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Determination Of Some Oxidative Stress Markers In Sheep Diagnosed With Anemia In Salah Al-Deen Governorate

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ABSTRACT

Background: The purpose of this study was to determine the changes of oxidation stress markers in sheep suffering from nutritional deficiency anemia. Additionally, the blood picture of these sheep was examined and compared to that of non-anemic sheep, which served as a control group.

Methods: Sheep aged 1-3 years who were clinically classified as anemic and non-anemic according to clinical symptoms were involved in the study. The study included 40 sheep divided to two groups, 30 animals as anemic group, and 10 animals as control group. The percentage of hemoglobin and the amount of red blood cells were also measured to validate this diagnosis. Superoxide dismutase, glutathione, and malondialdehyde were among the oxidation indicators.

Results: The findings demonstrated that the levels of each of the three (Hb, RBC, and HCT) were significantly Lesser ($p \leq 0.05$) in the groups of animals identified as anemic, as verified by blood picture tests, when compared to healthy animals. The levels of super-oxide dismutase and glutathione in anemic animals were significantly Lesser ($p \leq 0.05$) than in the control group, but the levels of malondialdehyde in anemic animals were significantly upper ($p \leq 0.05$) than in the control group.

INTRODUCTION

In addition to being exposed to numerous chemical toxins or toxic plants, sheep are thought to be susceptible to a number of diseases that result in anemia due to infection with internal and external parasites or nutritional deficiencies that affect the production of iron, copper, and cobalt, which are essential components of blood (1).

Blood is a red, liquid living tissue with a distinctive odor and a slightly salty taste. It is composed of formal elements: erythrocytes, leucocytes, platelets and a fluid medium, plasma. These formal elements are constantly being destroyed due to their functional activity, and new elements are being formed in quantities equal to what is destroyed. Therefore, their quantities remain approximately constant under normal conditions (2).

Iron deficiency is by far the utmost prevalent cause of nutritional anemia worldwide, despite the fact that numerous nutrients are involved in the synthesis of red blood cells and hemoglobin (3) A lack of vital nutrients, namely iron, vitamin B12, and folic acid, causes the body to create insufficient amounts of healthy red blood cells, a disease known as nutritional anemia (4). The creation of hemoglobin and red blood cells depend on these substances. The most common Symptoms of nutritional Anemia are Fatigue, weakness ,Pale skin (pallor),Shortness of breath, Dizziness or lightheadedness, Cold hands and feet, Brittle nails, Irregular heartbeat (in severe cases) (5)

Oxidative stress occur when there is an imbalance among reactive oxygen species (ROS) and the body's antioxidant resistances. In anemia, this can affect red

blood cells (RBCs) in several ways: Hemolysis: ROS can damage the red blood cell membrane, leading to premature destruction (hemolytic anemia) (6). Hemoglobin damage: ROS can oxidize hemoglobin, converting it to methemoglobin, which cannot carry oxygen efficiently (4). Bone marrow suppression: Chronic oxidative stress can impair erythropoiesis (the production of red blood cells) by damaging bone marrow progenitor cells. Iron metabolism: Oxidative stress alters iron balance, often leading to functional iron deficiency despite adequate iron stores (7).

MATERIALS AND METHODS

Animals groups:

The study included 40 Hamdaniya breed sheep's from the agricultural fields of some areas of Sallah-Addin governorate, Sheep aged 1-3 years who were clinically classified as anemic and non-anemic according to clinical symptoms, The percentage of hemoglobin and the amount of red blood cells were also measured to validate this diagnosis, the sheep's divided to two groups, 30 animals as anemic group and 10 sheep's as non-anemic group (control).

Blood 10 ml was drawn from the jugular vein of Hamdaniya sheep that were close in age (between one and three years) and were suspected of having anemia based on the color of eye membrane. The sample was taken with a sterile syringe once its yellow hue was confirmed, the first halve were put in an anticoagulant test tube with heparin for CPC.

The other halve was placed in a centrifuge to separate the blood serum. The serum was placed in a refrigerator at 5°C and then transported to the central laboratories

and analysis within 24 hours of Tikrit University, where tests were conducted on oxidation markers. CBC test was performed laboratory hematology analysis device of physiology department /collage of veterinary medicine.

Oxidative marker measurement:

glutathione:

Serum glutathione levels were measured using the Ellman reagent method.

Malondihyde:

The researchers Guuidet and Shah employed a modified thiobarbituric acid (TBA) reaction technique to quantify malondialdehyde (MDA), a byproduct of lipid peroxidation. The level serves as an indicator of this process, as the measurement relies on the reaction between lipid peroxides, specifically malondialdehyde, and TBA in an acidic pH media (8).

superoxide dismutase:

The method relies on the capacity of the enzyme SOD to prevent the reduction of nitroblue tetrazolium (NBT) to blue formazine by superoxide radicals produced by the reaction between xanthine and xanthine oxidase (9).

Statistical Analysis:

The current study's data were statistically analyzed using SPSS version 23, employing mean and standard deviation calculations, along with the T-test to assess the differences between the two groups at probability levels of 0.05 and 0.01.

