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Comparative evaluation of erythrocyte osmotic fragility between anemic and chronically iron exposed sheep in Iraq

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Abstract

The experiments were conducted with the aim of determining which of the two groups, anemic and chronically iron exposed sheep, is more fragile erythrocyte using Erythrocyte Osmotic Fragility as an index. A total of 20 ewes were used for this study, comprising of 10 anemic and 10 chronic iron exposed eweswhich were injected IM of10mg/kg BW iron dextran weekly for (12week).Approximately 5 ml of blood was collected from each animal for blood smear and erythrocyte fragility test. The result of blood smear showed iron accumulation in erythrocyte when stained with giemsa and Prussian blue stains, also presence of pallor center, anisocytosis and poikilocytosis in anemic sheep. The result of fragility test showed a significant differences at ($P < 0.05$) between anemic and iron exposed ewes, which was higher in chronically iron exposed ewes as compared with the anemic group. In conclusion, the chronic iron exposed animals have an erythrocyte more fragile than the anemic animal so that anemic animals must be treated with recommended dose.

Keywords: Erythrocyte osmotic fragility, ewes, anemic, chronic iron exposed.

Introduction

Erythrocyte Osmotic fragility (OF) refers to the degree or proportion of hemolysis that occurs when a sample of red blood cells are subjected to osmotic stress by being placed in a hypotonic solution. Osmotic fragility is affected by various factors, including membrane composition and integrity as well as the cells' sizes or surface-area-to-volume ratios (Fischbach& Dunning, 2008).

The uses of erythrocyte mechanical fragility can include diagnostic testing, calibrations to aid comparisons of hemolysis caused by blood-handling devices, or assessment of sublethal (i.e., non-hemolysing) damage caused to cells from devices that manipulate blood (such as for dialysis or intraoperative autotransfusion). It can also help in assessing damage of stored RBC product (so-called "storage lesion"), leading to applications in blood transfusion and blood banking(Yazer*et al*., 2008).

The role of iron in blood formation became apparent in the 17th century when it was shown that iron salts were of value in treating chlorosis, now known as iron-deficiency anemia, in young women; The first clinical description of iron overload was reported in 1871 (Fairbanks, 1994).

Iron is an essential component not only for hemoglobin synthesis erythropoiesis but also for many enzymes and hormones, iron deficiency had negative effect on the sheep flock, deficient iron causes anemia in all animals, Iron deficiency is common finding in

ruminant, where low dietary intake, starvation, gastrointestinal parasites, blood parasite infection increased incidence of infectious disease, inadequate gastrointestinal absorption, hemorrhage, effect of pregnancy and lactation, all these causes influenced on the level of essential blood constituents especially iron , cobalt , copper and many of biochemical functions such as iron utilization and hemoglobin synthesis, (Weiss and Wardrop, 2010).

Iron's toxicity is largely based on its ability to catalyze the generation of radicals, which attack and damage cellular macromolecules and promote cell death and tissue injury, however excessive iron accumulation results in tissue damage and organ failure, pathological iron accumulation in the liver has also been linked to the development of hepatocellular cancer (papanikolaou and pantopoulos, 2005).

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The aim of this study is to determine which of two groups are more susceptible to erythrocyte fragility.

Materials and Methods

Experimental animals:

The research was conducted to study the erythrocyte fragility in anemic and chronically iron exposed animal on 10clinically anemic and 10 chronically iron exposed ewes which given 10mg/kg weekly for 3 monthsin the veterinary medicine college of Al-Fallujah university. The animals were selected pregnant at the days (75-100) of pregnancy, at age (2-4) years, with body weight (25-43) kg.

Animals selected depending on complete blood picture, serum iron, blood smear and bone marrow smear stained fore iron, were divided randomly into two groups including (10) animal in each group as fallowing:

- The 1st group (anemic) was left on its feed only.
- The 2nd group (chronic exposed) were injected IM of10mg/kg BW iron dextran weekly for (12week).

Clinical examination.

Clinical examination wascarried to all animals for any changes appetite, wool loosing, diarrhea or any other signs such as pulse rate, rectal temperature, respiratory rate and mucous membranes which were examined and recorded according to Famacha anemia chart.

