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Full Length Research Paper

The Lysosomal Enzymatic Activities and the Digestive Disorders on Foot-Mouth Disease "FMD" in Egyptian Dairy Cows; Buffalo; Sheep "Impaction, Diarrhea and liver Cirrhosis" of Goats in Serum

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Abstract
It was investigated the activities of some lysosomal enzymes in serum of "FMD" in Egyptian dairy animals both vaccinated and infected. Twenty cows; Buffalo, and sheep were surveyed for estimation the activity of lysosomal acid hydrolases. Results revealed that ACP was highly increased in both vaccinated and infected sheep as well as vaccinated cows, while the activity reduced in infected cows and Buffalo. β-NAG exerted lower in Buffalo and cows either in vaccinated or infected, on the other hand, β-GAL appeared to be less in buffalo and cows either vaccinated than infected. As revealed, the activity was altered in either vaccinated or infected. β-NAG exerted a highly activity in the infected more than vaccinated for sheep. Also, ACP approved to be highly activity in vaccinated cows than infected. β-GAL in sheep exerted a highly % in cows either vaccinated or infected as compared to control dairy cows. Also, It was found that these enzymes under investigation in goats having diarrhea and liver cirrhosis diseases showed that ACP have the highest activity, also, β-GAL and β-NAG appeared to be less increased as compared to control group. Results indicated that the activity of ACP much higher in β-GAL, then β-NAG. It was concluded that the enzyme activity appeared to be highly increased in sheep and buffalo more than in cows either vaccinated or infected. Also, the effect appeared to be enzyme dependent in the case of digestive disorders. So, this effect was dependent on the animal species and a type of enzyme

Key words: *Foot-Mouth Diseases, Lysosomal enzymes, dairy Cows; buffalo; sheep and goat, liver cirrhosis, diarrhea diseases.*

Introduction

Lysosomes are organelles central to degradation and recycling process in animal cells, whether the lysosomal activity is coordinated to respond to cellular needs remains unclear (Sardiello et al., 2009). Lysosomes have a central role in cellular homeostasis as sites for digestion of foreign materials and for degradation of intracellular components undergoing autolytic processing (De Duve, 1983; Bhagavan, 2002 and Meijer and Codogno, 2009). In many pathological conditions, changes in the lysosomes take place, the loss of integrity of the lysosomes membrane and subsequent discharge of enzymes into the blood stream is a characteristic feature of hepatic diseases (Stvolinskaya et al., 1992 and Premalatha and Sachdanandam, 2000). The intracellular release of the lysosomal enzymes precedes cellular death by initiating the cellular injury process ultimately causing tissue necrosis. The compounds which antagonize the effect of labializes and prevent or reduce the release of the lysosomal enzymes are designated stabilizers (De Duve, 1963 and Abdel Gawad et al., 2005).

FMD is often confused with foot and mouth (also called hoof and mouth) disease, a disease of cattle, sheep and swine, however the two diseases aren't related and they are caused by different viruses (Chhabra et al., 2004 and Knox et al., 2005). Human don't get the animal disease, and animals don't get the human disease (NCIRD, 2008). Foot and mouth disease is a highly contagious and sometimes fatal viral disease of cloven-hoofed animals, including domestic animals such as cattle, water buffalo, sheep goats and pigs. It is caused by foot and mouth disease virus (Bhattacharya et al., 2005 and Martinez-Salas et al., 2008). Rafiei et al. (2015) found that the biochemical consequences of gene mutations in different patients of disease have served to reinforce the relevance of the pathways to pathogenesis, previously characterized, for example mitochondrial dysfunction, oxidative stress and protein misfolding and aggregation.

