

Hemato-Biochemical Response with Highlights on the Role of Oxidants and Antioxidants in Pneumonic Sheep

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Received August 05, 2019; Accepted August 22, 2019; Published October 08, 2019

ABSTRACT

This study was aimed to determine hemato-biochemical changes, the oxidants biomarkers including malondialdehyde (MDA), nitric oxide (NO) and to evaluate the total antioxidant capacity (TAC) and Glutathione peroxidase (GPX) as antioxidants in pneumonic sheep. This study was conducted on a total of adult twenty sheep including sheep suffered from pneumonia (n=10) and healthy sheep were confirmed by physical examination (n=10). Blood and tissue samples were collected from the diseased and healthy sheep during field study, for hematological, biochemical analysis and determine the oxidant/antioxidant in blood and tissues. The results showed statistically significant increased MDA and NO and reduced TAC and GPX in pneumonic sheep than healthy ones ($P<0.01$). Red blood cells (RBCs), total leukocytic counts (TLC), Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) were significantly increased in diseased group compared to control ones ($P<0.05$). Young age pneumonic sheep <2 years old were more likely to have elevated MDA and decreased TAC than old ones ($P<0.05$). It was concluded that reduced concentrations of TAC and GPX and elevated MDA and NO could suggest sever oxidative stress in pneumonic sheep. Moreover, oxidative stress was more associated with young age pneumonic sheep.

Keywords: Sheep, Malondialdehyde, Nitric oxide, Total antioxidant capacity, Glutathione peroxidase, Pneumonia

INTRODUCTION

Sheep are small ruminants that have specific properties rather than other livestock resources. They are more acclimatized to various ranges of environment, have short generation cycles and reproductive rate which lead to high production efficiency and impoverished people can buy them with less cost [1].

Pneumonia is multifactorial disease which always involves a combination of infectious causes as well as predisposing environmental and managerial factors [2]. Pneumonia is a respiratory disease resulted from an inflammatory response of the bronchioles and alveoli in the lungs to any causative agents; this is lead to consolidation of the lung tissue. It is a common disease of sheep in all sheep-producing countries [3,4].

Oxidative stress is an imbalance between free radical production and radical-removal mechanism resulting from increased production of pro-oxidants and/or a decrease in antioxidant status in the body [5]. Free radicals, which include reactive oxygen species (ROS) and reactive nitrogen species (RNS), are reactive chemical products that may result in oxidative damage by affecting macromolecules like

lipids, carbohydrates, proteins and nucleic acids [6]. Antioxidant is the first defense mechanism against free radicals and has a major role in prevention the hazard effects of oxidants in organs as lung, kidney [7].

Lipid peroxidation is a major factor involved in cellular damage and is used as oxidative stress marker in body fluids, cells and tissues. Lipid peroxides are not stable compound and when rapidly dissociated to form reactive carbonyl compounds. Polyunsaturated fatty acid peroxides generate malondialdehyde during the decomposition [8].

Nitric oxide is generated by different cells in the lung and plays a crucial physiological role in regulating pulmonary

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Citation: Hassan HY, Kamr A, Abdelazeim A & Khaled H. (2019) Hemato-Biochemical Response with Highlights on the Role of Oxidants and Antioxidants in Pneumonic Sheep. J Vet Marine Sci, 1(1): 7-14.

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vasomotor tone through many recognized factors [9]. Nitric oxide has various functions in the body including vasodilator, neurotransmitter, immunomodulator, antioxidant and pro-oxidant in many systems [10]. Nitric oxide plays a major role in mediating cytotoxic effects against virulent agents such as viruses, bacteria, fungi and protozoa and is generated from L-arginine by the catalytic action of nitric oxide synthase (NOS) [11,12]. Beside its Physiological properties, nitric oxide has a significant role in the immunity by activation of macrophages [13].

The antioxidants consider the first defensive line in the body against free radicals includes some antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione reductase (GR) [14,15]. Antioxidants have a vital role in preventing or repairing the damage caused by reactive oxygen species (ROS), in addition to regulation of redox-sensitive signaling pathways [16]. The neutralization process of hydrogen peroxide and organic peroxides is usually obtained by glutathione peroxidase activity [17].

The cumulative action of all the antioxidants can be indicated by measurement of TAC level in plasma and body fluids, thus providing an integrated parameter rather than the single sum of measurable antioxidants [18].

The aim of the present study were to focus on the possible effect of pneumonia on oxidants and antioxidants markers in addition to alteration in hematological and biochemical dynamics in hospitalized pneumonic sheep.