Blood sampling:

Blood samples were collected from jugular vein into EDTA tubes for hematological parameters. All samples collected after 12 hours of fasting at the morning before breakfast according to (Coles, 1986).

Blood smear:

Blood film was made and stained by Giemsa stain and Prussian blue stain according to the method by (Coles, 1986).

Erythrocyte Osmotic Fragility Determination:

Sodium chloride solution was prepared according to Faulkner and King (1970) in concentration ranging from 0.0 to 0.9 at pH 7.4. Aset of 10 test tubes (containing 5 ml of sodium chloride solution) where arranged serially in a test tube rack to analyze each sample. The test tubes were labeled with corresponding sodium chloride concentrations. One ml pipette was used to transfer0.02 ml of blood into each of the ten test tubes. The content was then mixed by gently inverting the testtubes for about 3 times. The test tubes were allowed to stand at room temperature (26 – 27°C) for 30 minutes. The contents of the test tubes were maintained at pH 7.5 thereafter the contents of thetest tube were centrifuged at 1,500 g for 20 minutes. The supernatant of each test tube was transferred into a cuvette. The concentration of haemoglobin inthe supernatant solution was measured at 540 nm using a spectrophotometer (CECIL / England) by reading the absorbance. The same procedure was repeated for every blood sample used for the study. The percent haemolysis was then calculated using the formula (Faulkner and King, 1970).

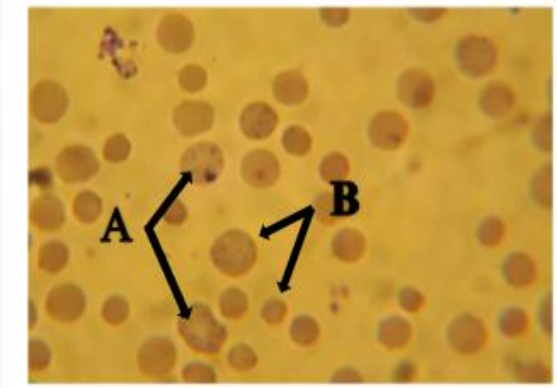
$$\text{Percent haemolysis} = \frac{\text{Optical density of test}}{\text{Optical density of standard}} \text{X100}$$

Statistical Analysis:

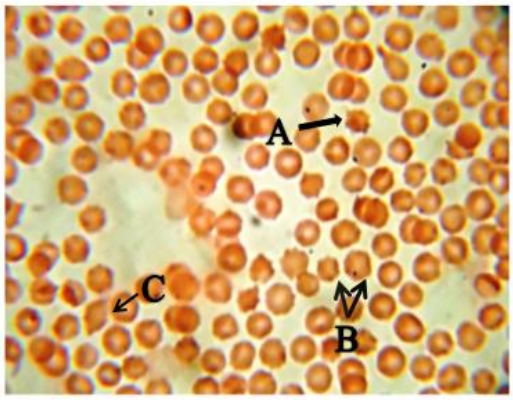
All data are represented as means + SE. One way analysis of variance (One-way ANOVA) by using SPSS program, followed by Least Significant Difference (LSD) test were used to determine differences among means of investigated groups. The level of statistical significant was set at ($P < 0.05$) (Snedecor and Cochran, 1989).

Results

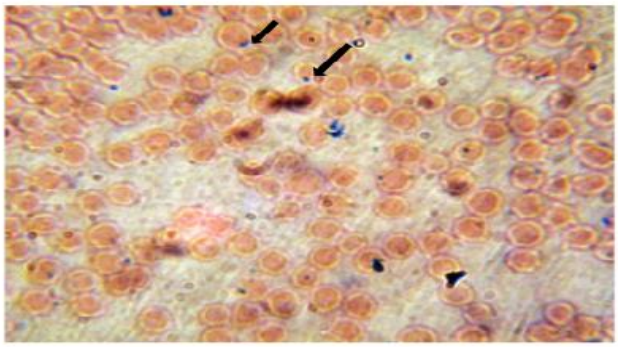
The result of blood smear showed toxic effects of iron in chronic exposed sheep which appeared in RBCs with variable shape and size due to iron accumulation (Figure 1), also there were presence of acanthocyte, Anisocytosis and poikilocytosis in anemic sheep (Figure 2).



