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Original Article

Clinical and Biochemical Study of Pregnancy Toxemia in Iraqi Ewes

Khames Mustafa, M[1], Shareef Saed, O[1 *], Abdulealah Ismaeel, M[1]

1. Department of Internal and Preventive Medicine, College of Veterinary Medicine, University of Tikrit, Tikrit, Iraq

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Corresponding Author: omar_med@tu.edu.iq

Abstract

Pregnancy toxemia (PT), also known as ketosis or twin lamb disease, is a group of in-sequence metabolic disorders usually observed in the last pregnancy period of ewes. Blood samples from 60 Awassi ewes were collected, including 50 ewes suffering from PT and 10 healthy ewes (2-8 years old) as a control group. All of them were in their final month of pregnancy from different regions of Salah Aldin Governorate, Iraq. The samples were collected between October 2021 and February 2022. Biochemical analysis of serum concentrations of all parameters was performed using the atomic absorption spectrophotometer, except for the beta-hydroxybutyrate and non-esterified fatty acids that were analyzed by enzyme-linked immunosorbent assay method. The results of the clinical criteria tests for temperature, respiration, and pulse showed nonsignificant differences ($P<0.05$) in the infected animals, compared to the healthy group. Clinical signs included depression, loss of appetite, weight loss, lying down, odor of ketogenic bodies through breathing, inability to walk, neurological signs, dental grinding, jaundice, blindness, bloat, dystocia, animal death, and fetal death. Based on the results of the biochemical parameters tests of the blood, a significant increase ($P<0.\ 05$) was observed in the parameters of the results of beta-hydroxybutyrate, non-esterified fatty acids, triglycerides, total bilirubin, and liver enzymes (ALT, AST, ALP, and GGT) in the animals affected by PT, compared to the control group. However, a significant decrease ($P<0.05$) was observed in the parameters of glucose, cholesterol, total protein, albumin, and globulin in the affected animals, compared to the healthy group. Concerning the association between disease and oxidative stress criteria, the infected animals showed a substantial ($P<0.05$) increase in malondialdehyde concentration and a significant ($P<0.05$) drop in glutathione and superoxide dismutase levels.

Keywords: Biochemical study, Clinical study, Ewes, Pregnancy toxemia

1. Introduction

Pregnancy toxemia (PT), also known as ketosis or twin lamb disease, is a group of in-sequence metabolic disorders usually observed in the last pregnancy period of ewes (1). It is initially associated with inappropriate nutrition in the last trimester of pregnancy, as most fetal growth occurs at this stage in pregnancy, which duplicates the requirements of energy, especially in the case of multifetal pregnancy (2). Pregnancy toxemia affects ewes during late gestation due to increased energy demand by the fetus and lactation due to increased energy required for milk production (3).

Pregnancy toxemia affects all ewes and ovine species worldwide, causing high morbidities and high mortalities among diseased ewes. The absence of early detection of the disease due to insufficient information about its pathogenesis and the lack of efficient diagnostic tools are the main obstacles to improving our prophylactic and therapeutic policies against the disease (4). Clinical ketosis is manifested by loss of appetite, depression, dehydration, decreased milk secretion, staying away from the herd, pointless exhibition movements, frequent press of the head against the wall or feeder, weak chewing muscles, seizure, tremor of the neck and the head, opisthotonus, teeth grinding, ataxia, convulsions sternal recumbency, loss of wool, and the back and lateral recumbency.

Chemically, PT is characterized by hypoglycemia and hyperketonemia, which prevent the animal from maintenance of an optimal energy balance (5). It should also be noted that almost 80% of fetal growth takes place in the final 6 weeks of pregnancy, with 40% of the maternal glucose supply being used by the fetal placental unit. If ewes do not receive at least half of the required energy during this period, large quantities of fat deposits are mobilized (6).

