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Research Article

Evaluation of hematological, antioxidant enzymes and oxidative stress parameters in buffaloes infected with babesiosis

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ABSTRACT

This study aimed to determine how the babesiosis infection affected hematology, oxidative stress, and antioxidant enzymes in buffaloes. A total of 41 babesia positive and 10 healthy buffaloes having different ages were investigated based on parasitemia. Blood and serum samples were collected from positive and healthy buffaloes for the laboratory examination. Animals had different clinical signs including pyrexia (104°F ± 1.0°F), chocolate color urine, edema of face and legs in 2-3 cases, congested and hyperemic eyes, lethargy, emaciation, watery exudate from eyes and pale mucous membrane of eyes were observed. Results did not show a significant association between buffaloes' age in occurrence of parasitemia. Results on hematological values indicated significantly increased total leukocyte counts, basophil counts, eosinophil and neutrophil counts while significantly reduced values of red blood cell counts, hematocrit, hemoglobin, monocyte and lymphocyte counts in infected buffaloes. The results regarding different antioxidant enzymes in babesia positive cases showed a significantly lower quantity of serum total proteins, albumin concentration, catalase, reduced glutathione and superoxide dismutase as compared to non-infected animals. Results on some oxidative stress parameters indicated significantly higher values of nitric oxide scavenging activity and malondialdehyde quantity in positive buffaloes. In conclusion, the findings of this study showed that babesiosis infection remarkably influences the hematology, oxidative stress and antioxidant enzymes in buffaloes.

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Introduction

In Pakistan, different livestock animals like camels, cattle, buffaloes and various small ruminants are commonly reared in small groups for meat and milk production (Ali et al. 2017; Hussain et al. 2020). The animals in Pakistan are usually and routinely kept under subtropical and tropical conditions and suffer from various viral (Hussain et al. 2020), bacterial (Mahmood et al. 2014; Hussain et al. 2019) and parasitic diseases (Zafar et al. 2019; Abbas et al. 2020; Ali et al. 2020; Zaman et al. 2020). Babesia parasite (*Babesia bovis*) is a tick-borne intracellular parasite responsible for causing babesiosis in animals including cattle (Hakimi et al. 2021; McFadzean et al. 2021). Babesiosis causes severe economic losses in livestock animals and is characterized by inducing high morbidity and mortality (Jacob et al. 2020). *B. bovis* completes its life cycle with sexual and asexual reproduction in vectors (ticks) while asexual replication in intermediate hosts. The disease starts with the entrance of sporozoites of parasite released from the tick's salivary glands, followed by growth and multiplication in the red blood cells of intermediate hosts. Bovine babesiosis is a tick-transmitted hemoprotozoan disease that is mainly caused by *B. bovis* and *B. bigemina* and is characterized by

significant morbidity and mortality worldwide. It is reported that the *B. bovis* parasite can be seen as binary or ring forms inside the erythrocytes of infected animals (Hakimi et al. 2021). The disease is prevalent in many parts of the world and threatens the production and development of different dairy animals like cattle and buffaloes in Central, and South Asia (Mahmoud et al. 2015; Elsworth and Duraisingh 2020; Jacob et al. 2020).

