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Original Article

Alteration in Clinical, Hemobiochemical and Oxidative Stress Parameters in Egyptian Cattle Infected with Foot and Mouth Disease (FMD)

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Abstract

The current study was carried out to study alteration in clinical, hemobiochemical and oxidative stress parameters in native cattle infected with foot and mouth disease (FMD). For this purpose twenty native cattle were used, divided into two equal groups: 10 apparently healthy cattle (control group), 10 cattle with FMD .All cattle were exposed to complete physical clinical examination, blood samples were taken. Hematological analysis revealed significant increase in MCV and MCH ($p \leq 0.001$); PCV ($p \leq 0.01$); while significant decrease in RBCs ($p \leq 0.001$) was ($p \leq 0.001$) in serum level of calcium, total protein and globulin; while significant increase in serum phosphorus ($p \leq 0.05$) and glucose levels ($p \leq 0.001$) were observed. Nitric oxide (NO), Malondialdehyde (MDA) and DNA fragmentation percentage were significantly increased ($p \leq 0.001$) in diseased cattle. However significant reduction in total antioxidant capacity ($p \leq 0.001$) and albumin as biomarker for antioxidant status were detected. The highest levels of MDA and NO indicate the occurrence of oxidative stress and lipid peroxidation. In conclusions cattle affected with FMDV experienced strong oxidative stress. So antioxidant drugs recommended during treatment of viral diseases as FMD.

Keywords: Clinical, hemobiochemical, oxidative stress, cattle, FMD, Egypt.

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Introduction

The field of oxidative stress in ruminant medicine is still in the early stages of development. There is a great deal to be discovered about its role in ruminant health and production (Celi, 2010). In the last few years the detection of free radicals damage and the body's defenses it have become increasingly important in clinical medicine as complementary tool in the evaluation of metabolic status of the animals (Castillo *et al.,* 2005). Research in oxidative stress has been associated in various pathological processes in veterinary medicine (Kataria *et al.,* 2010).Viral infection activates the immune system. It causes the release of reactive oxygen species (ROS) and reactive nitrogen species (RNS) with the potency of inducing oxidative stress (Zelnickova *et al.,* 2008). Foot and mouth disease (FMD) is the most contagious animal disease. It affects all the hoof stock animals (Alexandersen and Mowat, 2005). The disease is economically important as it causes heavy losses for the livestock industry in terms of high morbidity in adult animals, sharp reduction in milk production, weight losses, reproductive inefficiencies and death in young animals (Belsham, 2005). Infectious diseases generally activate macrophages to synthesize large quantities of nitric oxide (NO) that plays an important role as a defense mechanism (Rockett *et al.,* 2007). It has cytotoxic effects on these activators when synthesized in large quantities (Kandemir *et al.,* 2011). ROS and RNS are capable of degrading numerous biomolecules including protein and nucleic acid. In addition, it can attack the polyunsaturated fatty acids of membrane lipids causing lipid peroxidation and the disorganization of cell structure and function (Halliwell *et al.,* 1992). Lipid peroxidation is a well-established mechanism of cellular injury and is used as an indicator of oxidative stress in cells and tissues (Magni *et al.,* 1994). The most abundant lipid peroxide by product is Malondialdehyde (MDA) (Heidarpour *et al.,* 2013). It used as an inductive marker for oxidative damage (Kandemir *et al.,* 2011). The body minimizes the cellular effects of ROS by production of antioxidants which depleted with increasing of ROS production (Zalba *et al.,* 2006). Few studies have reported the alterations in clinical and biochemical parameters in cattle with FMD (Yeotikar *et al.,* 2003 and El-Saied *et al.,* 2007). Consequently the present work was carried out to investigate the effect of infection with FMDV on clinical, hemobiochemical and oxidative status in local cattle under the prevailing Egyptian field conditions.

Materials and Methods

Animals

Twenty native breed dry cattle aged 3 - 5 years, weighted 350-450 kg, its BCS 3-3.5 and feed on concentrate and green ration were used throughout this study. These cattle were belonging to different private farms in different localities in Egypt during FMD outbreak of 2012. These cattle were divided into two equal groups. The first group represented apparently healthy cattle (control) from all farms whereas cattle of the second group were affected with FMD (diseased). All animals were exposed to complete physical and clinical examination including (rectal temperature, respiratory rate and pulse rate) according to (Radostits *et al.,* 2007).

Samples

Blood samples were collected from the jugular vein into plain and EDTA vacationers both groups. Whole blood was used for determination of cellular blood constituents and blood indices. Sera was harvested from the plain tubes after centrifugation at 10 minutes at 3000 Xg and used for determination of the biochemical parameters.

