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Serum Concentration of Some Inflammatory Cytokines, Chemokines and Proteins in Holstein Dairy Cows Affected with FMDV

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Abstract

Foot and mouth disease (FMD) is one of the most contagious diseases affecting cloven hoofed livestock. In order to evaluate serum concentration of some inflammatory mediators which may contribute in FMD pathogenesis, total number of thirty Holstein dairy cows was selected during FMD outbreak in Egypt (summer, 2022) to carry out this study. Fifteen of them were affected with FMD and the remaining was healthy. Serum samples were obtained and analyzed to detect the level of some biomarkers which included tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), interleukin-6 (IL-6), interleukin-4 (IL-4), interleukin-10 (IL-10), interferon-gamma (IFN- γ), in addition to serum level of serum amyloid A protein (SAA), mannose binding lectin (MBL), lactoferrin and monocytes chemo-attractant protein-1 (MCP-1). All the previously listed mediators were significantly elevated in serum of FMD-affected cows, as compared to healthy cows, except the serum level of IL-4 and IL-10; their concentration was significantly decreased in the affected cows. In conclusion, these cytokines, chemokines and proteins are involved and play a key role in pathogenesis of FMD.

KEYWORDS
FMD, Cattle, Cytokines, Chemokines, Pathogenesis.

INTRODUCTION

Foot and mouth disease (FMD) is classified as one of the most serious viral communicable diseases which affect both of domesticated and wild cloven- hoofed animals (Blancou, 2002). The causative agent is FMD virus which has seven main serological types (O, A, C, Asia1, SAT1, SAT2 and SAT3) and more than 65 alternative serotypes, so vaccination against one type does not offer immunity against the others (Biswal *et al*., 2014). Clinical Signs of the disease include fever, anorexia, and decreased milk yield, in addition to the characteristic blister on different body areas as, mouth, tongue, teats and between toes (Baluka, 2016). The disease is endemic in Africa and Middle East (Constable *et al*., 2016), and it is a very contagious disease, as the virus is ultimately presents in all types of excretions and secretions of the affected animals, on the other hand, it can spread directly and indirectly and even through air route (Poonsuk *et al*., 2018). The hostile site for primary FMD virus replication is thought to be pharyngeal mucosa, and then it is transferred through blood vessels and lymphatics to secondary sites of replications as oral mucosa; as a result, viremia will occur (Alexandersen *et al*., 2001). Diagnosis of the disease depends mainly on clinical signs, but reverse transcriptase-polymerase chain reaction (RT-PCR) can confirm the presence of the disease (the most accurate method for early diagnosis). The disease-related mortalities are low in adult animals (Admassu *et al*., 2015), but it usually cause massive economic losses due to decreased animals productivity (Lubroth, 2002). Serum analysis is very important tool as it help in understanding of diseases pathogenesis, but, literature about immune-inflammatory molecules and their probable role during course of FMD in cattle is very scarce, so, this study aimed to elucidate the alterations in serum concentration of some inflammatory cytokines, chemokines and inflammatory proteins in FMD-infected dairy cows and their relation with pathogenesis and clinical presentation of the disease during FMD outbreak in Egypt (Summer, 2022).

MATERIALS AND METHODS

Animals

During FMD outbreak in Egypt during May 2022, thirty Holstein cows aged between 4-6 years from a private dairy farm on desert road of Cairo-Alexandria Road were selected to accomplish this study. Fifteen cows were affected with FMD (FMD-affected group) based on clinical signs which include feet and mouth vesicular lesions, lameness, fever, rope salivation, anorexia, tachycardia and recumbency in some cases. These clinical signs were clearly obvious on the animals about 2-3 days before sampling. The other fifteen cows were apparently healthy without any signs

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of those mentioned above (Control group). All the animals were tested for presence of antibodies against non-structural protein (NSP) of FMD virus in serum using rapid screen test (Herdscreen®, GlobalDX, UK) to confirm infection. According to farm records, vaccination program against FMD was applied regularly.

Sampling

From both two groups, blood samples were drawn from tail vein in plain vacutainer tubes, samples were left to coagulate for 30 minutes and then centrifuged for 10 minutes at 3000 rpm to separate serum. Serum aliquots were kept frozen at -20°C till analysis time.