Studies have reported that the supply of antioxidant enzymes during the pathogenesis becomes insufficient, and the generation of free radicals gets unregulated, leading to oxidative stress in the host (Esmaeilnejad et al. 2020). The tissue damage induced by free radical production is characterized by lipid peroxidation (LPO) and has been reported in many pathological conditions. Similarly, in the present investigation, a considerable elevation in RBC's osmotic fragility was seen in infected calves with varied parasitemia rates. During parasitemia, RBCs elimination through the antioxidant system increases due to lipid peroxidation and reactive oxygen metabolites production. Membrane lipids are the primary targets of reactive oxygen species (ROS) during cellular damage (Su et al. 2019). The interaction of ROS with the abundant polyunsaturated fatty acids (PUFA) leads to the production of malondialdehyde (MDA) at the cellular level, which interacts with the components of the cell membrane, resulting in increased enzyme activity and cell permeability (Jaiswal et al. 2019). MDA levels beyond a certain threshold indicate that antioxidant defense mechanisms are insufficient to mitigate oxidative stress. As a result, oxidative stress-induced erythrocyte damage could be linked to lipid peroxidation (Esmaeilnejad et al. 2018). This process can also result in decreased symmetry of membrane and increased permeability, leading to increased osmotic fragility and alteration in the surface of RBC. As a result, these altered erythrocytes would be vulnerable to erythrophagocytosis which is a typical occurrence in severe anemia (Jalali et al. 2018). Antioxidant enzymes, particularly catalase (CAT), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) can be affected by oxidative damage. Lipid peroxidation has also been linked to erythrocyte oxidative degradation and anemia, and it could be the cause of RBC structural abnormalities and osmotic fragility, making them vulnerable to phagocytosis by reticulo-endothelial cells (Dhanasree et al. 2020). Antioxidant enzymes are synthesized using microminerals including selenium, zinc and copper, which are key components of the antioxidant defense mechanism against free radical-induced tissue damage (Esmaeilnejad et al. 2016). Due to a lack of information on the role of the inflammatory process and oxidant/antioxidant interactions in bovine babesiosis, this study was planned to clarify the oxidative stress by measuring the activities of key anti-oxidases.

Materials and Methods

Animals and samples

This research was carried out in two districts (Lodhran and Bahawalpur) of Punjab Province, Pakistan, where bovine tick-borne diseases are common during the hot seasons (April to late September). This study included a total of 375 buffaloes kept in different villages of the two districts. The blood samples were collected from morbid animals showing fever and apparently healthy. History regarding various clinical signs like dullness, less feed intake, increased rectal temperature, dyspnea, lethargy, icterus and chocolate color urine was recorded.

Blood collection and parasitological examination

Blood sample without any anticoagulant was collected from the jugular veins of buffaloes. Fresh blood films were immediately prepared from each buffalo for parasitological examination. All the blood smears were stained with Giemsa stain for microscopic examination of babesia parasite. An equal number of blood samples were obtained from buffaloes to estimate various hematological parameters like RBC count, packed cell volume (PCV), haemoglobin (Hb). Total and differential white blood cell counts were done using automated haematology analyzer according to previous procedure (Qayyum et al. 2016).

Oxidative stress and antioxidant enzymes in erythrocytes

To assess different oxidative stress parameters and antioxidant enzymes, all the collected blood samples were centrifuged at 5000rpm for 5 minutes, and the plasma was discarded. After that, the separated erythrocytes were washed two times and the cleaned red blood cells were hemolyzed using ice-cold distilled water ultimately yielding 10% hemolysate of erythrocytes. Various oxidative stress biomarkers including MDA and Nitric oxide (NO) were measured according to previously published protocol (Sreejayan and Rao 1997; Ghosh et al. 2015). Serum albumin and total serum proteins were measured as reported in earlier protocol. Some antioxidant enzymes in erythrocytes such as superoxide dismutase, catalase, and reduced glutathione were estimated using previous procedures (Madesh and Balasubramanian 1998; Esmaeilnejad et al. 2018).

Statistical analysis: For statistical analysis, where appropriate, the standard error of the mean was determined to express the collected data. All the data of this study were analyzed by statistical software (SAS 2004). Data regarding some epidemiological traits were subjected to chi-square analysis while data regarding hematology, antioxidant enzymes and oxidative stress parameters were compared by t-test.

Results

The results on an overall prevalence of babesiosis are presented in Table 1. Results revealed an overall 9.86% of prevalence of disease in buffaloes by microscopic examination. Different clinical signs including pyrexia (104°F ± 1.0°F), chocolate color urine, edema of face and legs in 2-3 cases, congested and hyperemic eyes, lethargy, emaciation, watery exudate from eyes and pale mucous membrane of eyes were observed. Results did not show significant association of age of buffaloes in occurring of parasitemia. A total of 37 babesia positive and 10 healthy buffaloes having different ages were investigated based on the presence of parasitemia. Blood and serum samples were collected from positive and healthy buffaloes for the estimation of different

blood and serum tests. Results on hematological values indicated a significantly increased total leukocyte counts, basophil counts, eosinophil, and neutrophil counts while significantly reduced values of red blood cell counts, hematocrit, hemoglobin, monocyte, and lymphocyte counts in infected buffaloes (Table 2). Babesia were identified microscopically on blood smears stained with Giemsa stain (Fig. 1). The results regarding different antioxidant enzymes in babesia positive cases showed significantly lower quantity of serum total proteins, albumin concentration, catalase, reduced glutathione and superoxide dismutase as compared to non-infected animals (Table 3). Results on some oxidative stress parameters indicated significantly higher values of nitric oxide scavenging activity and malondialdehyde quantity in positive buffaloes (Table 3).