The liver plays a vital role in blood glucose metabolism for the glucose tissue supply, and since it is almost the only organ where gluconeogenesis takes place (7), it is good to measure liver function tests. However, it should be noted that the kidney makes small contributions as well (8). The ketone body can generate superoxide radicals, which can subsequently become hydroxyl radicals. These free radicals exert their deadly impact by initiating peroxidation of the phospholipid membrane, leading to the buildup of lipid peroxidation products. These products are known to crosslink membrane components causing alterations in membrane permeability and lipid organization, cellular dysfunction, and membrane damage. However, normal cells can detoxify superoxide radicals using antioxidant enzymes, such as superoxide dismutase, glutathione, and catalase, that help maintain the intracellular concentration of reduced glutathione and reduced nicotinamide adenine dinucleotide phosphate, which is necessary for the optimal function of antioxidant defense system (9). This research aimed to study the clinical signs and effects of PT on the biochemical parameters, oxidant, and antioxidant status of ewes.

2. Materials and Methods

Blood samples were collected from Awassi 60 ewes, including 50 ewes suffering from PT and 10 healthy ewes (2-8 years old) as a control group. All of them were in their final month of pregnancy and from different regions of Salah Aldin Governorate, Iraq. It should be mentioned that the data were collected between October 2021 and February 2022. Case history information was collected from breeders, and the most important clinical signs appearing on the studied animals were recorded with vital clinical parameters (pulse, respiratory rate, and temperature) (10). Blood samples were collected from the jugular vein of all study subjects in an amount of 10 ml according to the method proposed by Pugh and Baird (11). Afterward, they were put in test tubes without anticoagulant to achieve serum isolation for biochemical values examination.

After keeping the serum-containing test tubes in a standing position for half an hour, they were centrifuged at 3,000 rpm for 15 min for proper separation of serum from coagulated blood. The serum was separated and stored in aliquots at -20 °C until examination. Blood samples were drawn from all animals for biochemical tests, including beta-hydroxybutyrate, non-esterified fatty acids (NEFA), glucose, cholesterol, triglycerides, total bilirubin, total protein, albumin, globulin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyltransferase, malondialdehyde, glutathione, superoxide dismutase.

Biochemical analysis determination of serum concentrations of all the above-mentioned parameters was performed using the atomic absorption spectrophotometer according to the method described by Rifai (12) and Ohkawa, Ohishi (13). Except for the beta-hydroxybutyrate (BHBA) and NEFA, the enzyme-linked immunosorbent assay (ELISA) was used in estimations. The collected data were analyzed in SPSS software for Windows (version 10. 01) and presented as mean±standard error (SE). The t-test determined the differences between the study groups. It should be mentioned that *P* values of less than 0.05 were considered statistically significant.

3. Results

3.1. Clinical Findings

Results of the clinical criteria tests for temperature, respiration, and pulse are summarized in table 1, which showed nonsignificant differences (P<0.05) between the infected animals and the healthy group in terms of these clinical criteria.

Pregnancy toxemia showed clinical signs on the affected ewes, which were represented by depression (n=50, 100%), loss of appetite (n=44, 88%), weight loss (n=36, 72%), lying down (n=38, 76%), odor of ketogenic bodies through breathing (n=34, 68%), inability to walk (n=12, 24%), neurological signs (n=22, 44%), dental grinding (n=12, 24%), jaundice (n=5, 10%), blindness (n=4, 8%), bloat (n=6, 12%), dystocia (n=10, 20%), animal death (n=16, 32%), and fetal death (n=14, 28%) (Table 2).

3.2. Biochemical Examination

According to the results of biochemical studies presented in table 3, there was a significant increase (P<0.05) in the concentrations of BHBA ketone bodies according to the ELISA test in serum, where their concentrations reached 7.15 ± 0.152 mmol/L in the affected animals, compared to the healthy group (0.85 ± 0.821 mmol/L). Moreover, there was a significant increase (P<0.05) in the concentrations of non-esterified fatty acids, which were 2.83 ± 0.093 and 0.42 ± 0.069 mmol/L for both infected and healthy animals, respectively. Results of the current study showed a significant decrease (P<0.05) in the rates of glucose concentration, as its concentrations in serum reached 34.20 ± 1.930 mg/dL and 68.94 ± 0.869 mg/dL in infected and healthy animals, respectively.

