Dicle Üniversitesi Veteriner Fakültesi Dergisi
Dicle University Journal of Faculty of Veterinary Medicine

e-ISSN:1308-0679
https://dergipark.org.tr/tr/pub/duvetfd

RESEARCH ARTICLE

Evaluation of Serum Iron, Total Iron Binding Capacity, Transferrin Saturation, Haptoglobin and Ceruloplasmin Levels in Cows with Reticuloperitonitis Traumatica (RPT) and Pericarditis Traumatica (PT)

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Received
25.10.2023

Accepted
04.10.2024

Published
31.12.2024

DOI

10.47027/duvetfd.1381264

How to cite: Sezer M, Akyüz E, Aydın U et al (2024). Evaluation of serum iron, total iron binding capacity, transferrin saturation, haptoglobin and ceruloplasmin levels in cows with reticuloperitonitis traumatica (RPT) and pericarditis traumatica (PT). *Dicle Üniv Vet Fak Derg.*, 17(2):124-129

Abstract

The aim of this study was to evaluate serum iron (Fe), total iron binding capacity (TIBC), transferrin saturation (TD), haptoglobin and ceruloplasmin levels in cows with reticuloperitonitis traumatica (RPT) and pericarditis traumatica (PT). Swiss brunettes and crossbreds between 2-6 years of age were used in the study. A total of 60 cows, 30 cows diagnosed clinically and radiologically as RPT and 30 cows diagnosed as PT, constituted the patient groups. The control group consisted of 20 healthy cows in the same age range. Serum Fe, TD and TDBK levels were significantly lower in PT and RPT groups compared to the control group (P<0.001); haptoglobin levels were significantly higher in PT and RPT groups compared to the control group (P<0.001); ceruloplasmin levels were significantly higher in RPT and PT groups compared to the control group (P<0.05). As a result, serum Fe level, total iron binding capacity, transferrin saturation played a role as negative acute phase reactivators while haptoglobin and ceruloplasmin changed as positive acute phase reactivators in cows with RPT and PT. These parameters are very important in determining the severity of inflammation.

Key Words: Acute phase response, cow, iron, pericarditis, reticuloperitonitis traumatica, transferrin saturation

Reticuloperitonitis Travmatika (RPT) ve Pericarditis Travmatika (PT)'lı İneklerde Serum Demir, Total Demir Bağlama Kapasitesi, Transferrin Satürasyonu, Haptoglobin ve Seruloplazmin Düzeylerinin Değerlendirilmesi

Öz

Sunulan bu çalışmada retikuloperitonitis travmatika (RPT) ve perikarditis travmatika (PT) teşhisi konulan ineklerde serum demir (Fe), total demir bağlama kapasitesi (TDBK) ve transferrin doyumu (TD) ile haptoglobin ve seruloplazmin seviyelerinin değerlendirilmesi amaçlanmıştır. Çalışmada 2-6 yaş aralığında, İsviçre esmeri ve melezleri kullanılmıştır. Klinik ve radyolojik olarak RPT teşhisi konulan 30, PT teşhisi konulan 30 toplam 60 inek hasta gruplarını oluşturmuştur. Kontrol grubunu ise aynı yaş aralığında ve sağlıklı olan 20 inek oluşturmuştur. Serum Fe, TD ve TDBK seviyesinin kontrole göre sırasıyla PT ve RPT'li grupta istatistiksel açıdan çok önemli düzeyde düşük olduğu (P<0,001); haptoglobin seviyesinin kontrol grubuna göre sırasıyla PT ve RPT'li grupta istatistiksel açıdan çok önemli düzeyde yüksek (P<0,001) olduğu; seruloplazmin seviyesinin ise kontrol grubuna göre sırasıyla RPT' li ve PT'li grupta önemli derecede (P<0,05) yüksek olduğu tespit edilmiştir. Sonuç olarak retikuloperitonitis travmatikalı ve perikarditis travmatikalı ineklerde serum Fe seviyesi, total demir bağlama kapasitesi, transferrin doyumu negatif akut faz reaktivatörü olarak rol oynarken; haptoglobin ve seruloplazmin ise pozitif akut faz reaktivatörü olarak değişim göstermiştir. Bakılan bu parametreler yangının şiddetinin tespit edilmesinde oldukça önemlidir.