MATERIAL AND METHODS

Animal's criteria

A completely randomized field study was carried out on a total of 20 adult female sheep aged between 1-3 years old were collected in different seasons throughout the year and were divided into two main groups according to their body condition score (BCS), their general inspection and physical examination. **Group 1:** Included 10 apparently healthy sheep showed no clinical signs of diseases and free from external and internal parasites. The body condition score (BCS) of this group was ≥ 3 and act as a control group [19,20]. **Group 2:** Included 10 diseased sheep admitted to veterinary clinics at different area at Menofya Governorate suffered from general debility, poor health conditions included signs of anemia, emaciation, dehydration, cough, in appetite, nasal discharge and harsh vesicular sound and confirmed by physical examination and their BCS was <3 [19,20]. Furthermore, hospitalized pneumonic sheep was categorized based on their age into pneumonic sheep >2 years ($n=12$) and <2 years ($n=8$).

Clinical information

The clinical history of sheep was obtained from their owners. Physical examination, serum biochemistry and hemogram were determined for all sheep. Body condition

scoring in sheep is determined by estimation of muscle and amount of fat cover the lumbar processes scaled on a scoring system of '0' to '5', The BCS of an animal is evaluated by the palpation of the lumbar region, specifically on and around the backbone (spinous and transverse processes) in the loin area, immediately behind the last rib and above the kidneys to detect the degree of sharpness or roundness [19,20].

Samplings

Blood samples: Two blood samples were collected from jugular vein of each sheep using sterile syringe. The first one was collected in dry clean labeled test tube contain anticoagulant, Ethylene diamine tetra acetic acid (EDTA) for complete blood count (CBC) and The second sample collected in dry clean centrifuge tube and kept in sloping position without agitation till coagulation. The clotted samples centrifuged at 3000 rpm for 10 min for separation a clear non-hemolysed serum for biochemical analysis. Sera were kept in deep freezer at -20°C till biochemical analysis.

Tissue samples: Lung tissue samples were collected before dissection, the tissue samples were perfused with a phosphate buffered saline solution with pH 7.4 containing 0.16 mg/ml heparin to remove any red blood cells then the tissues were homogenized in 5-10 ml cold buffer (i.e., 100 mM potassium phosphate, pH 7.0, containing 2 mM EDTA) per gram tissue and centrifuged at 4,000 rpm for 15 min at 4°C till obtaining the supernatant. These are for determination of glutathione peroxidase activity and nitric oxide.

Hematological and biochemical measurements

Hematological examinations included Complete Blood Count (CBC), Red Blood Cells Count (RBCs), Packed Cell Volume (PCV), Hemoglobin (Hb), Mean Corpuscle Volume (MCV), Mean Corpuscle Hemoglobin (MCH), Mean Corpuscle Hemoglobin Concentration (MCHC), Total Leukocytic Count (TLC) and differential Leucocytic Count (DLC) were carried out on apparently healthy and diseased sheep according to methods described by Meyer et al. [21].

Total protein, albumin, ALT and AST were measured in serum by UV-colorimetric spectrophotometric method using special kits according to Doumas [22] and Reitman and Frankel [23], respectively. Globulin was calculated by subtraction of albumin value from total protein value and then A/G ratio was calculated by dividing albumin on globulin concentrations [24].

Determination of oxidants and antioxidants status

MDA in serum samples was measured by using a special kit in which MDA reacts directly with thiobarbituric acid at optimum pH (3.5) to produce a red color that was measured spectrophotometrically [25]. NO in tissue samples was measured by using a special kit [26]. Total antioxidant capacity was determined according to the method of using a

special kit in which a Fe-EDTA complex reacts with hydrogen peroxide by a Fenton-type reaction, leading to the formation of hydroxyl radicals that degrade benzoate, followed by release of thiobarbituric acid reactive substances [27]. GSH activity was evaluated by using special kit at which Glutathione peroxidase catalysis the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and NADPH, the oxidized glutathione is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP⁺. The decrease in absorbance at 340 nm was measured [28].

STATISTICAL ANALYSIS

Data from healthy and diseased sheep were compared by means of independent t test by using the statistical package for social science (SPSS) for windows (Version 16.0; SPSS Inc., Chicago, Ill.). Results were expressed as the mean $\pm$ standard error (SEM), Significance was set at $P < 0.05$. Univariate analysis was done based on age of hospitalized sheep with reference to 5-95% confidence interval from healthy sheep. Receiver operating curve (ROC) was determined using GraphPad Prism 8.