Table 1. Clinical examination parameters (Mean ±SE) in healthy and pregnancy toxemic ewes

Groups parameters	Healthy Ewes (n:10) Mean ± SE	Ewes with PT (n:50) Mean ± SE
temperature	0. 041^{a}±39.5	0. 050^{a}± 39.6
Respiratory rate/min	1. 032 ^{a}±28.5	0. 894 ^{a}± 30.2
Pulse rate/min	1. 965 ^{a}±80.6	1. 713 ^{a}± 83.1

Different letters in each row refer to a significant difference at the level (P<0. 05)

Table 2. Clinical signs appearing on ewes affected by pregnancy toxemia (50 ewes), frequency and percentage

Clinical signs	frequently	Percentage (%)
Depression	50	100
Appetite loss	44	88
Emaciation	36	72
Recumbency	38	76
Fruity odor	34	68
Unable to work	12	24
Nervous signs	22	44
teeth Grinding	12	24
Jaundice	5	10
Blindness	4	8
Bloat	6	12
Dystocia	10	20
Death	1٦	32
fetal death	10	20

Table 3. Biochemical examination parameters (Mean ±SE) in healthy and pregnancy toxemic ewes

Group parameters	Healthy animals (n:10) Mean ± SE	Ewes with PT (n:50) Mean ± SE
BHBA (mmol/l)	0. 821 [b] ±0. 85	0. 152 [a]± 7. 15
NEFA (mmol/l)	0.42 [b] ±0.069	0. 093 [a]±83.2
Glucose (mg/dl)	94.68 [a] ±0.869	1. 930 [b]±20.34
Cholesterol (mg/dl)	5.62 [a] ±5824	3. 020 [b]±7.40
Triglycerol (mg/dl)	7.52 [b] ±3.662	2. 544 [a]±4.82
Total bilirubin (mg/dl)	0.75 [b] ±0.031	0. 020 [a]±4.41
Total protein (g/dl)	0.254 [a] ±6.55	0. 228 [b]±5. 31
Albumin (g/dl)	0.151 [a] ±3.22	0. 181 [b]±2. 60
Globulin (g/dl)	0.135 [a] ±3.33	0. 121 [b]± 2. 71
ALT (IU/L)	442[a]. ± 37.22	2. 141 [a]±48. 4
AST (IU/L)	4. 176[b]± 6.87	5.822[a] ±152
ALP (IU/L)	6. 230[b]± 2.75	4.066[a] ± 255
GGT (IU/L)	2.620[b] ± 6.32	2. 310 [a]± 58.7
MDA (nM/ml)	1. 450[b]± 212.6	2. 805 [a]±36. 10
Glutathione (nM/ml)	1. 335 [a]±22. 94	1. 518 [b]±14. 42
SOD (IU/L)	18. 023[a]±441	16. 162 [b]± 412

Different letters in each row refer to a significant difference at the level (*P*<0. 05)

Findings of the current study indicated a significant decrease (*P*<0.05) in cholesterol levels of infected animals (40.7 ± 3.020 mg/dL), compared to healthy animals (62.5 ± 5.824 mg/dL). Furthermore, there was a significant increase (*P*<0.05) in the average triglyceride concentrations of infected and healthy animals which were 82.4 ± 2.544 and 3.662 ± 52.7 mg/dL, respectively. Results of the current study also showed a significant (*P*<0.05) increase in the total bilirubin concentration rates, which were 1.44 ± 0.020 mg/dL and 0.75 ± 0.310 mg/dL in the infected and healthy animals, respectively. The results also showed a significant decrease (*P*<0.05) in the levels of total protein, albumin, and globulin, which were 5.31 ± 0.228, 2.60 ± 0.181, and 2.71 ± 0.121 g/dL, respectively, in the affected animals, and 6.55 ± 0.254, 3.22 ± 0.151 and 3.33 ± 0.135 g/dL, respectively, in the healthy animals.

