

Relation between Level of Some Immunological Markers and Liver Functions with Respiratory Bacterial Infection in Sheep

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Abstract

Sheep pneumonia remains an important cause of morbidity and mortality worldwide. The aim of the study report to determine Interleukine-6, IL-10, and liver function test , and kidney function test of sheep suffering from respiratory disease. Blood samples were collected from the diseased and healthy sheep during field study, for biochemical analysis. The results of 50 diseased animals were compared with results of 50 healthy animals. Data of selected biochemical parameters implicated a significant increase in Interleukin-6, alanine aminotransferase and aspartate aminotransferase, and Gamma glutamyltransferase in the diseased group compared to the healthy sheep's. On the other hand, a significant decrease was seen in the levels of IL-10, lactate dehydrogenase and total protein in the diseased group compared to the control. The results of this study could conclude that respiratory infections in sheep were associated with significant biochemical and immunological alterations which upon understanding can provide us with rapid diagnostic tools for pneumonia process in sheep and thus lead to better management and proper treatment. Future research is warranted to determine the response of individual animals to this disease and to describe the metabolic changes which occur during the course of respiratory diseases.

Keywords: Sheep, Pneumonia, blood, clinical biochemistry

Introduction

Sheep represent an important aspect of livestock in Iraq and the Arab world, the number of sheep in the Arab world is more than 105 million, while the number of sheep in Iraq is estimated at about (9) million ⁽¹⁾. It is a great wealth that deserves attention, care and development. Sheep constitute a major source of meat in the Arab world about 33.3% of the total meat, as is the case in developed countries. In addition to being an important source of 16.74% milk, leather and wool ⁽¹⁾. The diseases caused by many microorganism has a negative role in limiting the prosperity and growth of livestock in most countries of the world, including Iraq. This is due to the economic losses resulting from the death of large numbers of animals and obstruction of their normal growth and the decrease in their production, as well as the costs of control and treatment ⁽²⁾. Bacterial infections are widespread among sheep flocks widely,

and at the forefront of this comes the infection of respiratory infections due to open breeding of sheep flocks provides direct and indirect contact among them and other animals for the transmission of these infections between different animals, and epidemiological and environmental factors play an important role in this field ⁽³⁾. Many epidemiological surveys indicated that sheep are exposed to many infections especially those that infected the respiratory system ⁽⁴⁾. These injuries also affect animal health, represented by weight loss, lack of milk production, poor wool, poor birth control and decreased reproductive efficiency, as well as economic damage from veterinary health services and the effort exerted for that ⁽⁵⁾. Consequently, infection with these germs can cause severe anemia in affected animals, loss of appetite, weight loss and a decrease in wool growth that in some causes leads to the animal's death. Young animals are more susceptible to infection while larger

animals are susceptible to resistance to some types of disease⁽⁶⁾.

Because of absence of a comprehensive study on the epidemiology and infection of respiratory infections in sheep in the Tikrit province, the purpose of this study was to determine Interleukine-6,IL-10,and liver functiontest , and kidney function test of sheep suffering from respiratory disease.

Materials and Methods

In The current study we used sheep that suffers from respiratory signs represented by coughing, sneezing, and respiratory secretions in the same time the temperature, pulse and respiratory speed has been measured and other pathological signs associated with respiratory injuries such as enlarged lymph nodes or joints, diarrhea or others were recorded. 60 ewes were used which were divided into two groups. The first group consisted of 30 healthy ewes that were considered as a control group, while the second group consisted of 30 ewes with respiratory diseases that represented the studied group.

Blood samples

Blood samples were obtained from the jugular vein of healthy and infected sheep using test tubes that do not contain anticoagulants to study some biochemical parameters in the serum. It was left at room temperature

for 20 minutes, then a centrifuge was used at 3000rpm for 10 minutes for the purpose of separating the serum and then kept in the Apendorf tubes for the purpose of conducting biochemical analyzes.

