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Investigation of Serum Insulin and Cortisol Concentrations in Foot and Mouth Disease- Infected Cattle in Relation to Changes in Serum Biochemical Variables and Protein Electrophoretic Fractionation Profile

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Abstract: The present study was conducted to monitor serum levels of insulin and cortisol in foot and mouth disease-infected cattle (FMD) in relation to possible alterations in some biochemical variables and protein electrophoretic fractionation pattern. The study was carried out on two groups of cattle: one group of 15 naturally FMD infected cattle and another group of 10 healthy cattle were used as control. Evaluating parameters included serum levels of insulin, cortisol, total protein (TP), albumin (Alb), glucose, cholesterol, calcium (Ca), phosphorous (P), blood urea nitrogen (BUN), creatinine, serum enzymatic activities of alanine amino transferase (ALT) and aspartate amino transferase (AST) as well as serum protein electrophoresis. Results showed a significant increase in serum levels of glucose, cholesterol, phosphorus, AST and cortisol and a significant decrease in serum concentration of total protein, calcium and insulin. Serum protein electrophoretic fractionation showed a significant decrease in albumin and gamma globulins. There was a significant negative correlation between insulin and serum levels of glucose, cholesterol, phosphorus and cortisol and a significant positive correlation with serum levels of calcium and total protein. Serum cortisol concentration was positively correlated with serum levels of glucose, phosphorus and AST and was negatively correlated with albumin, calcium and insulin. Our results indicated that FMD infection in cattle results in hypoinsulinemia and significant increase in serum cortisol levels. Further, alterations in the biochemical variables and protein electrophoresis pattern seen in FMD-infected cattle are likely seem to be related to changes in serum concentrations of insulin and cortisol providing an importance of considering these hormones when interpreting blood biochemical changes in cattle infected with FMD. Finally, the present data may provide a better understanding of the disease process and clinical pathology of FMD in cattle.

Key words: Cattle · Insulin · Cortisol · Foot and mouth disease · Hypocalcemia · Hyperglycemia · Protein electrophoresis

INTRODUCTION

Foot and mouth disease (FMD) also known as aphthous fever is a highly communicable disease and one of the most serious livestock diseases that affects all cloven-footed domestic and wild animals including cattle, buffalo, camels, sheep, goats, deer and pigs [1, 2]. It is caused by one of the smallest disease producing viruses known as Aphthovirus or foot and mouth diseases virus (FMDV) which is a member of the Family Picornaviridae [2]. The disease is characterized by blister-like lesions on the tongue, nose, lips, in the mouth, on the teats and between the toes which then burst, leaving painful ulcers. Affected animals usually have high fevers, stop eating, give less milk and become lame [3, 4]. On most continents, cattle are usually the most important maintenance hosts for FMDV, but some virus strains are primarily found in pigs, sheep or goats [2, 5]. FMD can cause severe problems for animals with cloven hooves with the potential of causing severe economic losses and trade disruptions in animals and animal products [5]. Many studies have addressed the cellular and humoral basis of immunity to FMDV or the influence of infection on the regulation of the immune response [6-8].

Few studies have studied the hematological and biochemical changes associated with FMD infection in cattle [9-11] but to the best of our knowledge effect of FMD on serum concentrations of insulin and cortisol as

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well as serum profile of protein electrophoresis in FMD infected cattle are not well documented. Consequently, the present investigation aimed to monitor the alterations that may occur in serum insulin and cortisol levels in FMD infected cattle in relation to possible changes in some biochemical variables and serum profile of protein electrophoretic fractionation in these animal species.

MATERIALS AND METHODS

Cattle: Two groups of cattle were used in this study, one consisted of 15 naturally affected FMD cases showed characteristic clinical signs of FMD based on routine clinical examination and observation of the characteristic lesions which included vesicles on the feet, in and around the mouth and on the mammary gland. In addition, affected animals showed depression, anorexia, excessive salivation and lameness. Animals without characteristic lesions for FMD were not used in the study. The other group consisted of 10 being clinically healthy cattle were used as controls.

Blood Samples: Blood samples were collected from the animals of both groups and serum samples were separated and stored at -20°C until used for different assays described in this study.

Evaluated Parameters

Serum Biochemical Variables: Serum samples were evaluated for the concentration of total protein (TP), albumin (Alb), glucose, cholesterol, calcium (Ca), inorganic phosphorus (P), blood urea nitrogen (BUN), creatinine and serum enzymatic activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Total globulin was determined by subtracting albumin from total protein. All biochemical parameters were determined by spectrophotometric method using commercial kits supplied by Randox (Randox Laboratories Ltd, Crumlin, Co. Antrim, UK).