Anahtar Kelimeler: Akut faz yanıt, demir, inek, perikarditis, retikuloperitonitis travmatika, transferrin saturasyonu

INTRODUCTION

Reticuloperitonitis traumatica (RPT) is a disease seen in cattle that occurs when sharp, pointed and piercing foreign bodies taken with feed penetrate the reticulum and cause inflammation and damage in various organs after leaving this organ. Factors that facilitate the ingestion of objects include poor sense of taste in cattle, gluttony of cattle, insufficient chewing of food, pica state that occurs as a result of mineral and trace element deficiencies, long lactation period, contamination of barns and pastures by foreign objects, and prolonged stay of animals in pasture. Pointed and sharp objects pierce the reticulum wall and cause peritonitis or penetrate the liver, kidney, spleen, lung and heart, causing damage to these organs (1). Inflammation of the pericardial sac occurs when objects in the reticulum penetrate the diaphragm and sink into the heart, a disease called pericarditis traumatica (PT) (2,3). Symptoms include fever, anorexia, rumen atony and recurrent tympani, abdominal distension, groaning due to pain, cachexia, tachypnea, tachycardia, fullness in the vena jugularis and positive venous pulse, friction and churning sound in the heart, swelling in the ventral part of the body due to circulatory failure, dull sound on heart percussion and cardiac arrhythmia (4-8). Clinical findings, ferroscopic and radiographic examinations, pericardiocentesis and ultrasonographic examinations are used in the diagnosis of the disease. (8,9).

Acute phase proteins (AFP) are proteins synthesized by the liver in response to an acute inflammatory response. These proteins are negligible in healthy animals but increase rapidly during inflammation and act as an indicator of inflammation (10). The main function of haptoglobin, an important acute phase protein for cattle, is to prevent Fe loss by forming stable complexes with free hemoglobin in the blood. Another important acute phase protein for cattle is ceruloplasmin, which is particularly useful in monitoring the inflammatory process (1).

The aim of the study was to evaluate serum iron, total iron binding capacity, transferrin saturation, haptoglobin and ceruloplasmin levels in cows with RPT and PT.

MATERIAL AND METHODS

Animal Material

The animal material of this study consisted of a total of 60 cows between 2-6 years of age, including 30 cows diagnosed clinically and radiologically with RPT and 30 cows diagnosed with PT. The control group consisted of 20 healthy cows in the same age range.

Blood Sampling and Biochemical Measurements

Blood samples were collected from sick cows after diagnosis and from healthy cows after diagnosis using a sterile needle tip (Vacuette®, Greiner Bio-One GmbH, Austria) compatible with the holder into vacuum gel serum tubes (BD Vacutainer®, BD, UK). Blood samples taken in vacuum tubes were centrifuged at 3000 rpm for 10 minutes (Hettich Rotina 380R®, Hettich, Germany) to obtain serum samples. Before blood samples were taken, the animals were also clinically examined and vital signs were evaluated and noted. Biochemical measurements were made from the serum samples obtained. Total iron binding capacity (TIBC) was calculated by summing serum iron (Fe) and unsaturated iron binding capacity (UIBC) levels. Serum transferrin saturation (TS) was determined by calculating the formula (TS (%)=Fe/TIBC x 100) from serum Fe and TIBC levels (11). Fe and UIBC were measured colorimetrically (Epoch, Biotek, USA) with a commercial test kit (Biolabo, France). Haptoglobin was determined as reported by Skinner et al. (12), ceruloplasmin was measured by the method of Colombo and Richterich (13).

Glutaraldehyde Test Procedure

Glutaraldehyde (GLA) testing is routinely used to assess the severity of inflammation and prognosis in patients diagnosed with RPT and PT. The advantages of the test are that it is easy to administer, inexpensive and provides results in a short time. The test is done using whole blood and provides information about the amount of fibriogen. In a sterile empty tube, a 1/1 ratio of blood and GLA solution is placed and mixed, then the clotting time is interpreted by looking at the duration of clotting by turning upside down every 30 s. If the clotting time is between 0-5 minutes, the inflammation is strongly positive, if it is between 6-10 minutes, the inflammation is moderately positive, if it is between 11-15 minutes, the inflammation is mildly positive, and if no clotting occurs within 15 minutes, the test is interpreted as negative (14-16).

