



Determination of Possible Potential Biomarker Between Plasma Arginase, Gelsolin and Cystatin C in Sheep Babesiosis: Based on Parasitemia Rate

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Abstract: The aim of this study was to assay arginase activity, gelsolin (GLS) and cystatin c (Cys c) levels and determination of potential biomarker in sheep babesiosis. The *Babesia* genus as pathogen blood parasites causes economic problems in livestock management. Many published studies have suggested babesiosis-induced biochemical parameters, but no one have determined those parameters alterations and clarification of sensitivity and specificity. Sheep with acute babesiosis were identified based on clinical signs and the observation of Piroplasm forms in red blood cells. After blood sampling from 25 infected and same number healthy sheep, above parameters measured in plasma. The results indicated significant increase ($P<0.01$) in arginase and Cys c and significant decreases ($P<0.01$) in the levels of GLS in different parasitemia rates compared with healthy group. In conclusion, the results suggested that sheep babesiosis causes cellular damage especially in kidney (owing to high level of Cys c) and high arginase activity can be considered as one of essential factor in *Babesia ovis* proliferation process. Finally, high sensitivity of Cys c and arginase in the different parasitemia rates than GLS could be clarified as potential biomarker of sheep babesiosis.

Keywords: Biochemical parameters, Potential biomarker, Sheep babesiosis.

Koyun Babeziozisinde Plazma Arginaz, Gelsolin ve Sistatin C Arasında Olası Potansiyel Biyobelirteç Tayini: Parazitemi Oranına Göre

Öz: Bu çalışmanın amacı, koyun babeziozisinde arginaz aktivitesi, gelsolin (GLS) ve sistatin c (Cys c) seviyelerini ve potansiyel biyobelirteç tespitini incelemektir. Patojen kan parazitleri olan *Babesia* türleri, hayvancılık yönetiminde ekonomik sorunlara neden olur. Yayımlanan birçok çalışmada babeziozis kaynaklı biyokimyasal parametreler önerilmiş, ancak hiç kimse bu parametrelerdeki duyarlılık ve özgüllüğü ve değişiklikleri açıklığa kavuşturmamıştır. Akut babeziozisli koyunlar, kırmızı kan hücrelerinde Piroplazma formlarının görülmesi ve klinik bulgulara göre belirlendi. Yirmibeş adet enfekte ve aynı sayıda sağlıklı koyunlardan alınan kan örneklemeinden sonra, yukardaki parametreler plazmada ölçüldü. Sonuçlar, arginaz ve Cys c'de anlamlı bir artış ($P<0.01$) ve sağlıklı gruba kıyasla farklı parazitemi oranlarında GLS seviyelerinde anlamlı düşüş ($P<0.01$) gösterdi. Sonuç olarak, koyun babeziozis'inin özellikle böbreklerde (Cys c'nin yüksek olması nedeniyle) hücresel hasara neden olduğunu ve yüksek arginaz aktivitesinin *Babesia ovis* proliferasyon sürecinde önemli bir faktör olarak göz önünde bulundurulabileceğini göstermiştir. Son olarak, GLS 'den farklı parazitemi oranlarında CYS c ve arginaz 'nin yüksek duyarlılığı, koyun Babeziozis 'in potansiyel biyomarkeri olarak göz önünde bulundurulabilir.

Ahtar Kelimeler: Biyokimyasal parametreler, Koyun babeziozis, Potansiyel biyomarker.

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INTRODUCTION

Babesiosis is known as a tick-borne hemoprotozoan disease with host-specific features carried by members of the *Babesia* genus. *Babesia ovis* induces the expression of severe pathogenic traits in sheep and causes sheep babesiosis, which is characterized by fever, anemia, icterus and hemoglobinuria (1,2). *B. ovis* has been observed in most regions of Iran (1). A high prevalence among sheep and goats in north-eastern of Iran was reported by Razmi et al., in 2003 (3). Studies of blood profiles changes in sheep babesiosis are scarce. Several studies have reported babesiosis-induced hematological and biochemical alterations (4,5) but no study has been conducted in this issue.