Hormonal Assays: Serum levels of insulin and cortisol were determined by ELISA technique using kits of Hellabio biokits company (USA) and following the manufacturer's instructions.

Serum Protein Electrophoresis: Electrophoretic separation of serum proteins was accomplished by an Polyaccrylamide Gel Electrophoresis using kits of Cobasintegra company (Roche, Germany) and following the manufacturer's instructions.

Statistical Analysis: All the values were presented as mean± standard deviation (SD). Mean values of FMD infected group and the control group were compared by Student's t-test at 0.05 level of probability [12]. Correlations between the monitored variables were determined with Pearson's simple correlation method. A difference was considered significant at $P < 0.05$.

RESULTS

Serum Biochemical Parameters: Table 1 reveals that there was a significant decrease in serum total protein ($P<0.05$) in FMD-infected group compared to the control one. Comparison of the mean values for blood glucose between the two groups showed a significant increase ($P<0.05$) in glucose level in the FMD-infected cattle. The mean values of serum cholesterol were significantly higher ($P<0.05$) in the FMD-infected group. Serum calcium concentration showed a significant decrease ($P<0.05$) in the FMD-infected cattle while, serum phosphorus was significantly increased ($P<0.05$). The mean values of serum BUN and creatinine in FMD-infected and healthy cattle were similar. Comparison of the mean values of serum enzymatic activities of ALT and AST demonstrated a significant increase ($P<0.05$) in serum AST and no significant changes were seen in serum ALT activity.

Hormonal Assays: Table 2 indicates that the mean values of serum insulin levels were significantly lower ($P<0.05$) in the FMD-infected group while serum cortisol levels showed a significant increase ($P<0.05$).

Serum Protein Electrophoretic Fractionation: Table 3 demonstrates that the major changes observed in the electrophoretic pattern of FMD infected cattle were the significant decrease in albumin, total globulins and gamma globulins ($P<0.05$).

Table 1: Some serum biochemical parameters in the FMD- infected cattle compared to the control group. (Values are means ± SD)

Variable	Control group (N=10)	FMD- group (N=14)
Total protein (g/dl)	7.85±0.11	6.58±0.07*
Glucose (mg/dl)	62.43±5.53	106.37±7.94*
Cholesterol (mg/dl)	125.40±17.09	196.12±21.83*
Calcium (mg/dl)	13.43±2.18	10.95±1.51*
Phosphorous (mg/dl)	5.30±0.30	6.39±1.13*
BUN (mg/dl)	17.57±1.41	18.18±0.73
Creatinine (mg/dl)	1.39±0.21	1.43±0.30
ALT (U/l)	58.27±1.32	57.17±3.14
AST (U/l)	137.00±4.95	153.84±6.30*

Significant differences in the values between the FMD and control groups were indicated by * $P < 0.05$.

Table 2: Serum insulin and cortisol concentrations in the FMD- infected cattle compared to the control group. (Values are means ± SD)

Hormone	Control group (N=8)	FMD- group (N=12)
Insulin (µIU/ml)	15.90±2.50	11.93±1.03*
Cortisol (µg/dl)	1.43±0.07	2.65±0.27*

Significant differences in the values between the FMD and control groups were indicated by * P < 0.05.

Table 3: Plasma protein profile (total and electrophoretic pattern) in the FMD- infected cattle compared to the control group. (Values are means ± SD)

Variable	Control group (N=8)	FMD- group (N=12)
Total protein (g/dl)	7.85±0.11	6.58±0.07*
Albumin (g/dl)	3.41±0.10	2.90±0.10*
Total globulin (g/dl)	4.44±0.01	3.68±0.01*
Alpha 1 globulin (g/dl)	0.50±0.09	0.36±0.11
Alpha 2 globulin (g/dl)	0.70±0.05	0.86±0.03
Beta globulin (g/dl)	0.80±0.00	0.83±0.07
Gamma globulin (g/dl)	2.40±0.18	1.65±0.09*

Significant differences in the values between the FMD and control groups are indicated by * P < 0.05.

Correlation Between the Measured Hormones and Other Parameters: Table 4 shows that there was a significant negative correlation between insulin and serum levels of glucose, cholesterol, phosphorus, AST and cortisol (R= 0.887, 0.839, 0.891, 0.931 and 0.902, respectively) and between cortisol and albumin, calcium and insulin (R = 0.859 0.911 and 0.902, respectively). Significant positive correlation was recorded between insulin and serum levels of calcium and total protein (R= 0.834 and 0.923, respectively) and between cortisol and serum levels of glucose, phosphorus and AST (R= 0.957, 0.976 and 0.957, respectively).