Radiographic Imaging Procedure

The radiological evaluation was performed in the Department of Radiology at XXX University School of Veterinary Medicine. The reticulum and diaphragm border were evaluated in the radiologic examination. A Dynamic brand ceiling static x-ray device and an FCR Prima brand (Fujifilm FCR T2 Veterinary Set, Medical Technology, Türkiye) imaging unit were used in the radiologic evaluation. For this purpose, irradiation doses between 35-40 mA and 85-90 kW were adjusted to the size of each cow. Radiographic images were taken by irradiation at a distance of 80 cm between the tube and the cassette (35x43 size).

Statistical Analysis

Data were presented as mean ± standard error of mean (SEM). The groups were showed normal distribution according to the Shapiro-Wilk test. The one-way ANOVA test was used for multiple comparisons, and the Tukey HSD test was used for post-hoc comparisons. The SPSS (SPSS Version 26.0®, Chicago, IL, USA) program was used for all statistical analyses. The differences between the groups in terms of the parameters were considered significant at the $p<0.05$ level.

RESULTS

Clinical Findings

In the clinical examination of the cows with RPT and PT, fever, anorexia, rumen atony and chronic tympani, abdominal tension, groaning, kyphosis posture, cachexia, loss of efficiency and positive ferroscopic examination were noted. However, it was also found that the group with PT had fullness and positive venous pulse in the vena jugularis, friction or churning sound in the heart on auscultation of the heart,

swelling in the ventral part of the body due to circulatory failure, dull sound on percussion of the heart and rhythm disturbance in the heart. Figure 1 shows the edema and positive venous pulse in the gerd region of 2 cows from the PT group of the present study. The severity of the inflammation was evaluated by GLA test on cows diagnosed with RPT and PT after clinical and radiological examinations.

Figure 1. Show the edema and positive venous pulse in the gerd region of cows from the PT group of the present study

Biochemical Findings

The vital and biochemical findings of the sick and healthy cows are given in Table 1 and Table 2, respectively. Among the biochemical parameters, Fe, TS and TIBC levels were statistically significantly lower in PT and RPT groups compared to the control group (P<0.001); haptoglobin level was statistically significantly higher in PT and RPT groups compared to the control group (P<0.001); ceruloplasmin level was significantly higher in RPT and PT groups compared to the control group (P<0.05).

Parameters	Groups (Mean ± SEM)			P value
	TRP (n:30)	TP (n:30)	Control (n:20)	
Rectal temperature (°C)	38.57 ± 0.28^{ab}	39.28 ± 0.38^{b}	37.89 ± 0.14^{a}	0.008
Breaths/min	25.78 ± 1.43	26.45 ± 1.27	22.30 ± 1.12	0.362
Heart beats/min	71.62 ± 2.47^{b}	61.31 ± 2.25^{a}	68.50 ± 2.26^{ab}	<0.001

a-b: The mean values with different letters in the same line represent the difference between patient and control groups (p<0.05). **n:** The number of cow in groups. **SEM:** Standard error of mean. **RPT:** Reticuloperitonitis traumatic group. **PT:** Pericarditis traumatic group.

Table 2. Biochemical findings of RPT, PT and control groups

Parameters	Groups (Mean ± SEM)			P value
	RPT (n:30)	PT (n:30)	Control (n:20)	
Iron (mg/dL)	78.80 ± 1.83^{b}	61.14 ± 1.92^{a}	112.36 ± 5.69^{c}	<0.001
Total iron-binding capacity (g/dL)	212.81 ± 3.84^{a}	200.14 ± 5.03^{a}	264.17 ± 6.21^{b}	<0.001
Transferrin saturation (%)	37.46 ± 1.13^{b}	31.37 ± 1.39^{a}	42.69 ± 2.05^{c}	<0.001
Haptoglobin (g/L)	0.29 ± 0.02^{b}	0.31 ± 0.02^{b}	0.09 ± 0.01^{a}	<0.001
Ceruloplasmin (mg/dL)	14.11 ± 0.84^{b}	13.92 ± 0.90^{b}	10.17 ± 0.86^{a}	0.011

a-c: The mean values with different letters in the same line represent the difference between patient and control groups (p<0.05). **n:** The number of cow in groups. **SEM:** Standard error of mean. **RPT:** Reticuloperitonitis traumatic group. **PT:** Pericarditis traumatic group.

Apart from this, the correlation of biochemical parameters in the study is given in Table 3. Total iron binding capacity and transferrin saturation were positively correlated with serum iron level. It was found that the level of haptoglobin was negatively correlated with serum iron level and total iron binding capacity. It was noted that ceruloplasmin level was negatively correlated with serum iron level and total iron binding capacity.