(Figure 1) Blood smear of chronic iron exposed animal (a) diffuse regular basophilic granule in RBC. (b) Anisocytosis (Giemsa stain) ×100



(Figure 2) Blood smear of anemic animal (A) acanthocyte (B) Anisocytosis, (C) poikilocytosis. (Giemsa Stain)×100



(Figure 3)Blood smear of chronic iron exposed animal show iron accumulation on RBC wall (Prussian blue stain) ×100

Table (1) Comparison between anemic and iron exposed groups

Concentration / groups	0.0	0.2	0.4	0.6	0.9
Anemic	1659.5±270.6b	1658±269.7b	1681.3±248b	1427±204.8b	59±27.4b
Iron exposed	1765±251.1a	1860.5±213.7a	1922±220.5a	1747.5±182.2a	105.25±66.8a

Discussion

The normal functionality of the erythrocyte is based on its ability to maintain its membrane integrity. The compromization of the erythrocyte membrane integrity resulting in increased erythrocyte fragility may have arisen from the increased lipoperoxidative changes which lead to the destruction of erythrocytes being more in the animals as a result of iron accumulation in the cell wall.

This is evidenced by increase in haemolysis which act as powerful free radical generators when found as free ions in high concentration (Adenkola *et al*., 2011).

When free radicals production exceed the capacity of natural tissue antioxidants enzymes (Miller and Brzezinska-Slebodzinzka, 1993; Nazifi *et al*., 2009) or as a result of depletion of endogenous antioxidant enzymes due to increased lipoperoxidative changes (Gultekin*et al*., 2001; Mansour and Mossa, 2009). The oxidative modification of the erythrocyte membrane has been shown to increase the fragility of the RBC (Langsdorf and Zydney, 1993). Increase free radical generation in the body has been shown to cause lipid peroxidation of cytomembranes, resulting in cell injury and, consequently, death (Padayatty *et al*., 2003; William *et al*.,2008), including the erythrocytes (Sumikawa*et al*. 1993; Avellni *et al*., 1995; Adenkola and Ayo, 2009) thereby exposing the erythrocyte to destruction by macrophages (Lichtensteiger and Vimir, 2003). Lipid peroxidation, which is the process of oxidative degradation of polyunsaturated fatty acids (PUFA) when it happens in biological membranes leads to impairment of membrane function and structural integrity (Gutteridge and Halliwell, 1988). The results of this study agree with the findings of Avellini *et al*.(1995) who showed that free radicals play a vital role in tissue damage and have adverse effects on erythrocytes. Although free radicals were not measured in this study it has been established that free radicals are generated in animals subjected to stress (Elsna, 1991; Altan *et al*., 2003; Tauler *et al*., 2003).

The constant exposure to high oxygen tension, high level of iron and richness in PUFA (Kollanjiappan *et al*., 2002) coupled with their inability to possess nucleus and other organelles (Dorđevic *et al*., 2008) have made erythrocyte a center of free radical attack. Process of lipid peroxidation decreases hydrophobic characteristics of bi-layer membrane of erythrocytes, altering affinity and interaction of proteins and lipids, thereby impairing the functioning and homeostasis of erythrocytes membrane (Dargel, 1991).

The presented result of anemic animals showed high fragility this is due to Iron role in in oxygen delivery to the tissues, also as a cofactor with several enzymes involved in energy metabolism and thermoregulation, mitochondrial iron enzymes are essential for oxidative production of cellular energy, aerobic metabolism depends on iron because of its role in the functional groups of most of the enzymes of the Krebs cycle, as an electron carrier in cytochromes, and as a means of oxygen and carbon dioxide transport in hemoglobin (Fairbanks, 1994).

In conclusion, the chronic iron exposure increase the erythrocyte fragility more than anemia, so that treatment of iron deficiency anemia must be according to recommended dose.

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