The IL-6 , and IL-10 were measured by using the commercial enzyme-linked immunosorbent assay (ELISA) kits. Serum AST, ALT TP, albumin, GGT levels were measured by spectrophotometric kit.

Statistical Analysis

Statistical analysis of data was performed using SAS (Statistical Analysis System - version 9.1). Independent t test was used to assess significant differences between means. P < 0.05 is considered statistically significant.

Results

In Tables 1,2&3 values of serum biomarkers are expressed as mean and mean of standard error (mean ± SEM) of the measured biochemical and immunological parameters in healthy and diseased sheep.

The obtained results revealed that a significant increase in IL-6 162.49±16.04 vs 71.42±6.16 pg/ml:<0.01 values while decreased in IL-10 (3.24±0.42 vs 5.39±0.65 pg/ml:<0.01)were observed in the diseased group compared to the healthy group as shown in Table(1).

Table1: Immunological studies in apparently healthy, and diseased groups.

Parameters	Serum		P-value
	Control	Diseased	
IL-6 (pg/ml)	71.42±6.16	162.49±16.04	<0.01
IL-10(pg/ml)	5.39±0.65	3.24±0.42	<0.01

The mean value of ALT, AST,LDH, and GGT were significantly increased(P<0.01)(127.58± 4.72 vs 84.82±4.91:<0.01),ALT(37±3.5 vs 20.13±2.21:<0.01), (61.07±5.23 vs 19.43±2.14:<0.01) whereas LDH(113.78±7.78 vs 781± 49.86:<0.01) significantly reduced in diseased pneumonic sheep than healthy ones (Table 2).

Table 2: Means (\pm SE) of ALT, AST,LDH, and GGT for two sheep groups (diseased and healthy).

Parameters	Serum		P value
	Diseased	Control	
AST	127.58 \pm 4.72	84.82 \pm 4.91	<0.01
ALT	37 \pm 3.5	20.13 \pm 2.21	<0.01
LDH	113.78 \pm 7.78	781 \pm 49.86	<0.01
GGT	61.07 \pm 5.23	19.43 \pm 2.14	<0.01

There was a significant decrease ($P < 0.01$) in the concentration of total proteins in infected (5.08 \pm 5.74 g/dl) as compared with healthy (9.11 \pm 0.59) and decrease in albumin (2.74 \pm 0.45 vs 3.97 \pm 0.20 g/dl ($P < 0.01$) in diseased pneumonic sheep than healthy ones (Table 3).

Table 3: Means (\pm SE) of total proteins, albumin for two sheep groups (diseased and healthy)

Parameters	Serum		P-value
	Control	Diseased	
Total proteins(g/dl)	9.118 \pm 0.59	5.08 \pm 0.57	<0.01
Albumin (g/dl)	2.74 \pm 0.45	3.97 \pm 0.20	<0.01

Discussion

Cytokines are a heterogeneous group of soluble small polypeptides with a wide range of inflammatory, metabolic and immunomodulatory properties and tissue homeostasis, that play a critical role in mediating inflammatory processes and tissue homeostasis underlying pneumonia^(7,8).

Serum biochemical alterations in respiratory diseases were common and might display reasonably predictable changes in response to inflammation. In this regard, the results of our study revealed higher serum levels of IL-6 in the diseased group versus control group, suggesting the implication of systemic inflammation in diseased groups.

Interleukin-6 (IL-6) is a multifunctional inflammatory cytokine produced in response to tissue damage and infections. Multiple cell types including fibroblasts, keratinocytes, mesangial cells, vascular endothelial cells, mast cells, macrophages, dendritic cells, and T and B cells are associated with the production of this cytokine. After targeting its specific receptor^(9,10).

In an animal trial, IL-6 was shown to affect megakaryocytopoiesis, leading to bigger platelets released into the circulation⁽¹¹⁾. The mechanism of action of IL-6 on platelet size in pneumonia is unclear.