According to the results of the present research, there were significant differences (*P*<0.05) among all liver enzyme levels (ALT, AST, ALP, and GGT), where the activity of alanine aminotransferase and aspartate aminotransferase were 48.4 ± 2.141 and 152 ± 8.225 IU/L, respectively, in infected animals and 22.7 ± 3.442 and 87.6 ± 4.176 IU/L, respectively, in healthy animals. As for alkaline phosphatase and gamma-glutamyltransferase, their values were 255± 4.066 and 45.7 ± 2.310 IU/L, respectively, in infected animals, and 75.2 ± 6.230 and 32.6 ± 2.620 IU/L, respectively, in healthy animals.

Results of the current study also revealed a significant increase (*P*<0.05) in the level of malondialdehyde (MDA) concentration in infected animals 36.10 ± 2.805 nmol/mL, compared to the healthy group 12.62 ± 1.450 nmol/ml. However, there was a significant decrease in the concentrations of both glutathione and superoxide dismutase which were 14.42 ± 1.518 nmol/mL and 412 ± 16.162 IU/L, respectively, in infected animals, and 22.94 ± 1.335 nmol/mL and 441 ± 18.023 IU/L, respectively, in healthy animals.

4. Discussion

Based on the results, it was found that there were large numbers of ewes infected with PT, and this agrees with the findings of the studies performed by Olfati, Moghaddam (14) and Gaadee and Gehan (6). It is considered one of the most important metabolic diseases, which causes significant economic losses in production. Metabolic diseases usually occur due to the lack or inability of the body to produce some necessary substances, or they may be due to some administrative and production factors or growth factors that lead to metabolic problems in the body.

As it was noted in the current study, all cases were in the last month of pregnancy, and this is in line with the results of a study conducted by Constable, Hinchcliff (10). In the last month of pregnancy or near the date of parturition, the endocrine glands prepare to secrete different types of hormones that directly affect the natural physiological processes in the body. It adds to the challenge of metabolic diseases associated with childbirth since a negative energy balance begins in the body as the food taken by the animal is not sufficient to meet the high energy needs necessary for the growth of the fetus, childbirth, and milk production.

Results of the clinical criteria tests for temperature, respiration, and pulse are summarized in table 1, which show a non-significant difference between the infected animals and the healthy group in terms of the rates of temperature, respiration, and pulse. These results agree with the findings of other researchers (6, 7), who noticed no significant differences between the ewes infected with PT and healthy ones.

Results of the current study are presented in table 2. The clinical signs that appeared in infected animals were depression, loss of appetite, weight loss, lying down, odor of ketogenic bodies through breathing, inability to walk, neurological signs, dental grinding, jaundice, blindness, bloat, dystocia, animal death, and fetal death. It should be mentioned that these results agree with those of other previous research (7, 11). The clinical signs of PT are depression, restlessness, grinding of the teeth, constipation, loss of consciousness, acetone smell from the mouth, and dystocia. Neurologic signs include blindness, opisthotonus, incoordination, stiffness, convulsions, tremors in the neck muscles, lateral recumbency, coma, and death.

As was observed in the current study, the affected ewes experienced weight loss. The animals with excess weight are more susceptible to infection as they suffer from increased resistance of the adipose tissue to insulin, which endows them with the movement of NEFA due to the increased decomposition of fats when the animal is in a state of negative energy balance (7). Bad breath in ewes infected with PT is one of the most important signs of the disease due to ketone bodies that increase during pregnancy (6). Acetone is volatile and therefore, causes the characteristic odor of breath in ruminants with clinical ketosis (15). Results of this study also agreed with those of a study carried out by researcher Balikci, Yildiz (15), which explained the emergence of neurological signs, such as frequent chewing, lethargy, salivation, and head twisting in this disease due to cerebral hypoglycemia.