RESULTS AND DISCUSSION

The mean value of RBCs, PCV and TLC were significantly increased in diseased pneumonic sheep than healthy ones

($P < 0.05$; **Table 1**). We have also shown that serum total protein and albumin concentrations were significantly increased in pneumonic sheep rather than healthy group as demonstrated in **Table 1** ($P < 0.05$). Our findings were agreed with the results that obtained by Maina et al. [29] and this due to that diseased sheep could be exposed to dehydration that accompanied by loss of fluid [29]. TLC was significantly increased in pneumonic sheep which is similar to a previous study that reported by Maina et al. [29] and Chirkena et al. [30] as in acute inflammatory diseases TLC increased especially those due to bacterial infections. This could be explained tissue injury due to acute inflammation during bacterial infections stimulate various types of cells to produce growth factors, cytokines, and other mediators of inflammation that in turn increase TLC and more production, proliferation, maturation and bone marrow release of mature and immature neutrophils [31] Additional explanation for this neutrophilic leukocytosis stress factors to which the animal exposed during the course of the respiratory diseases that lead to endogenous production and increase of corticosteroids which play a vital role in regulation of circulating concentration of leukocytes in moderate and severe pneumonia [32].

Table 1. Hemato-biochemical parameters in healthy and pneumonic sheep.

Variables	Healthy sheep (n=10)	Pneumonic sheep (n=10)
RBCs ($10^6/\mu\text{l}$)	10.88 ± 0.47^b	14.71 ± 0.66^a
Hb (g/dl)	11.42 ± 0.5^b	13.46 ± 0.77^a
PCV (%)	34.25 ± 1.65^b	42.25 ± 2.21^a
TLC ($10^3/\mu\text{l}$)	10.10 ± 1.18^b	14.5 ± 1.67^a
ALT (U/L)	29.28 ± 2.10^b	37.0 ± 1.74^a
AST (U/L)	38.57 ± 1.52^b	45.57 ± 3.16^a
Total protein (g/dl)	6.30 ± 0.67^b	7.9 ± 2.85^a
Albumin (g/dl)	3.28 ± 0.17^b	4.44 ± 0.32^a
Globulin (g/dl)	3.01 ± 0.71^b	5.21 ± 1.17^a
A/G ratio	1.08 ± 0.49	0.84 ± 0.36

n: number; RBCs: Red Blood Cells; Hb: Hemoglobin; PCV: Packed Cell Volume; TLC: Total Leukocytic Count; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase
Means with different letter superscripts in the same column are significantly different at ($P < 0.01$)

ALT and AST were increased in diseased pneumonic sheep rather than control one ($P < 0.01$; **Table 1**). Similar to findings observed by El-Deeb et al. [33] and Donia et al. [34]. This might be due to the changes in the antioxidant abilities that occurred in liver and in the phospholipid

structure of the cell membrane which lead to high levels of ALT and AST as markers of liver damage according to Deger et al. [35]. Of interest, serum globulins concentrations were increased in pneumonic sheep compared to healthy ones. Globulins are serum protein produced by liver and

categorized into α -, β - and γ -globulins in ruminants which are produced and increased in response to acute inflammation which is the case of pneumonic sheep of this study suggesting sever oxidative stress on the liver [36].

The present study showed a significant increase in the levels of MDA pneumonic sheep when compared to its levels in the healthy ones ($P<0.01$; **Table 2**). TAC concentrations were inversely correlated with MDA concentrations in pneumonic sheep ($r_s=-0.6$; $P<0.01$; **Figure 1**). Moreover, MDA and NO were associated with severity of disease in pneumonic sheep (ROC=0.95; $P<0.05$; **Figure 2**). Additionally, young age pneumonic sheep <2 years old were

more likely to have increased oxidant MDA (OR= 1.97; 95% CI= 1.02-15.6; $P=0.04$; **Table 3**) than old one >2 years old. The increase in MDA level could be explained by the risk effect of cellular damage and inflammation which are associated with bronchopneumonia and broncho-interstitial pneumonia in addition to destruction of epithelial cells and fibrinous reaction resulted from vascular damage. This clearly clarified that the measurement of MDA level, is closely linked to the inflammatory reactions and mechanisms of cellular response [37]. MDA is the best marker of oxidative stress [38], its level increases during any stressful condition.

Table 2. Serum levels of oxidant/antioxidant levels in healthy and pneumonic sheep.