Table 3. Correlation of biochemical parameters in the study

Parameters	Iron (mg/dL)	Total iron-binding capacity (g/dL)	Transferrin saturation (%)	Haptoglobin (g/L)
Total iron-binding capacity (g/dL)	0,564**			
Transferrin saturation (%)	0,794**	-0,038		
Haptoglobin (g/L)	-0,397**	-0,441**	-0,182	
Ceruloplasmin (mg/dL)	-0,293**	-0,256*	-0,148	0,183

**. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed).

Radiological Findings

In cases with RPT, foreign bodies sinking into the reticulum may have different orientations and the problem in the abdomen is usually related to the direction of sinking and whether or not they pass into the abdominal cavity after sinking. In most of our cases, the objects that penetrated the reticulum were either oriented towards the diaphragm or craniovetrale. In cases with RPT, it was observed that the foreign

body penetrated into the base of the reticulum and formed a lesion in this region and the reticulum border was regular. (Figure 2). However, in cases with pericarditis, it was observed that most of the foreign body left the reticulum, moved towards the chest cavity and the diaphragmatic border became more irregular (Figure 3).

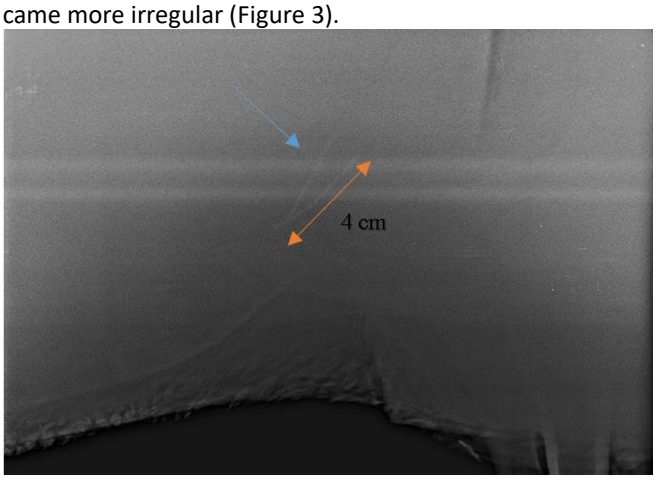

Figure 2 In cases with RPT, it was observed that the 4 cm long foreign body penetrated into the base of the reticulum and formed a lesion in this region and the reticulum border was regular.

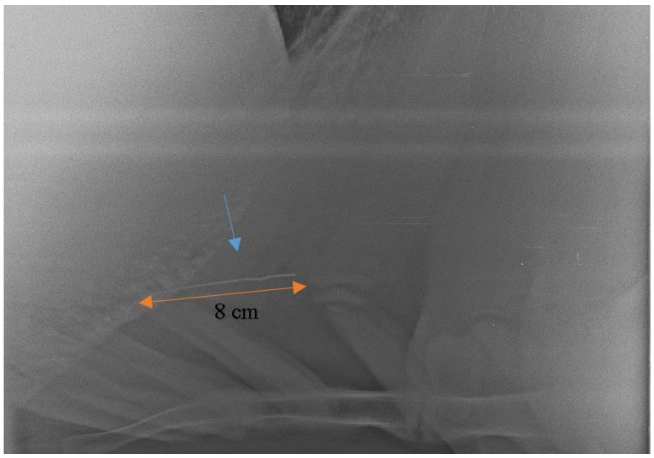

Figure 3. In cases with pericarditis, it was observed that most of the 8 cm long foreign body left the reticulum, moved towards the chest cavity and the diaphragmatic border became more irregular.

Glutaraldehyde Test Findings

In the group diagnosed with RPT and PT, the clotting time of the glutaraldehyde test was between 0-5 minutes, while this time was longer than 15 minutes in the control group (Table 4). This is an indication that the inflammation is severe in the patient group.

Table 4. Comparison of GLA test times between PT, RPT and control group

Parameters	Groups (Mean ± SEM)			P value
	RPT (n:30)	PT (n:30)	Control (n:20)	
GLA (minute)	2 ± 0.20	4.5 ± 0.32	16.5	<0.001

In the group diagnosed with RPT and PT, the clotting time of the glutaraldehyde test was between 0-5 minutes, while this time was longer than 15 minutes in the control group. **n:** The number of cow in groups. **SEM:** Standard error of mean. **RPT:** Reticuloperitonitis traumatic group. **PT:** Pericarditis traumatic group.

