

Investigation of Levels of Haptoglobin, Serum Amyloid A, Ceruloplasmin and Albumin in Cattle with Foot-and-Mouth Disease

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ABSTRACT

In this study, it was aimed to determine haptoglobin, serum amyloid A (SAA), ceruloplasmin and albumin levels in cattle with foot-and-mouth disease (FMD). Twenty cattle with FMD and 10 healthy female Brown Swiss cattle were used in this study. Blood samples were collected from the Jugular veins into plain tubes and then used to prepare serum samples. These serum samples were used to measure haptoglobin, SAA, ceruloplasmin and albumin levels colorimetrically. Serum haptoglobin, SAA and ceruloplasmin levels were significantly higher and albumin levels were lower in cattle with FMD compared to those obtained from the control group. The degree of the changes in acute phase proteins were found to associate with the severity of the clinical symptoms observed in cattle with FMD. In conclusion, acute phase proteins such as haptoglobin, SAA and ceruloplasmin synthesis were increased, while albumin synthesis was decreased in response to acute phase responses develop in cattle with FMD.

Keywords: Acute Phase Proteins; Cattle; Foot-And-Mouth Disease.

INTRODUCTION

Foot-and-mouth disease (FMD) is an acute viral disease, which affects domestic and wild cloven-hoofed animals and causes weight loss and decline in milk production. The disease is highly contagious and also has zoonotic features (1, 2). Because it is a zoonosis, professional groups such as veterinarians, animal keepers and breeders, butchers and herders are at risk (3). Following entrance through the mucosa of digestive system and respiratory system and damaged skin, the virus forms primary vesicles in the primary entry site and then forms secondary vesicles in oral mucosa, nail skin, nipple and vulva after being transported hematogenously during the viremic period. The myotropic virus settles in the heart and skeletal muscle especially in young animals, and leads to death due to myocarditis and acute heart failure (3-5).

The proteins that are synthesized by the liver in response to stimuli such as inflammation, tissue damage, infection and neoplastic growth that lead to acute phase response (APR) are referred to as acute phase proteins (APP) (6). While the serum concentrations of some APPs increase during APR, others decrease (6, 7). Although the serum concentrations vary according to species, their levels generally begin to rise within first 4-6 hours of stimulation, reaching peaks in 24-48 hours and gradually decreasing to normal levels within 4-7 days (6, 8). Important APPs for cattle are haptoglobin and serum amyloid A (SAA) (7, 9). In several studies, APPs were reported to be used in discrimination between bacterial and viral infections, differential diagnosis of clinical and subclinical diseases, in determining the prognosis in sick animals and in monitoring patients (6, 8, 10).

Although there are many studies (11-17) on hematological and biochemical changes in cattle with FMD, there are limited numbers of studies on APPs (18, 19). In this study, it was aimed to determine the levels of APPs in cattle with FMD. We believe that the obtained data will contribute to better understanding of the pathophysiology of the disease in cattle with FMD.

MATERIALS AND METHODS

Animals

Twenty cattle from the Kars region in Turkey with FMD at 1-2 years of age and 10 healthy female Brown Swiss cattle at similar ages which served as controls were used in this study.

It was learned from the anamnesis of the animals with FMD used in the study that there has been loss of appetite, stagnation and excessive saliva production for about 3-5 days. The clinical examination revealed that there were vesicles and erosions in the interdigital space and on the tongue, with high fever, loss of appetite, excessive secretion and drooling of stringy or foamy saliva. Additionally, samples obtained from sick animals were sent to the Republic of Turkey Ministry of Food, Agriculture and Livestock; Institute of FMD for the investigation of the agent and strain.

Samples

Blood samples obtained from Jugular veins of animals were collected, centrifuged at 3000 rpm for 10 minutes and the obtained sera were stored at -20°C until analyzed.

Determination of acute phase protein levels

SAA levels were measured by ELISA (Serum amyloid A assay, Tridelta Development Limited, Ireland) and haptoglobin levels were measured (Haptoglobin assay, Tridelta Development Limited, Ireland) colorimetrically based on hemoglobin binding capacity (Epoch, Biotek, USA). Ceruloplasmin analysis was performed according to p-phenylenediamine oxidase activity method reported by Colombo and Ricterich (20), and albumin levels were measured spectrophotometrically (Epoch, Biotek, USA) using a commercial test kit (Erba Diasis Diagnostic Systems Inc., Turkey).

The cases were classified as symptomatic and the severity of the disease was determined. In the classification, high fever, mouth smacking, drooling of stringy saliva, and lesions between the mouth and nail were considered. Those with

Table 1. Mean values and standard error of the means (mean±SE) of acute phase proteins in clinically healthy and animals with foot-and-mouth disease (FMD)

Acute phase proteins	Control (n=10)	Moderately infected (n=10)	Severely infected (n=10)	P
Haptoglobin (g/L)	0.084±0.006 ^a	0.308±0.043 ^b	0.387±0.035 ^c	<0.01
SAA (µg/mL)	4.86±0.30 ^a	28.80±3.37 ^b	45.44±8.77 ^c	<0.01
Ceruloplasmin (mg/dL)	7.76±0.59 ^a	9.66±1.64 ^a	15.98±2.19 ^b	<0.01
Albumin (g/dL)	3.43±0.15 ^a	3.39±0.25 ^b	2.49±0.49 ^c	<0.05

^{a,b,c}: The groups in the same line labeled different letters are statistically significant (P<0.05, P<0.01).

all of the symptoms were assessed as severe, and those with one or some of these symptoms were classified as mild to moderate.