Interleukin 10 (IL-10) is considered an immunoregulatory cytokine produced by monocytes, macrophages, B and T lymphocytes, brain cells such as neurons and microglia that capable of inhibiting proinflammatory responses. Despite the previous fact,

IL-10 is a pleiotropic cytokine that can exert either immune-stimulatory or immunosuppressive properties on many cells⁽¹²⁻¹⁴⁾. In the present study, the IL-10 in affected sheep in farm (A) showed significant decrease which was similar to Fernández *et al.*⁽¹⁵⁾ who reported a significant interaction between the microorganisms and cytokine markers that leads to significant decrease in IL-10 in diseased group.

Liver is one of the largest organs in the body. It has many important metabolic functions. Liver tissue has a relatively large amount of enzymes activity and produces inflammatory cytokines^(16,17).

Data obtained from the present study implicated a significantly high serum enzymatic activity of AST, ALT, ALP in the diseased group. These changes could be changes in the antioxidant abilities that occurred in liver, may be attributed to cellular damage in the liver alters their transport function and membrane permeability, leading to leakage of enzymes from the cells. For it, the marked release of AST and ALT from liver cytosol into circulation refers to the extensive damage of hepatic tissue membranes^(18,19), which attributed to dysfunction of various organs including liver due to hepatic degenerative and necrotic changes caused by bacterial infection and toxins. Similar to findings observed by Hassan HY⁽²⁰⁾, El-Deebet *et al.*⁽²¹⁾ and Donia *et al.*⁽²²⁾. Furthermore, a significant increase in the ALP may be resulting from the elevated biliary pressure and acute cell necrosis caused the release of ALP from its membranes bound site and its entry into blood⁽²³⁾. LDH is one of the potential variables presented in the literature as a possible indicator of lung damage. Increased LDH activity in sheep in the diseased group probably resulted from increased respiratory rate and muscle work during prolonged duration or severe cases of respiratory disease⁽²⁴⁾. Similar finding were recorded by Nagy *et al.*⁽²⁵⁾.

As markers of renal function, serum concentrations of creatinine and blood urea were measured. Creatinine is a breakdown metabolite of creatine phosphate in muscle. It is eliminated by kidneys, mostly via

glomerular filtration and partly via secretion at proximal tubule. Therefore, creatinine clearance could be used to estimate the glomerular filtration rate (eGFR) by measuring serum creatinine (SCr), urine creatinine, and volume. Additionally, SCr can be a reflection of muscle mass and diet⁽²⁶⁾. Blood urea nitrogen (BUN) is a common marker of kidney function, and hence its blood concentrations vary according to change in glomerular filtration rate (GFR). BUN levels are increased by the catabolism of lean body mass and lower levels of BUN may be a marker of the cessation of catabolism, which is certain for respiratory muscle recovery and a well-known predictor of weaning⁽²⁷⁾.

The results revealed that the mean values of serum creatinine and urea blood urea concentrations were significantly higher in the diseased ewes compared to control. The obvious implication of this observation is that, the high level of serum urea has been attributed to excessive tissues protein catabolism associated with protein deficiency and could be explained also by the accelerated catabolism of body protein and could result as a response to infection. The present study demonstrate that the increasing level of serum creatinine in diseased group than control group 1.35 & 1.09 mg/dl respectively. High creatinine is indicative of poor protein and amino acid metabolism that can lead to impaired renal function or might be attributed to kidney dysfunction after infection, which would be promoted by a reduction in the glomerular filtration rate^(28,29). Creatinine is formed by the degradation of phosphocreatine for energy release in the skeletal muscle⁽³⁰⁾.

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References

1. FAO. Global Forest Resources Assessment 2010: Main report. FAO Forestry Paper 163;2013.
2. Abaza, S.H. Heat shock proteins and parasitic diseases. *Bio Med Res Int*:2017;46-50.