The heart samples obtained at necropsy from five cattle which dead during the outbreak used for DNA fragmentation and compared with 5 apparently healthy slaughtered cattle (control group).

Hematological Analysis

The blood samples used to establish cellular blood constituents according to (Schalm *et al.,* 1986) and blood indices as described by (*Willard et al.,* 1989).

Serum Biochemical Analysis

The commercial Kits were used for determination of some biochemical parameters. The total protein, albumin, glucose, cholesterol, urea,

creatinine, calcium, phosphorus, Aspartate Aminotransferase, alanine aminotransferase, alkaline phosphatase, and Creatinine phosphokinas were measured. A selective chemistry analyzer (Abbott Alcyon 300I, USA) was used in measuring as previously described by (Grunwaldt *et al.*, 2005). All Kits were performed according to the manufacturer's recommendations.

Determination the Level of Serum Malondialdehyde (MDA)

MDA level was measured by the double heating method (Draper and Hadley, 1990). Briefly, 1.25 mL of 10% TCA and 0.25 mL of serum were added into tubes, and then the tubes were boiled for 15 minutes. The mixture was centrifuged at 3000 Xg for 10 minutes. One milliliters of the supernatant was taken and 0.5 ml of 0.675% TBA was added. The tubes were boiled for 15 minutes. The optical density was measured at 532 nm.

Determination the Level of Serum Nitric Oxide (NO)

Serum NO concentration was determined according to (Miranda *et al.*, 2001). Initially, serum samples were deproteinized with 30% zinc sulphate and serum nitrate was reduced to nitrite by vanadium (III) chloride. Total nitrite, an indicator of NO was then determined calorimetrically using Griess reagent by developing a purple color measured at 540 nm.

Determination the Level of Total Antioxidant Capacity (TAC)

It performed according to (Koracevic *et al.*, 2001) using kit supplied by Biodiagnostic- Egypt, and depending on colorimetric technique. It performed according to the manufacturer's recommendations.

Genomic DNA Fragmentation

Quantitation of DNA fragmentation was determined by colorimetric diphenylamine assay as described by (Shen *et al.*, 1992). Heart samples were lysed in hypotonic lysis buffer pH 8.0. Lysates were centrifuged at 13000 X g for 10 minutes and divided into two portions one for electrophoretic analysis using agarose gel electrophoresis. The second portion and pellet were precipitated with TCA and then were centrifuged. After centrifugation the supernatant containing small DNA fragments was separated from the pellet of intact DNA and divided into two portions one for electrophoretic analysis using agarose gel electrophoresis. The second portion and pellet were precipitated with TCA and then resuspended in two volumes of diphenylamine solution. Samples were stored at 4°C for 48 h and measured spectrophotometry at 578nm.

Statistical Analysis

All values are given as the mean ± S.E. Significant difference between the means of the two groups was statistically analyzed by independent t test and person correlation was carried out on diseased group. The significance levels was set at $P^* \leq 0.05$ for all the tests. Statistical analysis was performed using SPSS 16.0 software package (SPSS Inc., Chicago, IL, USA).

Results and Discussion

Characteristic signs of FMD as high fever, depression, dullness, anorexia, salivation, lameness and vesicular eruptions on buccal mucosa and inter digital space were observed in all infected animals. Cattle with FMD showed a significant increase ($P \leq 0.01$) in temperature, pulse rate and respiratory rate as represented in (Table 1).

Hematological analysis in FMD group revealed significant reduction ($P \leq 0.001$) in RBCs and significant increase in MCV, MCH ($P \leq 0.001$) and PCV ($P \leq 0.01$) as reported in (Table 2).

Serum biochemical analysis of FMD group revealed a significant reduction in total protein, albumin, globulin, calcium ($P \leq 0.001$) and cholesterol levels ($P \leq 0.05$) as detected in (Table 3). In addition a significant increase in glucose ($P \leq 0.001$) and phosphorus and AP levels ($P \leq 0.05$) were detected.

Serum analysis for oxidative stress parameters in FMD group revealed a significant increase in NO and MDA levels ($P \leq 0.001$) and significant reduction in TAC level ($P \leq 0.001$) (Table 4).

Significant differences were found in the percentage of DNA fragmentation ($p < 0.001$)

between cattle infected with FMDV and normal control group (Table 5).

Negative significant correlation between level of MDA and total protein and globulin levels were detected. Also there was negative correlation between albumin level (well known as antioxidant) and all oxidative stress biomarkers (MDA& NO) (Table 6).

Table 1: Physical clinical examination in the FMD and control groups.

Parameters	FMD group(n=10)	Control group(n=10)
Temperature(°C)	39.50 ± 0.23**	38.76 ± 0.13
Pulse rate (/ min)	66.9 ± 2.31**	51.30 ± 1.90
Respiratory rate(/min)	22.8 ± 1.28**	18.10 ± 0.82

*Denote means values significant at $P^{*} \leq 0.05$, $P^{**} \leq 0.01$, $P^{***} \leq 0.001$.