DISCUSSION AND CONCLUSION

Reticuloperitonitis traumatica and its complications are among the important digestive system diseases in cattle (17). In studies conducted in cattle with RPT and PT, fever, anorexia, rumen atony and chronic tympani, abdominal tension, groaning and kyphotic posture due to pain, cachexia, loss of yield and positive ferroscopic examination; However, in the group with PT, fullness in the vena jugularis and positive venous pulse, friction or churning sound in the heart on auscultation of the heart, swelling in the ventral part of the body due to circulatory failure, dull sound on percussion of the heart and cardiac arrhythmia are among the clinical symptoms reported (2,18,19). In this study, similar clinical findings were observed in the RPT and PT groups in accordance with the literature.

GLA Test is used to detect the severity of inflammation in many diseases, especially RPT and PT in cattle. This test is a practical method that can be applied very quickly. It is based on the detection of increases in serum fibrinogen and globulin concentrations. Glutaraldehyde forms a clot by primarily reacting chemically with free amino groups in fibrinogen and immunoglobulin. The clotting time in this test allows estimating the amount of protein produced in response to the inflammatory process (19). In studies conducted in cattle with RPT and PT, it was reported that blood clotted between 0-5 minutes in the GLA test due to increased fibrinogen concentration in patients with severe inflammation and the test was strongly positive (1,16). In this study, which was conducted in accordance with the aforementioned literature, blood clotting within the first 5 minutes in the GLA test performed in the patient group is an indication of the severe course of the inflammation.

Acute phase proteins are very useful in the evaluation of infection, inflammation, trauma, etc. occurring in the organism (10). Haptoglobin and ceruloplasmin are among the positive acute phase proteins synthesized by the liver in cattle (20-22). Studies have reported that haptoglobin and ceruloplasmin levels increase in severe inflammation, trauma and infectious conditions (19,22). In this study, haptoglobin and ceruloplasmin levels were significantly higher in cows diagnosed with RPT and PT compared to the control group. Authors think that the probable cause of this is trauma due to foreign body, severe inflammation and tissue damage.

Iron is an element with very important functions for living organisms. Iron acts as a building block of many proteins, especially hemoglobin (23). One of the most important conditions affecting iron metabolism is severe inflammation (24). Therefore, Fe levels are often examined to determine the severity of the inflammation. It has been reported in studies that Fe levels decrease in severe inflammatory conditions (25-27).

Edema occurs in the intestines as a result of loss of appetite and circulatory disorders due to heart failure. Iron deficiency occurs because this situation causes a decrease in iron intake with food and a decrease in the absorption of iron. Additionally, ferroportin binds and breaks down as inflammation-related cytokine levels increase. This condition impairs the absorption of iron, causing iron to be retained especially in the liver and reticuloendothelial cells and decreasing its level in the blood (28).

One of the most important reasons for this situation is loss of appetite and nutritional deficiency due to severe inflammation, and the other reason is hypoferremia caused by interleukin-6 (IL-6), one of the inflammatory mediators (23,29). It has been stated that total iron binding capacity decreases in inflammatory conditions (30). The organism's defense mechanism aims to retain Fe, which is necessary for the replication of pathogenic agents in inflammatory situations (31). Transferrin saturation is an indicator of how much Fe in serum is bound to transferrin. Transferrin saturation decreases in iron deficiency (32). In the present study, serum Fe, TIBC and TS were found to be significantly lower in the PT and RPT groups compared to the control group, respectively.

Authors think that the main reason for this is the severe course of inflammation due to both trauma and infection in the patient group and the activation of the body's defense system and retention of iron stores in the body to prevent the use of iron by pathogenic agents, as well as the development of iron deficiency as a result of the lack of food intake due to digestive problems and anorexia that occur with the deterioration of the general condition due to severe inflammation.

FINANCIAL SUPPORT

No support was received from any organization in the conduct of this research.

CONFLICTS OF INTEREST

The authors have declared that there are no conflicts of interest associated with this study or its results.

AUTHOR CONTRIBUTIONS

The writing of the study and final checks were carried out with the contributions of all authors

ETHICAL STATEMENT

The study was started after approval was obtained from Kafkas University Animal Experiments Local Ethics Committee (KAÜ-HADYEK/2023-003).

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