Sero-typing of FMDV

FMD virus serotype was detected using ELISA FMD diagnostic kits (antigen detection), (I.Z.S.L.E.R, Bresica, Italy), using serum according to manufacturer instructions. According to this test, all the tested animals were infected with O serotype of FMDV.

Serum immune-biochemical analysis

Highly specific enzyme linked immune-sorbent assay (ELISA) kits were used to evaluate serum level of TNF-α, IL-1β, IL-6, IL-4, IL-10 (Abcam, USA) , IFN- γ (BIO-RAD, USA), lactoferrin (Bethyl Laboratories, USA), serum amyloid-A protein (Abbexa, UK), MBL (Maxisorp; Nunc, Roskilde, Denmark) and MCP-1(RayBiotech, USA), with the aid of an automatic micro-plate reader (Stat Fax, USA).

Statistical analysis

The statistical differences in means of the serum concentration of the tested parameters between both two groups were measured using independent samples t-test using SPSS 16.0 for windows.

RESULTS

As shown in Table 1, serum concentration of TNF-α, IL-1β, IL-6 and MCP-1 was significantly elevated ($P < 0.01$) in FMD-infected group when compared to control group. Table 2 illustrated that serum concentration of IFN- γ showed a significant increase ($P < 0.01$) in diseased animals when compared to control group. On contrary, serum level of anti-inflammatory cytokines (IL-4 and IL-10) recorded a significant decrement ($P < 0.01$) in the affected animals as compared with control group. Also, the level of evaluated inflammatory proteins (SAA, MBL and lactoferrin) in serum of infected cows was increased significantly ($P < 0.01$) in comparison with the control group as present in Table 3.

DISCUSSION

FMD could be considered as one of inflammatory disease (Shang *et al*., 2017), inflammation is the expected response of the tissues to any injury, which followed by an increase in vascular permeability, blood flow, accumulation of inflammatory fluids, leukocytes recruitment, along with release of inflammatory mediators. These mediators are generated in a signaling cascade in response to micro-organisms invasion (Rosenbloom *et al*., 2005) to activate tissues resident cells (as mast cells and macrophages), call up the other immune cell (as neutrophils, monocytes and lymphocytes) and/or produce systemic response to inflammation (as leukocytosis, fever and synthesis of acute phase proteins) to finally get rid of the causative agent (Gallin *et al*., 1992). Selectively from these mediators, pro-inflammatory cytokines as TNF-α, IL-1β and IL-6 are released firstly to mediate acute inflammatory reaction (Feghali and Wright, 1997), activate both cellular and humoral immune response including lymphocyte

Table 1. Mean values of some inflammatory cytokines and chemokines among healthy and FMD affected animals.

	Healthy cows (Mean±SE)	FMD-affected cows (Mean±SE)	*t-value*
TNF-α (pg/ml)	119.53±5.99^{b}	218.13±10.59^{a}	8.10**
IL-1β (pg/ml)	5.01±0.40^{b}	14.43±1.34^{a}	6.74**
IL-6 (pg/ml)	142.80±5.74^{b}	232.13±10.87^{a}	7.26**
MCP-1 (pg/ml)	29.60±2.58^{b}	71.97±3.93^{a}	9.02**

Means within the same row of different superscript litters are significantly different at ($P < 0.01$); **: Significant at ($P < 0.01$)

Table 2. Mean values of interferon gamma, IL-4 and IL-10 among healthy and FMD affected animals.

	Healthy cows (Mean±SE)	FMD-affected cows (Mean±SE)	*t-value*
INF- γ (pg/ml)	22.20±2.15^{b}	57.47±3.33^{a}	8.89**
IL-4 (pg/ml)	438.80±20.60^{a}	196.13±22.90^{b}	7.87**
IL-10 (pg/ml)	43.33±3.37^{a}	25.20±2.74^{b}	4.17**

Means within the same row of different superscript litters are significantly different at ($P < 0.01$); **: Significant at ($P < 0.01$)

Table 3. Mean values of some inflammatory proteins among healthy and FMD affected animals.