Discussion

Babesiosis, a tick born disease, is caused by intra-erythrocytic protozoan parasite like Babesia which is the most common and costly disease of dairy animals reared across the globe. Recently the disease has gotten more attention in public medicine due to its zoonotic potential (Savic et al. 2014). Results revealed an overall 9.86% disease prevalence in buffaloes by microscopic examination. Previously, various prevalence rate of babesiosis including South America (64%) and Asia (19%) with pooled global prevalence (29%) have been recorded. In Pakistan, the prevalence (± standard error of mean) of babesiosis in cattle (5.7 ± 4.4 %) and buffaloes (7.5 ± 1.5 %) has been recorded by Jabbar et al. (2015). Similarly, increased prevalence of *B. bovis* and *B. bigemina* in dairy animals (14.7%) has also been recorded in Thailand (Terkawi et al. 2011), in cattle (1.49%) reared in Philippines (Herrera et al. 2017) and prevalence of *B. bigemina* (31.6%) and prevalence of *B. bovis* (24.2%) in Columbia (Jaimes-Dueñez et al. 2018) in cattle and buffaloes. In present study, different hematological parameters like total leukocyte counts, basophil counts, eosinophil and neutrophil counts increased significantly while red blood cell counts, hematocrit, hemoglobin, monocyte and lymphocyte counts decreased in infected buffaloes.

Previously, a significant drop in different hematological parameters like erythrocyte count, hemoglobin quantity and PCV in infected cattle due to intracellular parasite showing anemia have also been recorded. In the present study, lower hematological parameters in infected buffaloes could be induction of hemoparasitic disorders in buffaloes ultimately resulting in inhibition of systemic inflammation inhibition (Das 2012; Doyle et al. 2016). In earlier published data, it has been investigated that different autoimmune events cause destruction of erythrocytes due to parasitic infections (Halliwell and Gutteridge 2007; Doyle et al. 2016). In present study, increased concentrations of lipid peroxidation product while significantly drop in concentrations of various antioxidant enzymes like SOD, RGHS and CAT in red blood cells of infected buffaloes were recorded. The increased quantity of lipid peroxidation product in this study might be linked with increased process of lipid peroxidation in erythrocytes of infected buffaloes. Previously, it was recorded that oxidative stress biomarker (MDA) significantly increased in erythrocytes of cattle infected with intracellular parasitic infection is suggestive of increased generation of free radicals. Previously it was reported that increased concentrations of lipid peroxidation product (MDA) in cattle due to parasitic infection (bovine theileriosis) causing damage to cellular membranes and depletion of antioxidant enzymes (Halliwell and Gutteridge 2007; Chaudhuri et al. 2008). Different earlier studies have highlighted that various antioxidant substances including various trace elements (manganese, copper, selenium and zinc) reduced significantly in cattle due to parasitemia. In infected buffaloes, the lower values of reduced glutathione and superoxide dismutase can be due to parasitemia, suggestive of induction of oxidative stress in exposed erythrocytes. It has been recorded that different antioxidant enzymes play important role in reduction of oxidative stress by decomposition of intracellular lipid peroxidation process (Chaudhuri et al. 2008; Esmaeilnejad et al. 2018). Moreover, it has also been recorded that SOD plays an important role for the protection of red blood cells from oxidative stress causing damage to cells and tissues (Chaudhuri et al. 2008).

The lower concentrations of SOD in infected buffaloes might be due to hemolysis and insufficient antioxidant capacity in association with parasitic anemia. In contrast to our results on SOD and CAT, previously increased values of SOD enzymes in dogs naturally infected with *Babesia gibsoni* have been recorded, suggesting reticulocytosis, because these cells have more SOD as compared to mature erythrocytes (Chaudhuri et al. 2008). Furthermore, the lower levels of different antioxidant enzymes in erythrocytes of infected animals could also be due to increased consumption of antioxidant enzymes which have free radical scavenger activity during the induction of oxidative stress (Doyle et al. 2016).

