherosclerosis. Haptoglobin, by binding hemoglobin and removing it from the circulation, prevents iron stimulated formation of oxygen radicals and has an important role as an antioxidant (Nazifi *et al*, 2011). On the other hand, Fibrinogen level was also significantly increased in diseased lambs, Since, Fibrinogen is a plasma protein that considers as an acute phase protein in most species, including cattle and sheep, Therefore, Evaluation of this protein was found to be particularly useful in detecting inflammatory diseases (Fasulkov *et al*, 2014).

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