Table 2: Hematological parameters in the FMD and control groups.

Parameters	FMD group(n=10)	Control group(n=10)
RBCs($\times 10^6$/µl)	4.09 ± 0.22***	5.50 ± 0.28
PCV(%)	33.70 ± 0.66**	31.50 ± 0.98
Hb(g/dl)	10.89 ± 0.24	10.39± 0.33
MCV(f)	83.81 ± 3.17***	59.02 ± 4.31
MCH(pg)	27.21 ± 1.33***	19.47 ± 1.44
MCHC(g/dl)	32.40 ± 0.82	32.98 ± 0.07
WBCs($\times 10^3$/ µl)	5.99 ± 0.14	5.88 ± 0.09

*Denote means values significant at $P^{*} \leq 0.05$, $P^{**} \leq 0.01$, $P^{***} \leq 0.001$.

Table 3: Serum biochemical parameters in the FMD and control groups.

Parameters	FMD group(n=10)	Control group(n=10)
Total protein (g/dl)	4.15 ± 0.28***	8.06 ± 0.32
Albumin (g/dl)	2.15 ± 0.19***	4.95 ± 0.25
Globulin (g/dl)	2 .00 ± 0.23***	3.11 ± 0.32
Glucose(g/dl)	70.44 ± 3.47***	52.36 ± 2.62
Cholesterol (mg/dl)	177.96 ± 5.53*	192.95 ± 3.92
BUN (mg/dl)	28.10 ± 0.56	28.82 ± 0.54
Creatinine (mg/dl)	3.14 ± 0.19	3.08± 0.16
Calcium (mg/dl)	7.87 ± 0.30***	9.71 ± 0.41
Phosphorus (mg/dl)	7.68 ± 0.90*	4.85 ± 0.26
AST(U/L)	93.91 ± 6.14	102.33 ± 5.58
ALT (U/L)	22.31 ± 2.04	22.31 ± 2.04
CPK (U/L)	244.47 ± 51.37	129.90 ± 21.21
AP(U/L)	86.32 ± 2.53*	76.95 ± 1.90

*Denote means values significant at $P^{*} \leq 0.05$, $P^{**} \leq 0.01$, $P^{***} \leq 0.001$.
Blood urea nitrogen (BUN), Aspartate Aminotransferase(AST), alanine aminotransferase(AST), alkaline phosphatase (AP), and Creatinine phosphokinase (CPK).

Table 4: Oxidative stress parameters in the FMD and control groups.

Parameters	FMD group(n=10)	Control group(n=10)
TAC (m mole/L)	0.250 ± 0.02***	0.463 ± 0.05
NO (µ mole/L)	38.58 ± 1.43***	19.99 ± 1.87
MDA (n mole/L)	11.33 ± 0.88***	6.86 ± 0.60

*Denote means values significant at $P^{*} \leq 0.05$, $P^{**} \leq 0.01$, $P^{***} \leq 0.001$. TAC: Total antioxidant activity NO: Nitric oxide MDA: Malondialdehyde.

Table 5: Effect of FMD infection on the percentage of DNA fragmentation.

Parameter	FMD (n=5)	Control (n=5)
DNA fragmentation %	42.6±3.3***	26.9±4.2

*Denote means values significant at $P^{*} \leq 0.05$, $P^{**} \leq 0.01$, $P^{***} \leq 0.001$.

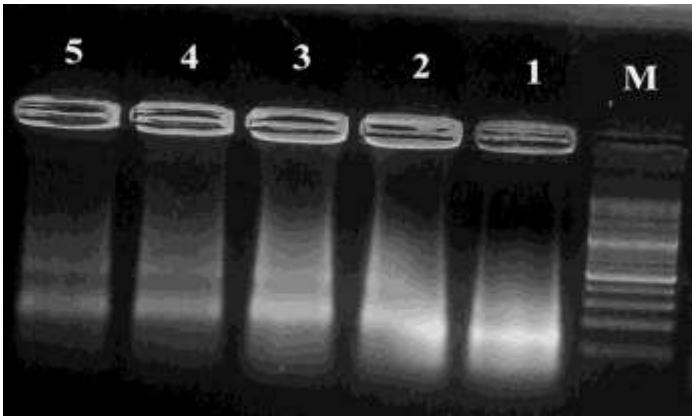

Fig. 1: which represent the infected group was increased in compared to lanes 4 and 5 which represent the control one.

M: 100bp DNA marker, lanes 4 and 5 represent normal heart tissue 1, 2and 3 represent the infected heart tissues. The level of DNA fragmentation (smear) were apparently observed in lanes 1, 2 and 3.

