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INFORMATIVENESS OF BIOCHEMICAL MARKERS OF CONNECTIVE TISSUE METABOLISM IN CANINE BABESIOSIS**Dmytro Morozenko, Yevheniia Vashchyk, Andriy Zakhariev, Seliukova Nataliia, Dmytro Bereznyi, Kateryna Glibova**

The aim: to analyze the diagnostic informativeness of biochemical markers of connective tissue in the blood of dogs with babesiosis.

Materials and methods. German Shepherd (n=7), Labrador Retriever (n=3) and Rottweiler (n=2) dogs aged 1 to 5 years and diagnosed with babesiosis were studied. As a control group, clinically healthy dogs were used, which came to the veterinary clinic for a preventive examination, the age of the animals was from 1 to 5 years (n=10). The animals were examined according to the following scheme: collection of anamnestic data, clinical examination according to generally accepted methods, general and biochemical blood analysis, urine examination. Glycoproteins in blood serum were determined according to Shteinberg – Dotsenko, sialic acids – according to the Hess method, chondroitin sulfates – Nemeth-Csoka in the modification of L.I. Slutsky.

Results. The biochemical examination of the blood revealed the presence of acute cytolytic syndrome and cholestasis in the animal's body. Cholestasis in sick animals was characterized by an increase in the content of bilirubin and the activity of alkaline phosphatase, as well as an increase in the content of β -lipoproteins. The increase in the content of total bilirubin in the blood was obviously due mainly to its unconjugated fraction, since intravascular hemolysis took place. In the blood serum of patients with babesiosis in dogs, there was an increase in the content of markers of connective tissue metabolism – glycoproteins by 1.63, sialic acids – by 1.36, and chondroitin sulfates by 1.8 times, respectively.

Conclusions. Glycoproteins have "acute phase proteins" in their composition, which are indicators for assessing the degree of the inflammatory process in the body of dogs with babesiosis. Sialic acids are components of sialoproteins, which are also markers of the inflammatory process and destructive changes in the body. An increase in the content of chondroitin sulfates in the blood during babesiosis indicates the development of a compensatory reaction, associated with the action of toxic hemolysis products on the endothelium of blood vessels. Thus, the increased content of biochemical markers of connective tissue in the blood of dogs with babesiosis indicates the presence of a systemic inflammatory-protective reaction in animals, which makes it possible to recommend them for assessing the acute phase of the inflammatory process in the body and protecting blood vessels from damage due to toxic hemolysis

Keywords: dogs, babesiosis, diagnosis, biochemical markers, connective tissue, metabolism, informativeness, glycoproteins, sialic acids, chondroitinsulfates

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1. Introduction

Babesiosis of dogs (Babesiosis canis) is an acute or chronic transmissible natural focal disease of dogs, characterized by lethargy, depression, anemia, jaundice of the mucous membranes, disorders of the functions of the cardiovascular system and digestive organs. Ticks are carriers of the disease. The causative agent of babesiosis enters the dog's body when the tick is attached to the an-

imal's skin. Babesia reproduce in the dog's body by simple division or budding, causing hemolysis of erythrocytes [1].

In animals with this disease, anemia and hemoglobinuria are observed, dystrophic and inflammatory processes develop in the kidneys, liver, and pancreas, and protein, water, and mineral exchanges and the acid-base state of the body are disturbed. In the blood serum of pa-

tients with babesiosis in dogs, the content of urea, creatinine, bilirubin, aminotransferase activity significantly increases, which confirms the presence of acute inflammatory processes in the pancreas, liver, kidneys and the development of acute renal failure [2].

Typical results of laboratory studies in babesiosis are: accelerated erythrocyte sedimentation rate, increased protein content in serum, hemolytic regenerative anemia with varying degrees of thrombocytopenia, pronounced leukocytosis with a shift of the nucleus to the left and monocytosis, hemoglobinuria, bilirubinuria, proteinuria with hyaline cylinders [3]. According to the results of modern research, the most likely cause of the serious condition of dogs suffering from babesiosis is impaired blood circulation [4]. The diagnosis of babesiosis is established by microscopic examination of capillary blood smears from the lower part of the ear, which are stained by various methods, as well as by polymerase chain reaction [5]. Pear-shaped merozoites are most often found in erythrocytes, sometimes it is very difficult to find the causative agent, so a molecular genetic method of diagnosis is used [6]. However, there are no data in the literature on the disorders of the metabolism of connective tissue during babesiosis in dogs, which determines the relevance of our research.

The aim of the research to analyze the diagnostic informativeness of biochemical markers of connective tissue in the blood of dogs suffering from babesiosis.

2. Materials and methods

The research was carried out in 2021 on the basis of the Department of Veterinary Medicine and Pharmacy of the National Pharmaceutical University, the private clinic of veterinary medicine "Terra-VET" in Kharkiv, biochemical studies – on the basis of the Department of Laboratory Diagnostics and Immunology of the State Institution "Sytenko Institute of Spine and Joint Pathology National Academy of Medical Sciences of Ukraine".