Variables	Healthy sheep (n=10)	Pneumonic sheep (n=10)
MDA (nmol/ml)	2.02 ± 0.19^b	5.1 ± 0.97^a
NO ($\mu\text{mol/L}$)	36.46 ± 2.48^b	47.04 ± 0.40^a
TAC (mM/L)	0.70 ± 0.06^a	0.28 ± 0.06^b
GPX (U/gT)	66.67 ± 6.33^a	35.64 ± 3.61^b

n: number; MDA: Malondialdehyde; NO: Nitric Oxide; TAC: Total Antioxidant Capacity; GPX: Glutathione Peroxidase Means with different letter superscripts in the same column are significantly different at ($P<0.01$)

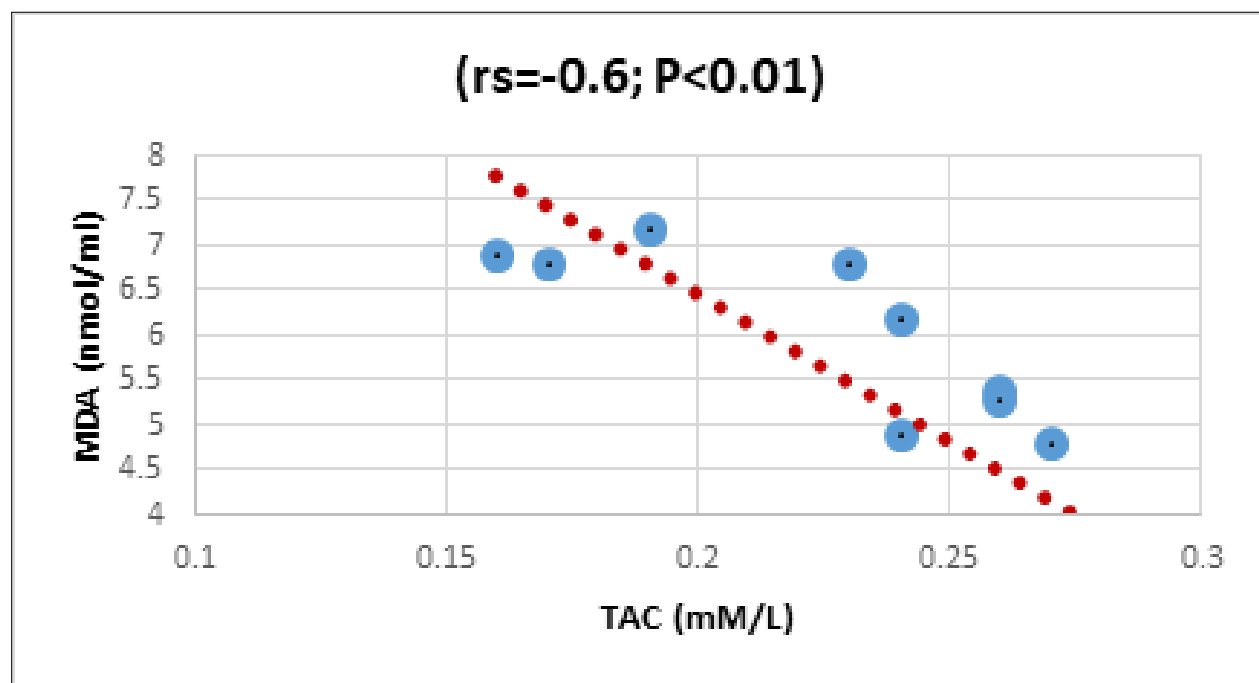


Figure 1. Pearson correlation between MDA and TAC showed inverse relationship in pneumonic sheep ($P<0.05$).