DISCUSSION

FMD is a severe, highly contagious viral disease which is most important in domesticated and wild cloven-hoofed animals, notably cattle, pigs, goats, buffalo and sheep [2].

The disease is characterized by fever and vesicles (blisters) on the feet, in and around the mouth and on the mammary gland. Vesicles often rupture rapidly, becoming erosions. Pain and discomfort from the lesions leads to a variety of symptoms including depression, anorexia, excessive salivation, lameness and reluctance to move or rise [2]. Whereas it is endemic, this disease is a major constraint to the international livestock trade because it has grave economic losses in the production of meat and milk as well as clinical consequences [5]. In this study, the characteristic clinical signs seen in the FMD-infected cattle comply with those findings recorded in previous reports [1, 4, 13, 14].

The results of the present investigation revealed that in the FMD group, serum concentrations of glucose significantly increased. Hyperglycemia is a common finding and well documented in cattle affected with FMD [9, 10, 15, 16]. Sustained increases in glucose can be seen with insulin deficiency due to pancreatic beta cell (β cells) dysfunction (type I diabetes mellitus) or insulin resistance (type II diabetes mellitus) [17, 18]. Like some other viruses, in both naturally occurring and experimental infections, FMDV has been implicated in the development of type 1 diabetes by 2 different mechanisms. First the virus can directly destroy insulin-producing pancreatic β cells in the pancreas due to viral replication [17, 19].

Table 4: The correlation between the selected hormones and biochemical variables in the FMD infected group (Pearson's correlation test)

Parameter	Insulin	Cortisol	Glucose	Cholesterol	TP	Albumin	Ca	P	ALT	AST	BUN	Creatinine
Insulin	1	-0.902*	-0.887*	-0.839*	0.923*	0.700	0.834*	-0.891*	0.671	-0.931*	-0.0434	-0.530
Cortisol	-0.902*	1	0.957*	0.788	-0.780	-0.859*	-0.911*	0.976*	-0.350	0.957*	0.654	0.590
Glucose	-0.887*	0.957*	1	0.867*	-0.843*	-0.836*	-0.953*	0.989*	-0.311	0.982*	0.671	0.430
Cholesterol	-0.839*	0.788	0.867*	1	-0.909*	-0.636	-0.848*	0.873*	-0.572	0.923*	0.248	0.082
TP	0.923*	-0.780	-0.843*	-0.909*	1	0.614	0.870*	-0.811	0.624	-0.882*	0.337	-0.329
Alb	0.700	-0.859*	-0.836*	-0.636	0.614	1	0.891*	-0.899*	-0.036	-0.843*	-0.855*	-0.540
Ca	0.834*	-0.911*	-0.953*	-0.848*	0.870*	0.891*	1	-0.911*	0.246	-0.918*	-0.620	-0.473
P	-0.891*	0.976*	0.989*	0.873*	-0.811	-0.899*	-0.911*	1	-0.369	0.989*	0.614	0.404
ALT	0.671	-0.350	-0.311	-0.572	0.624	-0.036	0.246	-0.369	1	-0.472	0.338	-0.118
AST	-0.931*	0.957*	0.982*	0.923*	-0.882*	-0.843*	-0.918*	0.989*	-0.472	1	0.536	0.372
BUN	-0.0434	0.654	0.671	0.248	0.337	-0.855*	-0.620	0.614	0.338	0.536	1	0.534
Creatinine	-0.530	0.590	0.430	0.082	-0.329	-0.540	-0.473	0.404	-0.118	0.372	0.534	1

Statistical significance of correlations * was recorded at (P < 0.05).

Second, an immune response against the virus infection may induce an autoimmune response in the host leading to destruction of the remaining β cells [17, 20, 21]. Both mechanisms will result in decreased insulin synthesis and thus hyperglycemia [17]. Therefore, to see whether hyperglycemia might be attributed to decreased insulin synthesis, serum levels of insulin were determined. The demonstration of significant lower levels of insulin in the serum of FMD- infected cattle than in appropriate controls and the significant negative correlation between serum levels of insulin and serum glucose concentration (Table 4) supports the contention that the FMD-induced hyperglycemia is, at least in part, secondary to insulin deficiency [17].