Foot-Mouth disease "FMD" is an extremely contagious viral disease of cloven hoofed animals that can lead to huge economic losses in the livestock production. No viral therapies are available for treating FMD virus "FMDV" infections in animals. The affected animals suffered from fever associated with depression, anorexia, loss of appetite, and excessive salivation, mouth and feet lesions which were attributed to exotic FMDV serotype A, where recognized picture of FMD clinical symptoms was noticed in January 2006. Followed this outbreak, many foci were appeared frequently with long or short intervals was investigated by (Ali et al., 2006; Martinez et al., 2008; Lu et al., 2007 and Vagnozi et al., 2007). The most affective FMD vaccines consisted of chemically inactivated FMDV and can only offer complete protection after seven days of vaccination because of the time needed to trigger an immune response (Grubman, 2005). It has been proposed that a combination of vaccine and antiviral agents can be more efficacious strategy to treat FMD-infected animals, limiting the spread of the disease and reduce the number of animals that need to be slaughtered during outbreaks (James and Rushton (2002); Lembo and Cavalli (2010); Ding et al. (2013); Ali et al. (2006) and Khadelwal et al. (2014)). It was provide evidence for necrosis near the center of the lesion and apoptotic-like cell death border, but in non-autophagy cells (Puyal et al., 2009). Recent evidence suggests that lysosomal storage impairs autophagy resulting in accumulation of polyubiquitinated proteins and dysfunctional mitochondria, ultimately leading to apoptosis (Teser et al., 2009).

The aim of this study was to investigate the effect of foot and mouth disease "FMD" in Egyptian dairy cows, Buffalo and sheep on the three lysosomal enzymatic activities of "ACP, β-GAL, and β-NAG" in serum both infected and vaccinated animals. Also, the activity of the lysosomal enzymes under investigation in serum of dairy animals of the digestive disorder of sheep and goats "Impaction, diarrhea and liver cirrhosis" were investigated.

Materials and Methods

Materials

All chemicals and reagents used in this experiment were purchased in pure and analytical grads from Sigma. Co.U.S.A.

First experimental animals

Twenty Cows, buffalo and sheep were supported from specific farm of Faculty of Veterinary Medicine, Zagazig University, (EGYPT). Dairy cows and buffalo ranged between 350-430 kg each, and sheep ranged 60 kg each (Age of animal ranged from 1-3 years), all animals were selected and fed on base diet containing wheat bran, cotton seed cake, yellow corn, molasses, sodium chloride (common salt), calcium carbonate (Lime salt) and crude protein. After 21 days, blood samples were taken from specific vein of each animal, sera separation to estimate the three lysosomal enzymatic activities and to describe the clinical findings.

Animal were divided into different groups:

a. Control groups "non-treated" including three groups from cows, buffalo and sheep (healthy).
b. Vaccinated animals "cows, buffalo and sheep" (Abdalla et al., 1993).
c. Infected animals.

Blood samples were collected from infected and vaccinated animals for sera separation and kept under -20 C until the estimation of the lysosomal enzymatic activities "ACP, β-GAL and β-NAG.

Second Experimental animals

Twenty goats average ranged 60 kg each, 10^{th} of them were healthy and the other 10^{th} were diseased (Abdalla et al., 1993), after 21 days blood samples were withdrawn of each animal and allowed to coagulate and then centrifuged at 3000rpm/20min, the sera separation were kept under -20 C until the estimation of the lysosomal enzymatic activities ACP, β-GAL and β-NAG.

The lysosomal enzymatic activities of ACP, β-GAL and β-NAG has been measured spectrophotometrically according to the method described by Van Hoof and Hers (1968) with some modification by Younan and Rosleff (1974), (Nermien, 2011 and Fouad et al., 2012) using different substrates (Koch-Light) for the following acid hydrolases:.

Acid hydrolase enzyme	Substrate
Acid phosphatase (Orthophosphoric-monoester-phosohohydrolases (ACP) *EC. 3.1.3.2*	p-nitrophenyl phosphate sodium salt pure
β-Galactosidase (β-GAL) *EC. 3.2.1.23*	p-nitrophenyl-β-D-galactopyranoside
N-acetyl-β-D-glucosaminidase (β*NAG) EC. 3.2.1.30*	p-nitrophenyl-2-acetamido-2-deoxy-β-D-glucopyranoside

Statistical Analysis

Levels of significance of different between means of treated samples and control were statistically evaluated by the use of the non-paired students (t) test (Goldstein, 1969).