Table 6: Person correlation between oxidative stress biomarkers and some biochemical parameters in FMD affected cattle.

Parameter	TAC (m mole/L)	NO (µ mole/L)	MDA (n mole/L)
Total protein (g/dl)	-.527-	0.541	-.777**
Albumin (g/dl)	.369	-.052-	-.340-
Globulin (g/dl)	-.321-	.679*	-.638*
Cholesterol (mg/dl)	-.096-	-.353-	-.311-
Glucose(g/dl)	-.024-	0.155	0.57
Calcium (mg/dl)	-.211-	0.464	-.387-
Phosphorus (mg/dl)	-.192-	0.444	-.111-
Creatinine (mg/dl)	0.508	0.203	-.054-
BUN (mg/dl)	-.271-	-.475-	0.037
ALT (U/L)	-.287-	0.302	0.174
CPK (U/L)	-.363-	0.049	-.401-
AP(U/L)	0.027	-.029-	0.611

Denote means values significant at $P^{*} \leq 0.05$, $P^{**} \leq 0.01$, $P^{***} \leq 0.001$.

Discussion

FMD is well known for the classic manifestations from which the name of the disease has been derived. Characteristic clinical signs of FMD were observed in all infected.

Animals with significant increase in temperature, pulse rate and respiratory rate which in agreement with (Lubroth, 2002 and Radostits *et al.,* 2007). The Alteration in hematological parameters in FMD group come in accordance's with findings were recorded by (Maddur *et al.,* 2008 and Ghanem and Abdel-Hamid, 2010).These alteration may be attributing to endocrinopathy (Radostits *et al.,* 2007). The significant reduction in total protein, albumin, globulin, calcium and cholesterol detected in our investigation were in agreement with (Ghanem and Abdel-Hamid, 2010).

Hypoproteinemia and hypoalbuminemia could be resulted from severe anorexia and off food. The reducing level of globulin indicates the immunosuppressive effect of FMDV. Hypocholesterolemia may be due to dysfunction in β cell from the pancreas induced by FMDV replication. Hypocalcemia could be attributed to hypoproteinemia resulting in decrease protein bounded calcium. Hyperglycemia and hyperphosphatemia were similar as that reported by (Yeotikar *et al.,* 2003 and Ghanem and Abdel-Hamid, 2010). Hyperglycemia may be attributed to implication of FMDV in the development of type 1diabetes through direct destruction of pancreatic B cell or through the autoimmune response (Clark, 2003).

ROS and nitrogen metabolites play a complex role in many infectious diseases; such metabolites influence the growth of viruses (Peterhans, 1997). We observed significant increases in MDA level which in agreement with (Ghanem and Abdel-Hamid, 2010). Macrophages, neutrophils and other phagocytic cells considered as the potent cells of immune response against viral and microbial infections. Those cells generated large amounts of ROS and RNS that considered as the main cause of lipid perixidation (Bozukluhan *et al.,* 2013) Lipid peroxidation is used as an indicator of oxidative stress in tissues. A significant high level of nitrate, an indicator of NO production suggests that FMDV induce the production of NO. This result agreed with previous studies on FMD in cattle (*Gulbahar et al.,* 2007and Bozukluhan *et al.,* 2013). Antioxidants play an important physiological role counteracting free radicals and preventing cellular damage. Because of the difficulty in measuring each antioxidant component separately and their interaction in the plasma, several methods have been developed to assess total antioxidant capacity (TAC). The measure of antioxidant capacity considers the cumulative action of all the antioxidants present in plasma and body fluids (Ghiselli *et al.,* 2000). The decreased level of TAC in our study was in accordance with the results reported by (Bozukluhan *et al.,* 2013). This reduction is related to overproduction of ROS leading to occurrence of oxidative stress. Oxidative stress has been implicated as major initiators of tissue damage and can affect enzymatic activity, signal transcription and gene expression, especially apoptotic gene (Sen and Packer, 1996). This may be the main cause for high DNA fragmentation percentage reported in our study in infected cattle. This result was confirmed by (Gulbahar *et al.,* 2007). The negative correlation between albumin level MDA and NO may be due to its antioxidant properties of albumin (Castillo *et al.,* 2005). This antioxidant function of albumin is attributed to multiple ligand-binding capacities and free radical trapping properties of it (Oettl and Stauber, 2007 and Guidet, 2009).

In conclusions, the results encountered in the present study revealed that cattle affected with FMDV experienced strong oxidative stress. The periodic assessment of oxidative status in ruminants is necessary to enhance body defense system for diseases, including FMD. So antioxidant and immune stimulant drugs recommended during treatment of viral disease as FMD.

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