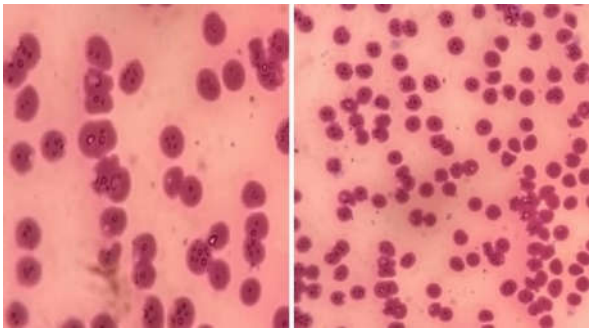

Fig. 1: Photomicrograph exhibiting the presence of babesia in red blood cells of buffalo (Giemsa Stained-1000X).

Table 1: Relationship of age and sex with prevalence of babesiosis in buffaloes

Species/sex/age	No. of animals	Positive		95% C.I.	Odd Ratio/ P value
		N	%		
Giemsa smear examination test					
Buffaloes					
Sex					
Male	120	13	10.83	6.16-17.38	1.29 [reciprocal = 0.78]
Female	255	22	8.62	5.63 -12.56	
Overall	375	37	9.86	7.15 - 13.20	
Age groups(Years)					
1-2	73	08	10.95	5.22 - 19.75	Mantel-Haenszel chi-sq. P = P = 0.922
3-4	113	13	11.50	6.55 - 18.41	
5-6	102	09	8.82	4.39 - 15.57	
>7	87	07	8.45	3.59 -15.27	

Table 2: Different hematological parameters of infected and healthy buffaloes

Parameters/Species	Healthy	Infected	p-value
Buffaloes			
Erythrocyte counts (10^6/µL)	7.11±1.203	5.17±0.17	<0.01
Hemoglobin quantity (g/dL)	12.77±0.33	9.11±0.19	<0.01
Hematocrit (%)	33.87±1.29	23.77±2.03	<0.01
Leukocyte counts (10^3 /µL)	31.7±1.11	49.13±3.1	<0.01
Neutrophil (%)	49.6±1.6	41.2±3.5	<0.01
Lymphocyte (%)	3.73±0.15	2.03±0.11	<0.01
Monocyte (%)	4.27±0.15	3.01±0.13	<0.01
Basophil (%)	0.73±0.09	1.69±0.13	<0.01
Eosinophil (%)	3.31±0.29	4.73±0.29	<0.03

Table 3: Status of antioxidant enzymes and oxidative stress profile of infected and healthy buffaloes

Parameters	Non infected	Infected	P-Values
Antioxidant Biomarkers			
ALB	2.93±0.17	2.03±0.07	<0.001
RGSH (IU/mgHb)	179.9±9.3	143.3±12.1	<0.001
SOD (IU/mgHb)	149.2±3.9	127.3±5.3	<0.001
CAT (IU/mgHb)	123.9±5.2	101.1±3.3	<0.001
Total Protein (g/dl)	5.03±0.15	3.15±0.19	<0.001
Oxidative stress biomarkers			
NO scavenging activity (%)	17.9±1.7	31.9±4.1	<0.001
MDA (nmol/gHb)	1.63±0.07	2.39±0.13	<0.001

In present study, lower amount of serum total proteins and hemoglobin may be associated with increased oxidative stress. It is observed that increased oxidation of red blood cells leads to rapid generation of free radicals resulting in infiltration of erythrocytes causing damage to cellular membranes and anemia. Due to prominent drop in hematological parameters suggestive of anemia, remarkable drop in different antioxidant enzymes and notably increased in lipid peroxidation product in babesia positive buffaloes, it can be suggested that the parasitic infections increase the process of lipid peroxidation which ultimately causes increased fragility of membranes of erythrocyte and reduces the antioxidant enzymes.

Authors contribution

All authors contributed equally

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