Results and Discussion

The present study was performed to evaluate the behavioral characterization of the three lysosomal enzymes in serum of the Egyptian dairy cows, buffalo and sheep such as acid phosphatase "ACP"; β-galactosidase "β-GAL", and β-N-acetyl glucosaminidase "β-NAG" in both vaccinated and infected animals. FMDV is known to be present in EGYPT, the strategy most commonly adopted in order to control the reading of the disease through vaccination three times yearly. Foot-Mouth disease (FMD) is a modifiable in most countries of the world, and any clinical suspicion of diseases should be reported to the appropriate authorities. The disease occur in most of the major livestock producing countries of the world, except North America, Central America, Australia, New Zealand, Japan and Ireland (Bhattacharya et al., 2005). A vaccination program for FMDV in order to be successful requires that a high proportion of the susceptible population in the area where the program is carried out with vaccine strains that match the circulating serotypes. This high level of immunity needs to be maintained in order to have a strong immune pressure on the virus. It appears evident that in order for a vaccination program against FMD to be efficacious the veterinary services need to carry out the program in an efficient way.

The lysosomal enzymes have been considered as the marker enzymes of the hepatic lysosomes by virtue of their presence in surplus amounts not only in secondary but also in primary lysosomes (Barret and Health, 1977). Also, these lysosomal enzymes are very important for liver lysosomal functions (De Roberts and De Roberts, 1980). As revealed from the results in Table (1) and Figure (1) that the activity of β-NAG appeared to be highly in the infected animals by 4691.5, 6077.3 and 5350.9 nmole/ml/hr for cows, buffalo and sheep respectively than in vaccinated animals by 4583.5, 747.0 and 1227.3 nmole/ml/hr respectively. β-GAL activity revealed the lowest values in vaccinated than in the infected animals by 842.4, 524.24 and 666.70 and 594.0, 907.6 and 1257.6 nmole/ml/hr for cow, buffalo and sheep respectively. Acid phosphatase "ACP" activity exerted a moderate effect then β-NAG and β-GAL activities, while the activity of the ACP in vaccinated animals appeared to be highly than the infected animals by (1263.6, 1203.0 and 1330.4) and (643.6, 785.7 and 998.9) nmole/ml/hr for cow, buffalo and sheep, respectively.

The percentage change of the enzymatic activities of these lysosomal enzymes seemed to be variable according to the effect of vaccinated and infected animals. The labilizing effect on the membrane of lysosomes may be due to increase in the synthesis by rough endoplasmic reticulum. Also, it may be due to an induction by regulatory genetic coding, which was accompanied by elevation in the enzymatic activities and the labialization which mediated by prostaglandin synthetase (Hope and Welton, 1983; Teleb et al., 1990 and Abdel Gawad et al., 2005).

Also, it was investigated that the membrane that surrounded cell and cell organelles such as lysosomes contain large amounts of polyunsaturated fatty acids so, they are a target for lipid peroxidation by free radicals to produced the cell injury and oxidative stress for many diseases. The oxidation of these biological membranes by the free radicals leads to a decrease in membrane fluidity and disruption of membrane structure and function as labialization and stabilization of membrane permeability (Haragushi et al., 1997).

These enzymes have been considered as the marker enzymes of the hepatic lysosomes by virtue of their presence in surplus amounts not only in secondary but also in primary lysosomes (Barret and Health, 1977). Also, these lysosomal enzymes are very important for liver lysosomal functions (De Roberts and De Roberts, 1980). Furthermore, activation of lysosomal proteases, nucleosides and other lipases cause degradation of biological molecules and subsequently cell death (Kappus, 1985 and Teleb et al., 2006). It was found that the increase in lysosomal activity and autophagosome formation together demonstrate increased autophagy, which occurred mainly in the border of the lesion, suggesting its involvement in delayed cell death. Also, it is provide evidence for necrosis near the center of the lesion and apoptotic-like cell death in its border, but in non-autophagic cells (Puyal et al., 2009).

Hence control of the disease relies on slaughter of the exposed animals and vaccination with chemically inactivated FMD vaccines. However, these vaccines typically provide protection against one or few of the 60 different FMDV serotypes. Moreover, they are unable to induce protection prior to 7 days post vaccination following the acute phase of FMDA infection in ruminant some animals may have experience a long asymptomatic persistent infection (Eble et al., 2006). In addition, animals which have been successfully vaccinated may also become persistently infected if exposed to infections virus (Grubman and Baxt, 2004). Following the acute phase of FMDV infection in ruminants some animals may experience a long asymptomatic persistent infection. These animals are referred to as carrier animals and the carrier state is a complication which can occur during outbreak situations (Grubman and Baxt, 2004).