Results of biochemical studies are summarized in table 3 which show significant differences in the concentrations of BHB and NEFA. Estimation of the level of BHBA and NEFA in the serum is one of the important and efficient methods for estimation of the state of negative energy balance or the occurrence of PT in ewes. Entry of animals into a state of negative energy balance will stimulate the movement of acids NEFA toward the liver. Lack of concentrated feed and hormonal changes are among the most important causes of negative energy balance and disease (15). Researchers have stated that the concentration of NEFA is related to lipolysis in adipose tissue, where its concentration rises due to problems in the energy balance. A compound, known as beta-hydroxybutyrate, is considered an essential indicator of the presence of metabolic disorders and a more important factor than NEFA in the diagnosis of the disease (16).

These results agree with those of several previous studies (17, 18). The reason for the high concentration of BHBA is the occurrence of negative energy balance, which is associated with a high demand for energy, fat, and protein in the last stage of pregnancy and immediately after birth. This leads to the movement of the stores of fat in the body and thereby, the rise of NEFA and their incomplete oxidation in the liver in response to the increasing demand for glucose and finally, the increase of ketone bodies (acetoacetate, BHB, acetate) to their abnormal and pathological levels in the blood. Preliminary research has indicated that a decrease in the production of glucose in the liver would reduce its concentrations in the blood and the secretion of insulin. This leads to an increase in the movement of fats from adipose tissue leading to a subsequent increase in the levels of NEFA and BHBA (19).

Results of the present study showed a significant decrease in the concentration of glucose in the serum. This is in line with the findings of other previous research carried out by Mohebbi, Lotfollahzadeh (20) and Paramesh, Kumar (21), which explained the reason that animals suffer from negative energy balance during the last period of pregnancy and early lactation period during which they have higher energy requirements. Its consumption leads to metabolic stress and the movement of fat from its stores in the body, as the regulation of the balance of lipolysis and fat formation takes place through the concentration of glucose and insulin. During the negative energy balance, its concentrations decrease and significantly increase the release of NEFA from the adipose tissue.

In the last period of pregnancy and precisely the last days before delivery, the production of lactose increases dramatically to form colostrum, which increases the demand for glucose. This accelerates the process of gluconeogenesis and reduces glucose consumption in most cases. Despite these compensatory efforts of the animal body, the concentration of glucose in the blood decreases, and energy production decreases (22). Alternatively, the decrease in glucose concentration during the last period of pregnancy may be due to the increased need of the fetus for glucose in relation to the increased development of the fetus during this period (23).

The decrease in cholesterol concentrations in the current study may be due to the role of the ovary in increasing the manufacture of sex hormones in the transitional or perinatal period. Alternatively, the decrease in cholesterol concentration may be attributed to its increased use by responsible tissues before and after birth in milk processing (24). The presence or increase in the concentration of triglycerides is conclusive evidence of the presence of lipolysis in the fatty tissues of ruminants and that its increase with the increase in the proportion of ketone bodies and NEFA to more than its average level is considered sufficient to be a predisposing factor for the occurrence of toxic seborrheic disease in ewes (25).

The increase in serum bilirubin level in ewes affected with PT in the current study is consistent with what was indicated by Singh, Randhawa (26). They used the measurement of bilirubin concentration as an indicator for the diagnosis of degeneration or inflammation of hepatocytes or fatty liver in ruminants, as it rises significantly in these infections. As mentioned by Constable, Hinchcliff (10), the high level of bilirubin is due to the lack of bile flow resulting from the enlargement of the hepatocytes infiltrating with large amounts of fat. They also declared that it could be due to the competition of NEFA and bilirubin for the binding sites in the hepatocytes, which reduces the take-up of bilirubin by the liver cells and casts it outside the body.