The research was carried out in accordance with the Law of Ukraine "On the Protection of Animals from Cruel Treatment" (2008), provision 3R in accordance with the general principles of experiments on animals, adopted at the 1st National Congress on Bioethics (Kyiv, 2001) and coordinated with the provisions of the European Convention on the Protection of Vertebrates animals, used for experimental and other purposes (Strasbourg, 1985). No experiments were conducted - all animals came to the clinic either with health complaints or for preventive examination. During the selection of biological material (blood and urine) for laboratory studies, all rules of asepsis and antiseptics were observed. The study was reviewed and praised by the bioethics committee "Sytenko Institute of Spine and Joint Pathology National Academy of Medical Sciences of Ukraine", protocol No. 17, date 09/15/2021.

German Shepherd (n=7), Labrador Retriever (n=3) and Rottweiler (n=2) dogs aged 1 to 5 years and diagnosed with babesiosis were studied. The diagnosis of babesiosis was established by microscopic examination of smears of capillary blood, taken from the inner surface of the ear, stained with the LEUKODIF 200 kit. As a control group, clinically healthy dogs that came to the veterinary clinic for a preventive examination were used,

the age of the animals was from 1 to 5 years (n=10). The animals were examined according to the following scheme: collection of anamnestic data, clinical examination according to generally accepted methods, general and biochemical blood analysis, urine examination. Blood for general clinical and biochemical studies was collected from the jugular and subcutaneous veins of the forearm. The number of erythrocytes and leukocytes was determined in the blood - in a chamber with a Goryaev grid; hemoglobin – by the hemoglobin cyanide method, erythrocyte sedimentation rate (ESR) – by the Panchenkov micromethod, leukogram – in smears, stained according to Romanovsky – Giemsa, hematocrit – by the Wintrobe method [7].

In the blood serum of dogs, the following were determined: total protein - by the biuret reaction, albumin – by the colorimetric method, the activity of alanine (ALT) and aspartate (AST) aminotransferases – according to Reitman-Frenkel, the activity of alkaline phosphatase – according to the Bodanski method, total calcium – spectrophotometrically, urea – with diacetylmonooxime, creatinine – by the Jaffe reaction (Popper's method), total cholesterol – by the Ilk method, β -lipoproteins – by the turbidimetric method according to Burshtein and Samai, total bilirubin – colorimetrically [8]. Glycoproteins in blood serum were determined according to Shteinberg – Dotsenko, sialic acids – according to the Hess method, chondroitin sulfates – Nemeth – Csoka in the modification of L.I. Slutsky [9].

The statistical processing was carried out by the Student's method with the calculation of the mean (M) and its error ($\pm m$) with a normal distribution of digital data.

3. Research results

During the clinical examination, all animals, diagnosed with babesiosis, were depressed, anemic mucous membranes and anorexia were noted. The body temperature of the sick dogs was higher than 40 °C, vomiting was observed in half of the animals, as well as hemoglobinuria and bilirubinuria. According to the results of the blood examination of animals suffering from babesiosis, a decrease in the number of erythrocytes was found, which indicates anemia. Anemic syndrome is associated with hemolysis of erythrocytes due to their destruction by pathogens. Also, in sick dogs, neutrophilic leukocytosis with a shift of the nucleus to the left, lymphocytosis, eosinopenia and an increase in ESR were detected, which indicates the development of an acute inflammatory process in the body (Table 1).

The biochemical examination of the blood revealed the presence of acute cytolytic syndrome and cholestasis in the animal's body. Cholestasis in sick animals was characterized by an increase in the content of bilirubin and the activity of alkaline phosphatase, as well as an increase in the content of β -lipoproteins. The increase in the content of total bilirubin in the blood was obviously due mainly to its unconjugated fraction, since intravascular hemolysis occurred, which is confirmed by the data of other authors [10]. Violation of the functional state of the liver in patients with babesiosis in dogs was not very pronounced, since hypoalbuminemia was not observed, although it is often typical for this disease [11]. In our study, the animals were diagnosed at the initial stages of

the disease, so changes in the biochemical composition of the blood had not yet occurred to a certain extent due to the timely appeal of the animal owners to the veterinary clinic. An increase in the level of markers of azote-

mia, as well as the level of cholesterol and hypoalbuminemia, which in combination with proteinuria can be considered a sign of nephrotic syndrome, was not established (Table 2).