Oxidative lipid damage, referred to lipid peroxidative, produces a gradual loss of cell membrane fluidity, reduces membrane potential and increase permeability to ions like Ca^{+2}. Oxidative stress has been proposed to be involved in the pathophysiology of many chronic diseases and viral infection is known to accelerate the aging process (De la Fuentes and Victor, 2000). For these reasons of membrane permeability, the lysosomal enzymes in drug-induced hepatotoxicity were significantly increased as compared with normal control (Nermien et al., 2009). As well as, it was investigated that the cell death is independent of autophagy and caspase activation. Instead, the drug-induced cell death are associated with large increases in autophagy lysosomes number and size which leads to lysosomal membrane permeability with a resulting leakage of hydrolases into the cytosol, which are then directly involved in cell death (Jahreiss et al., 2009).

It was found that autophagy, was conserved mechanism for lysosomal degradation of cytoplasmic components, and it has received much attention recently owing to its importance in tissue remodeling and innate immunity, and because it has been proposed that autophagy protects against cancer and neurodegenerative disease (Rusten and Stenmark, 2009).

Also, the activity of these lysosomal enzymes as a marker in liver "ACP, β-GAl, and β-NAG" were studied in serum of animals " goats" for looking at the effect of digestive disorders, impaction, diarrhea and liver cirrhosis on the activity of the lysosomal enzyme permeability and release in serum of animal. As revealed in Table (2) and Figure (2) that the three lysosomal enzymes under investigation appeared to be enhanced by a variable percentage change. Acid phosphatase activity exerted a highly significant increase of the enzyme release by 89.7% then, β-GAl activity by 13.7%, while the enzyme activity of β-NAG exerted a lowest activity increase by 6.0%. These results indicated that ACP enzyme was most pronounced activity for labilizing effect then β -GAl and β –NAG, so the labialization effect approved to be high in ACP> β-GAL> β-NAG. This may be due to the effect of the type of enzyme dependent.

It was found that, continued oxidation of fatty acid side chains of the lysosomal membrane and their fragmentation to produce aldehydes and hydrocarbons will eventually lead to loss of membrane integrity. Rupture of the membranes of lysosomes will spill hydrolytic enzymes into the rest of the cell to cause damage amplification (Dotan et al., 2004).

Beal (1998) and Beal (2005) found that the products of lipid peroxidation from the membranes can inhibit the protein synthesis and the activity of certain enzymes. At the liver injury, oxidative stress produced a potential contributor to the development of complications for increase free radical production or reduced antioxidant defense response. Consequences of oxidative stress are adoption or cell injury i.e. damage to DNA, proteins and lipids disruption in cellular homeostasis and accumulation of damage molecules Jakus (2000). The elevation levels of lysosomal enzymes may be due to increase in the levels of prostaglandins E_1, E_2, $F_{2\alpha}$, and D_2 in liver, kidney and brain (Rajakrishnan et al., 2000).

Table 1: Effect of Foot-Mouth disease (FMD) of Egyptian dairy Cows, Buffalo and Sheep in serum on the three marker lysosomal acid hydrolases enzymes as: Acid phosphatase (ACP), β- Galactosidase (β-GAL) and β-N- acetyl glucosamonidase (β-NAG) after (30) minutes of incubation period *in-vitro*.

Lysosomal enzyme	Control of dairy cows	Control of dairy buffalo	Control of dairy sheep	The lysosomal enzymatic activities by nmole/ml/hr (Mean ±S.E)(n=10)					
				Vaccinated			*Infected*		
				Cow	Buffalo	Sheep	Cow	Buffalo	Sheep
β-NAG	4438 ± 1.90	5336 ± 0.09	616.7 ± 0.011	4583.5 ± 0.065	747.0 ± 0.013	1227.3 ± 0.036	4691.5 ± 0.31	6077.3 ± 0.060	5350.9 ± 0.150
% Change				*↑3.3%†*	↓86.0%*	*↑99.0%**	*↑5.7%†*	↑13.9%†	*↑767.7%**
ACP	228 ± 15.6	340.6 ± 0.81	146.9 ± 0.006	1263.6 ± 0.051	1203.0 ± 0.02	1330.4 ± 0.068	643.6 ± 0.046	785.7 ± 0.020	998.9 ± 0.091
% Change				*↑454.2%**	↑253.2%*	*↑806.0%**	*↑182.3%**	↑130.7%*	*↑580.2%**
β- GAL	384 ± 17.0	420.9 ± 0.011	98.89 ± 0.003	842.4 ± 0.0043	524.24 ± 0.02	666.7 ± 0.007	594.0 ± 0.028	907.6 ± 0.023	1257.6 ± 0.041
% Change				*↑119.4%**	↑24.6%†	*↑574.2%**	*↑54.7%**	↑115.6%*	*↑1171.7%**