The increase in serum proteins level in ewes affected with PT in the current study is in agreement with what was indicated by other researchers. Accordingly, it can be said that protein deficiency may occur when the liver obtains large amounts of fat, which leads to an increase in the oxidation and storage of fats in the liver tissues in the form of triacylglycerol. This leads to a decrease in hepatic metabolism or impairment in its function and also a decrease in protein synthesis.

In addition, protein deficiency in the kidney and albumin may be due to the increased growth of the fetus and the need for proteins in the last month of pregnancy, as well as the increased use of amino acids by the mother for the manufacture of the muscles of the fetus. It may also be due to kidney failure caused by fatty infiltration, degeneration of its cells, and leakage of proteins outside the body with urine (27). Furthermore, the decrease in the concentration of total protein in the body may be due to the transfer of albumin and globulin from the blood to the udder to produce colostrum in the last days of pregnancy. This is because of the lack of proteins with the increase of ketone bodies which occurs due to fatty infiltration in the liver tissues and its degeneration and function impairment inside the body (28).

High levels of ALT and AST liver enzyme activities in PT may be caused by the movement of body fat toward the liver due to negative energy balance and damage or lipid damage to hepatocytes (27). The infiltration of fat in hepatocytes leads to the destruction of hepatocytes and tissues and the emergence of pathological lesions, as the rise in the level of hepatic enzymes, such as ALT, AST, and GGT, is attributed to inevitable cell. Tissue breakdown by hepatic AST enzyme is considered the most responsive to histopathological changes in the liver. Among some researchers, an increase in the level of hepatic enzymes in the blood serum is conclusive evidence of liver injuries, as it is accompanied by the accumulation of fat in hepatocytes, mitochondrial dysfunction, and the destruction of cellular organelles (29).

A significant increase in the concentration of BHBA may play an important role in the induction of oxidative stress (lipid peroxidation) in ewes with PT (6). Glutathione represents the strongest factor in controlling lipid peroxidation, as the fat moving towards the liver in negative energy balance causes a decrease in antioxidants, such as glutathione and superoxide dismutase in liver tissue and blood (2). Constable, Hinchcliff (10) indicated that lipid peroxidation inhibits glutathione in the liver of sheep and thus reduces its concentration in the blood.

It is known that MDA is a byproduct of lipid peroxidation after exposure to active oxygen species and free radicals. It is used as an indicator of damage to cell membranes, as the increase in the level of malondialdehyde in the liver tissue leads to an increase in the activity of superoxide dismutase, glutathione peroxidase, and catalase, which decreases the concentrations of these antioxidants in the blood due to exposure to their inhibition by lipid peroxidation (30). Jorritsma, Jorritsma (31) in their study declared that the infiltration of triglycerides in the region of the anterior lobes of the liver near the hepatic vein is distributed to the rest of the liver regions. In the blood plasma, the increase in bile components in the plasma is considered a toxic condition that leads to the production of free radicals in the liver tissue, which leads to an increase in malondialdehyde, inhibition, and a decrease in antioxidants, such as glutathione, superoxide dismutase, and catalase. According to them, this condition is predisposed to inflammation and damage in liver tissue and other tissues in the body.

The present study showed that ewes were infected with a high rate of PT in the last month of pregnancy and investigated the effects of this disease on the health status of the ewes, which leads to a great economic loss due to the death of fetuses and mothers. This study considered a high percentage of BHBA and NEFA and a decrease in glucose in serum as important indicators for the diagnosis of PT and the negative impact of the disease on some biochemical parameters and oxidative stress.

Authors' Contribution

Study concept and design: M. K. M.
Acquisition of data: O. S. S.
Analysis and interpretation of data: M. A. I.
Drafting of the manuscript: M. A. I.
Critical revision of the manuscript for important intellectual content: O. S. S.
Statistical analysis: O. S. S.
Administrative, technical, and material support: M. K. M.

Ethics

The ethical approval was obtained from the University of Tikrit, Tikrit, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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