

Research Paper

Hyperglobulinemia and increased erythrocyte membrane osmotic instability of intermediate hosts infected with the zoonotic parasite, *Linguatula serrata*

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Received: 2021-05-30

Accepted: 2021-08-10

Introduction

Linguatula serrata is a worldwide zoonotic parasite. The adult parasite is found in the respiratory system and especially in the nasal cavities of dogs (the final host). The eggs are released through the nasal secretions of these animals and then eaten by herbivores (intermediate hosts). The larvae hatch in the intestines of these animals and migrate to the mesenteric lymph nodes, liver, and lungs and become nymphs. Humans can be the intermediate and accidental final host of this parasite.

Given that the nymph of the parasite attacks important organs, it is thought that the parasite affects the functioning of the lymphatic system, blood, and tissues through its physical presence and possibly secretions. One of the most important biochemical factors in the circulatory system is proteins. Parasites affect the concentration of serum proteins directly or indirectly by invading target tissues, impairing cell membrane permeability, or producing their own products and

secretions. In addition, in many cases, the feeding mechanism and migration of parasitic agents are responsible for biochemical changes in the blood. The most important serum proteins include albumin, immunoglobulins, haptoglobin, transferrin and lipoproteins. Any disorder or loss of balance in the concentration of these proteins can be the cause or result of some diseases. Osmotic instability of erythrocytes membrane is one of the measurable values that estimates hemolysis under hypoosmotic pressure. The osmotic instability test of erythrocytes is used to evaluate membrane permeability in pathological conditions. Considering the mentioned cases, the present study aimed to investigate the prevalence of *linguatulula serrata* in Khuzestan Province and evaluate the possible effect of this parasite on serum proteins, and the osmotic instability of red blood cells in Intermediate hosts (sheep and goats) were performed.

Materials and methods

From September 2018 to March 2019, 791 samples of mesenteric lymph nodes and blood of goats (n= 335) and sheep (n= 456) were collected from the slaughterhouse in Ahvaz, Khuzestan Province. In this study, all the sampled animals were healthy based on antemortem and postmortem inspection. Any animals with signs of diseases and abnormalities were excluded. The infection with *L. serrata* was diagnosed by two methods: 1) observation and counting of parasitic nymphs in the mesenteric lymph nodes by using a stereomicroscope and 2) haemagglutination test.

The Biuret method was used to determine the concentration of total serum protein. Then serum albumin was measured using a special albumin kit (Man Company, Iran). Serum globulin was also determined by subtracting total protein and albumin. Osmotic instability of erythrocytes is an experiment that evaluates the stability of red blood cell membranes against osmotic stress. For this experiment, the usual method was used using different concentrations of 1% sodium chloride. Data were analyzed using SPSS version 24 and paired (dependent)-t-test. The significance level was considered $p < 0.05$. The results of different experiments were expressed as mean with standard deviation.

Results & discussion

The overall prevalence of *L. serrata* in goats and sheep was 10.7% and 5.7%, respectively. The number of *L. serrata* nymph in mesenteric lymph nodes of goats was more than in sheep. The mean intensity of infection in goats and sheep was 10.6 and 4.9 nymphs per lymph node, respectively. The mean length of nymphs was 4.8 ± 0.7 mm. From the animals sampled, 15

positive and ten negative blood and lymph node samples from each animal (goats or sheep) were used for subsequent experiments.

Total protein and albumin concentration, as well as albumin to globulin ratio, decreased significantly and globulin levels increased significantly in the infected group compared to the non-infected group in both animals ($p \leq 0.05$). In general, changes in serum proteins may indicate some pathological conditions. Serum total protein, albumin, and globulin levels are one of the serum biochemical parameters that affect the immune response. Low serum protein concentrations are seen in many cases, including liver and kidney disease. Serum proteins are mainly secreted by the liver. The migration of parasite germs into the liver, followed by damage to this organ and reduced function, may be one of the reasons for the decrease in serum proteins. Other reasons for the decrease in total protein levels in the serum of animals infected with this parasite include the invasion of the parasite larvae into the intestinal wall and the leakage of plasma from the damaged intestine. Albumin depletion may be exacerbated by increased albumin catabolism and protein malabsorption through the damaged intestinal mucosa. Elevated serum globulin levels in parasitic infections may also be due to the humoral immune response to parasitic infection. Thus, the presence of infection stimulates the host immune system and thus increases globulin synthesis.

The amount of membrane osmotic instability of erythrocytes increased in the groups infected with *L. serrata* ($p \leq 0.05$). Overall, the present study showed that sheep and goats are infected with *L. serrata* in Iran. This parasite also causes changes in the serum proteins of small ruminants. Infection with this parasite may cause oxidative stress and damage the structure of red blood cells and increase their membrane instability. Increased osmotic instability of the membrane was expected due to the life cycle of the parasite in the host medium and the presence of the new parasite in the lymph nodes, because this parasite is present in the lymph nodes, destroying these glands physically and possibly with its secretions and release. Secretions into the lymphatic system and blood are likely to damage the red blood cell wall, causing the globules to rapidly slide when exposed to hypotonic environments. In addition, red blood cells are the main cells that circulate oxygen in the circulatory system and are very sensitive to oxidative damage caused by free radicals or reactive oxygen species (ROS) produced under pathological conditions. According to the results of this study, it is also possible that this parasite causes oxidative stress to sensitize the wall of host red blood cells and consequently increase the osmotic fragility of red blood cells in this condition.

Conclusion

Overall, the present study shows that sheep and goats are infected with *Linguatula serrata* in Iran. The parasite also causes changes in the serum proteins of infected small ruminants. Infection with this parasite may cause oxidative stress and damage the red blood cell structure and increase the instability of their membrane. Further studies are needed to confirm the hypotheses. Knowing how the parasite becomes pathogenic in intermediate hosts can make us somewhat familiar with its possible side effects in humans. It is also important to know the pathogenicity of the parasite for therapeutic and preventive purposes.

Keywords: *Hyperglobulinemia, Intermediate hosts, Linguatula serrata, Membrane osmotic instability*

Acknowledgement: *-This study was financially supported by Shahid Chamran University of Ahvaz, Iran.*

Conflict of Interest: *The authors declare that they have no conflict of interest.*