Insulin resistance can be a result of increased cortisol concentration that opposes the action of insulin on peripheral tissues resulting in hyperglycemia [22-25]. Because cattle tend to produce marked stress hyperglycemia [26], the significant increase in serum cortisol levels seen in the present work may provide another reason for the significantly higher glucose levels in FMD- infected group. This explanation is further supported by the significant negative correlation between cortisol and insulin levels and the significant positive correlation between cortisol and glucose levels. There is also a hypothesis that the increase in blood glucose concentration in FMD-infected cattle may be a response to hypocalcemia because an adequate amount of calcium ions in extracellular fluids is essential for insulin secretion in response to blood glucose so, hypocalcaemia interferes with the secretion of insulin from the pancreas [10, 26, 27]. We reported a significant positive correlation between calcium and insulin levels that may support this hypothesis.

A significant reduction in serum TP and albumin was recorded in the FMD group. Protein requirement as well as protein catabolism increase in the presence of infection or any lesions on the body [23, 24]. Anorexia and off food due to mouth lesions that characterize cattle with FMD may be in part a possible cause [10]. It is also well known that glucocorticoids are closely involved in the protein metabolism either by their antianabolic effect reducing protein synthesis or catabolic action increasing breakdown [26]. Albumin degradation also is increased in the presence of increased glucocorticoids and may exceeds synthesis which will lead to a decrease in serum total protein and albumin concentrations [22, 26].

Consumption of protein has also been found to be associated with hypoinsulinemia and diabetes mellitus [10, 24, 25]. Therefore, hypoinsulinemia may in part explain the decrease in total protein concentrations observed in this study as detected by the significant positive correlation between serum TP and insulin levels.

Serum activity of AST was significantly increased. Stressful conditions and glucocorticoid excess can increase serum AST activity [26] so increased serum AST activity can be attributed to increased serum cortisol levels (significant positive correlation between cortisol and AST was indicated).

A significantly high level of cholesterol was detected in serum samples obtained from cattle with FMD. Abnormalities in lipid metabolism may be secondary to insulin deficiency [24]. In the absence of insulin, lipolysis is enhanced and plasma free fatty acids concentrations rise [26]. Very low density lipoproteins (VLDLs) accumulate in plasma because its catabolism requires insulin for optimal activity which are converted in the bloodstream to low density lipoproteins (LDLs). The rate of cholesterol synthesis is increased with an associated increase in plasma LDLs concentration [26]. This explanation is further strengthen by the significant negative correlation between insulin and cholesterol (Table 4).

Serum calcium values were significantly decreased in FMD group compared to those in the control one. Hypoproteinemia and hypoalbuminemia resulting in decreased protein bound calcium and may contribute to the hypocalcemia [10]. This suggestion could be supported by the significant positive correlation detected between calcium and TP).

Cortisol also has been found to produce marked depression of Ca uptake from gut due to inhibition of vitamin D [26]. Therefore, hypocalcemia could be attributed in part to increased serum cortisol levels (significant negative correlation was found between cortisol and Ca).

Serum phosphorous was significantly increased in the FMD group may be due to hypocalcemia based on the mass law of interaction between calcium and phosphorus, hypocalcemia leads to a reciprocal increase in the serum phosphorus concentration [24]. Significant negative correlation between Ca and P was indicated. In the present study a significant high serum concentration of cortisol was recorded. Stress due to febrile conditions, systemic infection and general body illness are associated with increase in adrenal activity resulting in increased glucocorticoid levels [28- 30]. Cortisol is the major glucocorticoid known as the critical stress hormone whose levels are increased in response to stressful conditions and is considered a part of the host's response to abnormal events particularly in the acute phase of the illness [31- 34].

The current findings of protein electrophoresis in FMD infected cattle revealed a significant decrease in total globulins and gamma globulins. Cortisol is known to weaken or suppress the activity of the immune system by inhibiting lymphoid mitosis and reducing immune cell number and function [31]. In the present study, significant negative correlation (results not shown) between serum levels of cortisol and gammaglobulins was recorded which indicates that excess cortisol may inhibit antibody production resulting in decreased gammaglobulins concentrations and thus total globulin, an effect that usually occurs before specific immunity is achieved [35].

In conclusion, infection of cattle with FMDV results in hypoinsulinemia which may indicate the development of some degree of pancreatic dysfunction in these animals. In addition, FMDV infection induces a prominent stress response as indicated by the significant increase in serum cortisol levels. Further, it seems likely that the alterations taking place in biochemical variables and protein electrophoresis in FMD-infected cattle seen in this study could be closely connected with changing in serum insulin and cortisol concentrations and appeared to be related to the magnitude of this alteration obviating the importance of considering or even the need for measurement of these two hormones when interpreting changes in blood biochemistry in cattle infected with FMDV. Finally, the present data may provide a better understanding of the disease process and clinical pathology of FMD in cattle.

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