	Healthy cows (Mean±SE)	FMD-affected cows (Mean±SE)	*t-value*
SAA (µg/ml)	12.57±1.28^{b}	35.03±2.27^{a}	8.62**
MBL (µg/ml)	14.50±1.58^{b}	33.53±2.33^{a}	6.76**
Lactoferrin (µg/ml)	9.93±1.10^{b}	22.50±2.22^{a}	5.07**

Means within the same row of different superscript litters are significantly different at ($P < 0.01$); **: Significant at ($P < 0.01$)

proliferation, antibody production in B cells, and production of acute phase proteins by hepatocytes, in addition to hypothalamic fever reaction (Zlotnik and Yoshie, 2000). On the other hand, IL-1β can stimulate histamine release from mast cells, which in turn increase vascular permeability which may share in vesicles formation in case of FMD. Also, both of IL-1β and TNF-α have the ability to increase IL-6 release (Warren, 1990). Additionally, oxidative stress state which has been proved to present during FMD infection (Khoshvaghti *et al.* 2014; Soltani *et al*., 2020) may share also in release of pro-inflammatory cytokines (as IL-6 and TNF-α) which modulate immune response to destroy causative agents (Lange *et al*., 1998). The former explanations may elucidate the reason of the increased serum level of these pro-inflammatory cytokines in FMD-infected cows. Interferon-γ is produced from antigen-stimulated T-lymphocytes (in response to presence of viral antigen) (Billiau and Matthys, 2009), and this may illustrate its increment in FMD infected animals. Interferon-γ may inhibit some virus replication through down regulation of viral genes transcription process and/or weakening of expressed viral genes stability (Kang *et al*., 2018), on the other hand, interferon-γ may prevent shedding of the virus from infected cells to protect the further infection of unaffected host cells (Mikloska and Cunningham, 2001). Acute phase proteins including SAA and MBL-1 can be produced in response to wide variety of stimuli including TNF, IL-1β, IL-6 and IFN-γ (Edbrooke and Woo 1989; Edbrooke *et al*.1991). SAA could prolong the lifespan of polymorph nuclear cells (PMNs) through inhibition of their apoptosis, which may prolong the participation of these cells in inflammatory process (El Kebir *et al*., 2007). In the same consistence, MBL is one plasma proteins which is produced by liver as a part of acute phase response (Thiel *et al*., 1992). It plays a central role in the innate immunity process as it helps in opsono-phagocytosis and activation of lectin complement pathway for elimination of microorganisms (Eisen and Minchinton, 2003), so, their level would increase in case of FMD. Lactoferrin present in almost all of mucosal secretions, in addition to neutrophils secondary granules (Ammendoliaa *et al*., 2007), increased concentration of lactoferrin in FMD-infected cows may be due to its antiviral activity against single stranded RNA naked viruses as Picornaviridae family viruses, which have been studied recently, as lactoferrin can inhibit virus replication through intracellular delivery of zinc ions after its saturation with these ions, this process could impair virus replication (Marchetti *et al*.,1999). In addition, lactoferrin would modulate immune and inflammatory process (Legrand *et al*., 2005) and increase the release of pro-inflammatory cytokines including (IL-6 and TNF-α), which recorded a significant increase in our study (Machnicki *et al*., 1993). MCP-1 is mainly secreted from epithelial cells, endothelium, smooth muscle, monocytes and fibroblast (Cushing *et al*., 1990; Standiford, *et al*., 1991) to direct migration of memory T-cells and macrophage to site of infection to promote immune response and inflammation (Deshmane *et al*., 2009) in response to oxidative stress evoked during the course of the inflammatory disease (Kumar *et al*., 2016; Singh *et al*., 2021) and this may justify its increase in serum of infected cows. Unfortunately, serum level of IL-4 and IL-10 was decreased in infected cows, this could occur due to effect of interferon-γ (IFN-γ), as it can inhibit production of anti-inflammatory cytokines including IL-10 (Hu *et al*., 2006) and IL-4 (Feghali and Wright, 1997), which are usually produced to inhibit progressive inflammation and tissues injury, and this may exacerbate the disease.

CONCLUSION

In conclusion, several inflammatory cytokines, chemokines and proteins may participate fundamentally in progression of FMD pathogenesis and occurrence of its related inflammatory lesions. Some of these molecules may share in virus clearance, and the others may exaggerate inflammation. Further studies are required to detect the role of the other inflammatory mediators in pathogenesis of FMD in cattle.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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