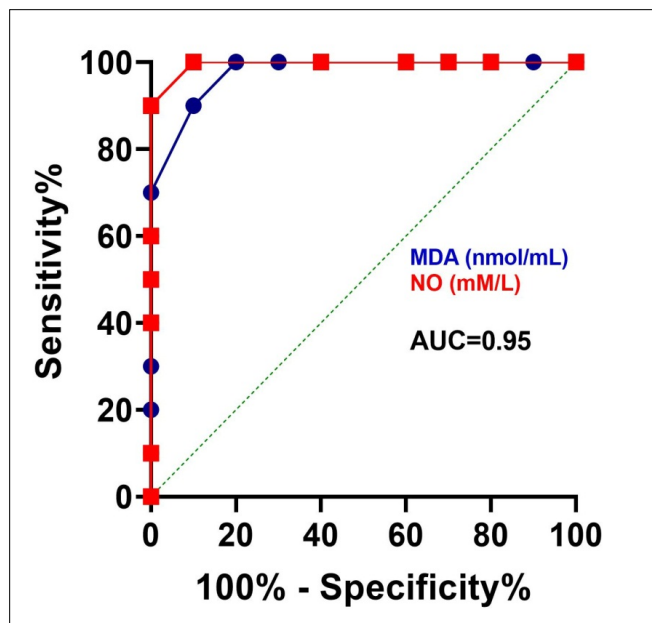


Figure 2. Receiver operating characteristic curve (ROC) for the association of MDA and NO with severity of disease in pneumonic sheep ($P < 0.05$).

Table 3. Univariate logistic regression for (MDA) and (TAC) in hospitalized pneumonic sheep.

MDA>2.02 (nmol/mL)	Range	B	S.E.	Wald Statistics	OR	95% CI	P value
Age	<2 years old	1.2	0.7	5.7	1.97*	1.02-15.6	0.04
	>2 years old	0.6	0.5	1.4	0.75	0.25-3.08	0.77
NO>36.46 (μmol/L)	Range	B	S.E.	Wald Statistics	OR	95% CI	P value
Age	<2 years old	0.4	0.6	1.2	0.4	0.20-2.4	0.8
	>2 years old	0.7	0.4	1.6	0.63	0.32-4.07	0.6
TAC<0.70 (mM/L)	Range	B	S.E.	Wald Statistics	OR	95% CI	P value
Age	<2 years old	1.6	0.8	5.9	2.04*	1.16-20.4	0.02
	>2 years old	0.5	0.4	1.2	0.754	0.18-4.2	0.9
GPX<66.67 (U/gT)	Range	B	S.E.	Wald Statistics	OR	95% CI	P value
Age	<2 years old	1.8	0.7	1.9	0.8	0.62-5.8	0.3
	>2 years old	1.5	0.6	1.5	0.62	0.45-3.85	0.7

MDA: Malondialdehyde; NO: Nitric Oxide; TAC: Total Antioxidant Capacity; GPX: Glutathione Peroxidase; OR: Odds Ratios; CI: Confidence Intervals; S.E: Standard Error; B: Regression Coefficients

* $P < 0.05$

The significant increase in NO level in diseased pneumonic sheep was also found by Yuksek et al. [39]. In case of respiratory diseases, NO played an important role in the immune system and its consumption is in surge with the severity of infection in a parallel way [40-47]. Alveolar macrophages are proven to have major roles for immunity in

the lower respiratory tracts and NO produced by alveolar macrophages has significant roles in respiratory system infections [48]. Moreover, previous studies clarified that the imbalance between oxidative compounds and local antioxidant system caused inflammation in the lungs,

increased alveolar capillary leakage and reduce the functions of surfactant [49-52].

There was a significant decrease in the concentration of TAC and GPX in diseased pneumonic sheep than healthy ones ($P < 0.01$; **Table 2**). Furthermore, we have documented that young age pneumonic sheep <2 years old were more likely to have decreased TAC (OR=2.04; 95% CI=1.16-20.4; $P=0.02$; **Table 3**). GPX was significant diminished in lung tissue of hospitalized sheep than healthy ones as recorded by Donia et al. [34], Jarikre et al [37] and Nisbet et al. [53]. Significant reductions were observed in broncho-interstitial pneumonia and bronchopneumonia [37]. Phagocytic cells and neutrophils are protected by (GSH-Px), from oxidative damage caused by free radicals, its decreased level shows that the host's defense system is affected by oxidative stress and that there is a severe damage of cells of the immune system [54-57].

CONCLUSION

Reduced TAC and GPX together with increased MDA and NO concentrations in hospitalized pneumonic sheep could indicate sever oxidative stress. Elevated MDA and reduced TAC in young age hospitalized pneumonic sheep suggest more likely oxidative stress rather than old ones. Pneumonic sheep were associated with alterations in hemato-biochemical dynamics during course of disease. Addition of antioxidants therapeutic strategy to routine pneumonia therapy might add value for enhancing recovery of pneumonic sheep [58-61].

ACKNOWLEDGMENT

Our grateful thanks to all veterinarians and technicians at The Faculty of Veterinary Medicine, University of Sadat City for supporting this study.

CONFLICT OF INTERESTS

No conflict of interests was declared.

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