** Significant: P<0.05: as compared with control group; † Insignificant: p>0.05: as compared with control group.*

Conclusion

It was concluded that the lysosomal enzymatic activities of ACP, β-GAL, and β-NAG of serum either infected or vaccinated animals appeared to be variable according to the enzyme type and the animal species. Also, the activities of the lysosomal enzymes were enhanced at the inflammation of liver cirrhosis and diarrhea of animals. These activities of the enzymes were altered according to the enzyme dependent.

Ethics

All the authors read and approved the manuscript and no ethical issues involved.

Table 2: The lysosomal Enzymatic activities of serum "ACP, β-GAL, and β-NAG" on digestive disorders of goats "Impaction, Diarrhea and liver Cirrhosis.

Acid hydrolases	The enzyme activities as nmole/ml/hr. (Mean ± S.E.)		
Source of serum samples	**Acid phosphatase (ACP)**	**β- Galactosidase β-GAL**	**β-N-acetylglucosaminidase β-NAG**
Serum samples of healthy sheep and goats	146.85 ± 0.0061	98.89 ± 0.0034	616.68 ± 0.011
Serum samples of Diseased sheep and Goats	278.57 ± 0.0066	112.43 ± 0.0045	653.77 ± 0.0099
% Change	↑ 89.7% *	↑13.7 %†	↑6.0 %†

% change in lysosomal enzymatic activities contents from corresponding initial estimates control.
** Significant: P<0.05 and † Insignificant: p>0.05: as compared with control group.*

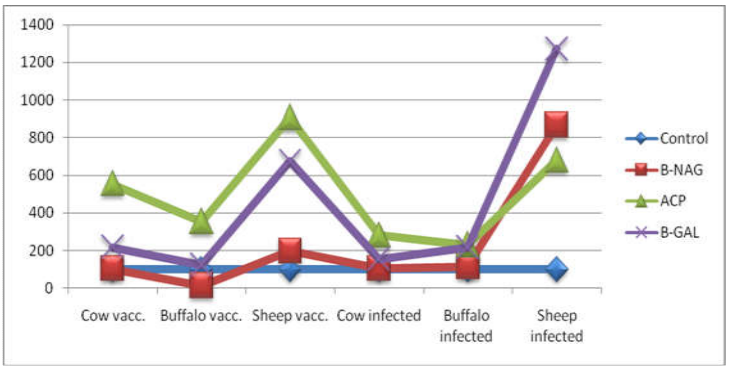

Fig 1: Relative change % of the effect of Foot-Mouth disease (FMD) in Egyptian dairy Cows, Buffalo and Sheep on serum lysosomal acid hydrolases.

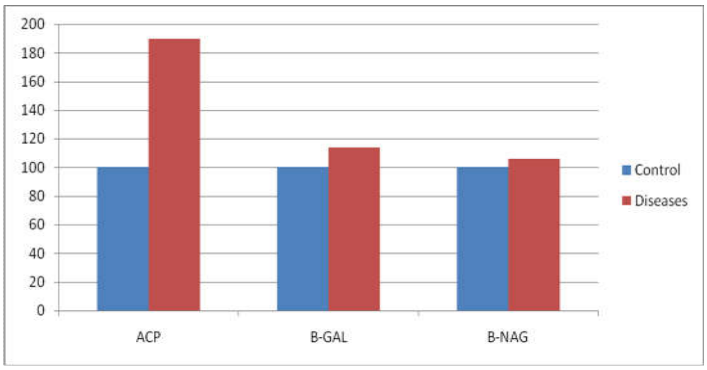

Fig 2: Relative change % of the effect of in Egyptian dairy Goats on serum lysosomal acid hydrolases as compared to control group.

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