3. Reneé,F.H. Review: Solutions For Grand Challenges In Goat And Sheep Production Industry. The Southeast Asian Journal of Tropical Biology. 2017; :46-50.
4. Levine,W.T. Isolation of *Lautropia mirabilis* from oral cavities of human immunodeficiency virus-infected children. J. Clin. Microbiol. 2011;20:433-436.
5. Anderson,K.L. and Hall,O.Y.Effect of age, weight, and metabolic rate on endurance, hyperthermia, and heatstroke mortality in a small animal model. Aviat. Sp. Environ. Med. 2015;57: 974–979 .
6. Ijaz,K.H.; Jacquet, P., J. Cabaret, F.Colas , M.L. Dia , D. Cheikhand A.Thiam.Infection rate and chemotherapy of various helminthes in diarrhoeic sheep in and around Lahore. Vet. Res. Commun.2015; ,16(6):437-
7. Zubaida N.M Albarzanji, Thikra Abdullah Mahmood ,Entedhar Rifaat Sarhat , Kasim Sakran Abas. Cytokines Storm Of COVID-19 And Multi Systemic Organ Failure: A Review. Sys Rev Pharm 2020;11(10):1252-1256
8. Sarhat ER, Rmaid ZJ, Jabir TH. Changes of salivary interleukine17, Apelin, Omentin and Vaspin levels in normal subjects and diabetic patients with chronic periodontitis, Ann Trop Med & Pub Health;2020; 23:S404. DOI: <http://doi.org/10.36295/ASRO.2020.23118>
9. Entedhar Rifaat Sarhat. Acute Myocardial Infarction: Melatonin, Apelin, and Visfatin as Predictors of Disease. Diyala Journal of Medicine.2017; 11(13): 11-17
10. Velazquez-Salinas L, Verdugo-Rodriguez A, Rodriguez LL and Borca MV . The Role of Interleukin 6 During Viral Infections. Front. Microbiol.2019;10:1057. doi: 10.3389/fmicb.2019.01057
11. Melisa Lilisari, Heda Melinda Nataprawira, Dida Akhmad Gurnida. Serum interleukin-6 and mean platelet volume in pediatric pneumonia. Paediatrica Indonesiana 2016;56:57-61.
12. Abdalla OA, Haidy GA, Yousseff FM, Rehab AY. Clinicopathological Alterations in Blood and Sera of Sheep due to Respiratory Affections. J Clin Pathol Forecast. 2019; 2(2): 1006.
13. Entedhar R. Sarhat ,Zubaidah N. M Albarzanji and Chateen I. Ali Pambuk. Estimation of Some Interleukins in Cerebrospinal Fluid in Children with Meningitis. Biomedical & Pharmacology Journal, December 2019. 12(4):2151-2155.
14. Intesar Jasim Mohammed, Entedhar Rifaat Sarhat ,Marwa Abdul-Salam Hamied, Thuraia Rifaat Sarhat. Assessment of salivary Interleukin (IL)-6, IL-10, Oxidative Stress, Antioxidant Status, pH, and Flow Rate in Dental Caries Experience patients. Sys Rev Pharm 2021;12(1):55-59.
15. Fernández S, Galapero J, Rey J, Pérez CJ, Ramos A, et al. Citokines Study in Lungs Infected with Pasteurellaceae and *Mycoplasma* spp. from Fattening Lambs. J Med Microbiol Immunol Res, 2018; 2:1.
16. Sayran Sattar Saleh ,Entedhar Rifaat Sarhat. Effects of Ethanolic *Moringa Oleifera* Extract on Melatonin, Liver and Kidney Function Tests in Alloxan-Induced Diabetic Rats. Indian Journal of Forensic Medicine & Toxicology, 2019;13(4): 1015-1015.
17. Nawal A. Al- Madany ,Entedhar R. Sarhat. Determination of Some Biochemical Parameters of Patients with Hepatitis B in Kirkuk City. Kirkuk University Journal /Scientific Studies (KUJSS):2018; 13(2):139 – 148.
18. Entedhar R. Sarhat ,Siham A. Wadi , Saba K. Ibrahim. The Influence of Lycopene on Interleukin-6, Tumor Necrosis Factor - α , Alanine Aminotransferase, Aspartate Aminotransferase Levels In Streptozotocin -Induced Diabetic Rabbits. 3 rd Scientific Conference - College of Veterinary Medicine - University of Tikrit 2,3 May 2016.
19. Entedhar Rifaat Sarhat, Siham A. Wadi, Ban I. Sedeeq, Thuraia R. Sarhat and Nawar A. Jasim.(2019). Study of histopathological and biochemical effect of *Punicagranatum L.* extract on streptozotocin -induced diabetes in rabbits. Iraqi Journal of Veterinary Sciences, 2019; 33(1): 189-194.
20. Hassan HY, Kamr A, Abdelazeim A & Khaled H. Hemato-Biochemical Response with Highlights on the Role of Oxidants and Antioxidants in Pneumonic Sheep. J Vet Marine Sci, 2019;1(1): 7-14.
21. El-Deeb WM, Tharwat M. Lipoproteins profile, acute phase proteins, proinflammatory cytokines and oxidative stress biomarkers in sheep with pneumonic pasteurellosis. Comp Clin Pathol. 2015; 24: 581-588.