Table 1

Results of general clinical blood analysis of dogs for babesiosis (M±m)

Indicators	Clinically healthy animals, n=10	Sick animals, n=12
Erythrocytes, T/l	5.6±0.12	4.3±0.24*
Hemoglobin, g/l	150.0±5.33	136.8±7.37
Leukocytes, G/l	11.5±0.35	6.2±0.44
Eosinophils, %	6.0±0.20	1.9±0.69 *
Neutrophils: %		
Young	0	0
Rod nuclear	2.0±0.10	9.8±1.37 ***
Segmented	67.0±1.50	41.3±1.98
Lymphocytes, %	21.0±2.10	41.0±3.10 ***
Monocytes, %	6.0±0.75	6.0±0.88
ESR, mm/hour	2.8±0.84	5.9±0.77 *
Hematocrit, %	46.0±1.56	40.3±2.18

Note: * – $p < 0.05$; *** – $p < 0.001$ compared to indicators in clinically healthy animals

Table 2

Results of a biochemical study of the blood of dogs for babesiosis (M±m)

Indicators	Clinically healthy animals, n=10	Sick animals, n=12
Total protein, g/l	68.5±3.1	73.3±2.84
Albumin, %	49.2±2.05	52.6±1.94
Total calcium, mmol/l	2.3±0.03	2.3±0.06
ALT, mmol/(hour×l)	0.77±0.10	1.40±0.12***
ACT, mmol/(hour×l)	0.73±0.05	0.70±0.04
Cholesterol, mmol/l	4.8±0.20	5.8±0.17
Glucose, mmol/l	4.4±0.20	5.1±0.22
β-lipoproteins, U	12.5±1.55	27.0±2.29***
Alkaline phosphatase, U. Bodansky	5.5±0.46	10.8±0.55***
Total bilirubin, μmol/l	4.9±0.64	11.4±0.79***
Urea, mmol/l	6.9±1.05	8.4±0.86
Creatinine, μmol/l	87.5±5.43	102.1±4.25

Note: *** – $p < 0.001$ compared to indicators in clinically healthy animals

In the blood serum of patients with babesiosis in dogs, there was an increase in the content of markers of connective tissue metabolism – glycoproteins by 1.63, sialic acids – by 1.36, and chondroitin sulfates by 1.8

times, respectively. As you know, glycoproteins are protein-carbohydrate compounds, their increased concentration in the blood is a sign of an acute inflammatory process (Table 3).

Table 3

Indicators of connective tissue metabolism in the blood of dogs with babesiosis

Indicators	Clinically healthy animals, n=10	Sick animals, n=12
Glycoproteins, g/l	0.64±0.016	1.04±0.032*
Sialic acids, mmol/l	1.87±0.048	2.54±0.077*
Chondroitinsulfates, g/l	0.155±0.02	0.287±0.01*

Note: * – $p < 0.05$ compared to indicators in clinically healthy animals

Sialic acids are components of sialoproteins, which are also markers of the inflammatory process and destructive changes in the body. Glycoproteins include "acute phase proteins", which are indicators for assessing the degree of the inflammatory process in the body of

dogs with babesiosis, which is confirmed by the research of other authors [12, 13].

An increase in the content of chondroitinsulfates in the blood may indicate damage to liver tissues due to the toxic effect of hemolysis products on its tissue due to

babesiosis. Recently, Japanese scientists have suggested that liver damage is mediated by chondroitin sulfate. This was established in an experiment when an increased content of chondroitinsulfate was found in rats after ligation of the bile ducts in the damaged liver [14, 15]. There is also evidence that chondroitinsulfate is able to protect endothelial cells of blood vessels from damage [16]. This fact allows us to make an assumption that the increase in the content of chondroitinsulfates in the blood during babesiosis indicates the development of a compensatory reaction, associated with the effect on the endothelium of blood vessels of toxic products of hemolysis. Thus, the increased content of biochemical markers of connective tissue in the blood of dogs with babesiosis indicates the presence of a systemic inflammatory-protective reaction in animals, which makes it possible to recommend them for assessing the acute phase of the inflammatory process in the body and protecting blood vessels from damage due to toxic hemolysis.

Research limitations. The research results, obtained by the authors, can only be used to assess the condition of animals that did not have complications of babesiosis.

Prospects for further research. A promising direction of research is the study of the new biochemical markers of the diagnostic of canine babesiosis.

4. Conclusions

Glycoproteins have "acute phase proteins" in their composition, which are indicators for assessing the de-

gree of the inflammatory process in the body of dogs with babesiosis. Sialic acids are components of sialoproteins, which are also markers of the inflammatory process and destructive changes in the body. An increase in the content of chondroitin sulfates in the blood during babesiosis indicates the development of a compensatory reaction, associated with the action of toxic hemolysis products on the endothelium of blood vessels. Thus, the increased content of biochemical markers of connective tissue in the blood of dogs with babesiosis indicates the presence of a systemic inflammatory-protective reaction in animals, which makes it possible to recommend them for assessing the acute phase of the inflammatory process in the body and protecting blood vessels from damage due to toxic hemolysis.

Conflict of interests

The authors declare that they have no conflict of interest in relation to this study, including financial, personal, authorship, or any other, that could affect the study and its results, presented in this article.

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