RESULTS

The findings in Table (1) demonstrated that, as determined by blood imaging tests

and comparison with healthy animals, there was a significant drop in the levels of each of the variables (Hb, RBC, and HCT) in the groups that were identified as having anemia, regardless of the reason.

DISCUSSION

Prior studies have indicated that a deficiency of minerals in animal feed and suboptimal nutritional status may contribute to this decline (10). Previous research indicates that parasitic diseases such as Theileria and Babesia can also cause anemia in sheep (11). Gastrointestinal worms, particularly those that cause anemia by extracting blood from their hosts, such as *Ostertagia* spp. and *Trichostrongylus*, are prevalent and economically important parasites in Iraq.

Several factors must be excluded when clinically addressing anemia in ruminants, including infectious agents (babesiosis, anaplasmosis, leptospirosis, clostridial toxins), metabolic issues (deficiencies in vitamins or minerals, concurrent chronic conditions, or neoplasms), and internal or external parasites (e.g., intestinal parasites, fleas, lice) (12). Bone marrow disorders, chemical poisoning (including heavy metals, pharmaceuticals, and rodenticides), hemorrhagic conditions (such as trauma, dystocia, abomasal ulcers, vena cava thrombosis, and hemorrhagic bowel syndrome), or, less frequently, immune-mediated anemia (for instance, administering cow colostrum to newborn lambs (*Ovis aries*))(13).

Studies have shown that the incidence of gastric and intestinal worms and liver flukes in sheep in Iraq is very high compared to other diseases. Infection with *Haemonchus* and liver flukes leads to hemorrhagic anemia. As for the intestinal protozoa of the genus *Eimeri*, there are at

least 15 species of *Eimeria* that infect sheep worldwide, many of which cause severe disease characterized by bloody diarrhea, emaciation, anemia and then death (14).

Anemia may be caused by iron deficiency and liver failure. Anemia associated with infections, copper deficiency (rarely), hereditary elliptocytosis and marrow tumors. All of the above-mentioned causes may be responsible for anemia, in addition to climatic factors that affect the blood picture in sheep (15).

The findings indicated a substantial reduction ($p \leq 0.05$) in the levels of both SOD and GSH in anemic animals relative to the control group, as illustrated in Table (2). Conversely, there was a notable elevation in the level of MDA in anemic animals compared to the control group, also at a significance level of $p \leq 0.05$.

Red blood cells are perpetually subjected to oxidative stress, originating both internally and from external sources, including other tissues and exposure to oxidants. Excessive oxidative stress surpassing the antioxidant capacity of red blood cells results in damage characterized by lipid and protein peroxidation, cytoskeletal crosslinking, oxidation of hemoglobin to methemoglobin, and the accumulation of distorted sulfhemoglobin as Heinz bodies (16).

Red blood cells receive antioxidants through both enzymatic and non-enzymatic mechanisms (17). Five enzymes play crucial roles: catalase, which converts hydrogen peroxide into oxygen and water; glutathione reductase, which utilizes NADH to reduce oxidized methemoglobin and superoxide; superoxide dismutase, which scavenges superoxide anions, producing hydrogen peroxide and oxygen;

and glutathione peroxidase, which employs NADPH to eliminate hydrogen peroxide and other organic peroxides in red blood cells (18).

Anemia results from diminished red blood cell synthesis or heightened breakdown or loss of red blood cells that compensation systems cannot adequately replenish (2).

Oxidative damage and anemia exhibit a bidirectional connection. A diminished red blood cell count results in compromised antioxidant defense and hypoxia. Hypoxia directly and indirectly adds to oxidative stress. Oxidative stress primarily impacts red blood cells because of the elevated levels of polyunsaturated fatty acids in their membranes. Red blood cells, characterized by a stiff membrane structure that precludes shape alteration, are phagocytosed by the spleen and macrophages (17). Oxidative damage thus reduces the lifespan of red blood cells and induces or worsens anemia (1).

CONCLUSION

This study concluded that although the animals being of similar ages and maintained on same diets, many were found to be anemic, while others were healthy, even with good body build. Anemia can have many causes, including nutritional, parasitic, or other diseases. Anemia is a condition of superoxide dismutase, which alters oxidation parameters compared to normal.

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TABLES

Table (1): The Value Of Some Blood Parameters In Sheep With Anemia Compared To The Control Group.

| Parameters Groups | Hb gm/dl | RBC/L× 10 ¹² | HCT % |
|----------------------|---------------|-------------------------|----------------|
| Anemic group | 6.6 ± 0.126 a | 3.79± 0.145 a | 16.582± 2.35 a |
| Control group | 9.4 ± 0.184 b | 6.84±0.198 b | 26.874± 3.74 b |

Table (2): Oxidative Markers in study groups

| Parameters Groups | SOD UN/ML | GSH µMol/L | MDA µMol/L |
|----------------------|----------------|-----------------|---------------|
| Anemic group | 1.66 ± 0.125 a | 16.6 ± 7.9 a | 1.29± 0.16 a |
| Control group | 5.13± 0.847 b | 25.7 ± 10.5 a b | 0.84 ± 0.49 b |