Statistical analysis

SPSS (21) for Windows 20.0 was used in the analysis of the study data. Kolmogorov-Smirnov test was used for assessing the normality of distribution. As the groups were normally distributed, ANOVA and Tukey multiple comparison test was used for comparison of the groups. The significance level was set at p≤0.05.

RESULTS

The clinical examination of animals with FMD revealed deterioration of general condition, loss of appetite, blisters inside the mouth and between nails, and stringy drooling saliva. A-NEP-84 (Nepal-84) type viruses were isolated from samples that were sent to the Republic of Turkey Ministry of Food, Agriculture and Livestock; Institute of FMD.

The mean haptoglobin levels were found as 0.308±0.043 g/L and 0.387±0.035 g/L in moderately infected and severely infected cattle with FMD, respectively. Serum haptoglobin and SAA (P<0.01) concentrations were significantly high in both moderately and severely infected cattle compared to those obtained in control group. Both haptoglobin and SAA concentrations in severely infected cattle were also higher than those of moderately infected cattle. Whereas ceruloplasmin concentrations in severely infected cattle were significantly higher than in concentrations obtained from control group (P<0.01). On the other hand, albumin concentrations were significantly lower in both moderately

and severely infected cattle compared to those of control group ($P < 0.05$). Results of the present findings indicated that the level of the increases in serum concentrations of haptoglobin, SAA and ceruloplasmin and decrease in albumin concentrations were associated with the severity of the clinical symptoms as obtained more severe alterations in severely infected animals than moderately infected cattle with FMD (Table 1).

DISCUSSION

In agreement with literature (5, 22), deterioration of general condition, loss of appetite, blisters inside the mouth and between nails, and stringy drooling saliva were found on clinical examination of cattle with FMD. A-NEP-84 type virus was identified from samples that were sent to the Institute of FMD. For the first time FMD A-NEP-84 type virus was classified as being epidemic in Turkey and Kars province. Nazifi *et al.* (19) have reported that the virus type influenced the response to viral virulence and clinical symptoms in addition it could lead to different interferon responses.

Inflammation, tissue damage and infection lead to an APR and APPs synthesized in the liver. While the serum concentrations of some infectious or non-infectious APPs increase, others decrease (6, 8).

Haptoglobin is composed of two subunits, namely α and β . Haptoglobin is either absent or present in small amounts (< 0.1 g/L) in the serum of healthy cattle (23). Haptoglobin concentrations of 0.2-0.4 g/L has been defined as indicative of a mild reaction while 1-2 g/L as severe infection (24). Serum haptoglobin levels were reported to be used in determining the prognosis of disease, and serum levels of 0.1-1 g/L were referred to as animals with a good prognosis and levels of > 1 g/L with a poor prognosis (23, 25). As haptoglobin levels were 0.308 ± 0.043 g/L and 0.387 ± 0.035 g/L in this study, all of the infected cattle with FMD were considered to have good prognosis.

As SAA levels increase in 2-5 hours and reach peak within 24 hours, it can be used in the earlier diagnosis of acute patients (26, 27). SAA levels were reported to increase in bacterial (28, 29) and viral (30, 31) infections, and starvation for more than 3 days (32). Ceruloplasmin is an α -2 globulin comprising a single polypeptide chain. Ceruloplasmin, which is used less than haptoglobin and SAA in cattle, is considered as an indicator of infection (33, 34). In a study in cattle with

FMD, Höfner *et al.* (18) detected increased haptoglobin levels. Nazifi *et al.* (19) have reported that increased levels of haptoglobin, SAA and ceruloplasmin in cattle with FMD. Similarly, haptoglobin, SAA and ceruloplasmin levels were found to be increased in this study. The likely reason for increased APPs is thought to be related to increased activity of phagocytic cells which are important components of the host immune response and to the extent of tissue damage.

Albumin, which is a negative APP, has been reported to decrease during intestinal malabsorption and during APR and starvation due to reduced synthesis in the liver (8, 35, 36). Albumin levels were significantly decreased compared to the control group also in this study and this was thought to be due to APR, liver dysfunction and/or anorexia in animals.

In conclusion, it was determined that haptoglobin, SAA and ceruloplasmin levels were increased and albumin levels were decreased in cattle with FMD in this study. Additionally, parallel to the severity of symptoms, the increases in serum haptoglobin, SAA, ceruloplasmin levels were found to be meaningfully higher in severely infected cattle than moderately infected cattle with FMD. It appears to indicate the relation between the severity of clinical diseases and the level of alterations in APP.

We consider that the data obtained from this study might contribute to the understanding of the pathophysiology and diagnosis of the disease. We suggest that more detailed studies are needed on this subject.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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