22. Donia GR, Wassif IM, Ebissy EL. Impact of some environmental factors and microbes causing respiratory diseases on antioxidant levels in small ruminants. *Global Veterinaria*, 2014; 12: 299-306.
23. Aziza Amin and Mona H. Ibrahim .Biochemical and Histopathological Effect of Ultrasound Waves on Hepatic Tissue of Rats' Neonates. *Arab J. Nucl. Sci. Appl.*2021; 54(1): 78-84.
24. Helena Šoltésová, VeronikaNagyová, CsillaTóthová, Oskar Nagy. Hematological and blood biochemical alterations associated with respiratory disease in calves. *ACTA VET. BRNO* 2015, 84: 249–256; doi:10.2754/avb201584030249
25. Nagy O, Tóthová Cs, Seidel H, Paulíková I, Kováč G. The effect of respiratory diseases on serum lactate dehydrogenase and its isoenzyme patterns in calves. *Polish J Vet Sci.*2013; 16: 211-218.
26. Thongprayoon, C., Cheungpasitporn, W., Chewcharat, A. *et al.* The Association of Low Admission Serum Creatinine with the Risk of Respiratory Failure Requiring Mechanical Ventilation: A Retrospective Cohort Study. *Sci Rep.*2019; 9, 18743.
27. Choudhary M., Yogi J.P., Fiza B., Sinha M., Rathor H.K., Chawdhary V., Evaluation of blood urea nitrogen, uric acid and uric acid/creatinine ratio in patients of chronic obstructive pulmonary disease (COPD). *Int.J.Med.Sci.Educ* 2020;7(2):21-24
28. Saleh, N. S. and Allam, T. S. Pneumonia in sheep: bacteriological and clinicopathological studies. *American Journal of Research Communication*, 2014;2(11): 73-88.
29. Mandal, R., Gupta, V., Joshi, V., Kumar, S., &Mondal, D. Study of ClinicoHematobiochemical Changes and Therapeutic Management of Naturally Infected Cases of Respiratory Disease in Non-Descript Goats of Bareilly Region. *International Journal of Livestock Research*,2017; 7(6): 211-218.
30. Carlos, M.M., Leite, J.H. Chaves, D.F. Vale, A.M. Façanha, D. A..Melo M.M. and Soto-Blancoc, B. Blood Parameters in the Morada Nova Sheep: Influence of Age, Sex and Body Condition score. *J. Anim. Plant Sci*,2015; 25(4): 950-955.