Arginase is known as essential enzyme in degradation of L-arginine to L-ornithine and urea and participates in polyamines biosynthesis and ornithine (6,7). Arginase possesses two distinct isoforms, arginase I and II. These two arginases indirectly regulate nitric oxide (NO) generation from nitric oxide synthase (NOS) (8,9). The depletion of arginine as essential substrate of arginase during activity of arginase, points out as limiting factor in the NO synthesis. Moreover, during arginase activity, L-ornithine synthesis as the precursor of L-glutamine and L-proline is elevated through the ornithine decarboxylase pathway (8). In addition, L-arginine depletion, through arginase, leads to T cell proliferation impeding (10). It is mentioning that in blood parasites diseases, polyamines simplify DNA and trypanothione synthesis, which recent one is substantial for intracellular redox system along with maintaining and presenting a defense against oxidative stress (11). Hence, elevated activity of arginase may participate to damage of host immune response and desirable for blood parasite proliferation. It should be noted that different factors such as reactive oxygen species, pro-inflammatory cytokines, and hypoxia induce high activity of arginase and leads to synthesis of high level of L-ornithine (12).

Gelsolin (GLS) belongs to proteins (82kd molecular weight) that can be found in the body, extracellular (plasma) and intracellular (cytoplasm and mitochondria). It was identified first time in 1979 in rabbit lung macrophages. GLS is known as actin-binding protein that involves in the removal of extracellular actin subsequently tissue damage (13). Various studies show that GLS participates in cell movement, regulation of apoptosis, regulation of tumorigenesis and removal of actin from blood and is proposed as prognostic marker (14,15).

Cystatin c is a small protein with a weight of 13 KD that belongs to cysteine protease inhibitors. This protein is continuously produced in all nucleated cells and released into the bloodstream and its half-life is 2 hours (16,17). This protein is independently filtered by glomeruli in the kidneys and re-absorbed and re-catalyzed in proximal tubules (18). Cystatin C has been known as the most valuable serum biomarker in determining renal function, and is especially a good choice for knowing the glomerular filtration rate (GFR) (19,20). Additionally, there is no change in Cys-C levels in different conditions, such as inflammation, infection, or malignancies (19). It should be noted that factors such as sex, age, diet, and body mass affect the concentration of creatinine, but does not affect Cys-C levels (19). It should be noted that in veterinary medicine, several studies have reported the importance of C-Cys as a valuable marker for assessing kidney function in dogs (21,22). But no study has been reported regarding cystatin c changes in sheep babesiosis.

To our knowledge, this study admittedly first one in respect of clarification of potential biomarker among Cys c, GLS (tissue damage marker) and arginase in sheep babesiosis.

MATERIAL and METHODS

Animals, Blood Sampling, Parasitological Examination and Biochemistry Analysis

This study was conducted in western Azerbaijan province (North west of Iran) in 2015 (from May to August) where *B.ovis* causes severe babesiosis in sheep. (Ethical Committee Decision Number: 2018-2/13/4402). Fifty sheep (25 babesiosis and 25 healthy ones) were appointed in this study. The animals (25 babesiosis sheep) were examined for the presence of ticks and clinical signs including hyperthermia (39.9-40.8), anorexia, petechial bleeding, icterus, hemoglobinuria and anemia, which is associated with pallor of the mucosal membrane. Blood samples were collected via the jugular vein and blood smear staining was performed with Giemsa solution 5%. Microscopic examination in the immersion objective (X100) revealed *Piroplasm*s in the same 25 sheep. The other 25 sheep without any clinical or paraclinical signs of babesiosis were selected as the healthy group. Parasitemia determination (as percentage) was performed by counting of infected red blood cells (RBCs) in 100 microscopic fields in the immersion objective (X100) (<1%, 1%, 2% and 3%) (23). After blood sampling and transferring to EDTA-contained tubes, plasma samples were prepared by centrifugation at 6000 RPM for 10 minutes at 4°C. The measurement of GLS and Cys c were performed by Elisa technique (Elisa, RA1000). Determination of plasma gelsolin with (Antibodies online Co kit, Germany), Cys-c with a dedicated kit (Mybiosource, San Diego, USA) and arginase activity was determined through modified method of thiosemicarbazide Diacetyl Monoxime Urea (TDMU) (39). (Spectrophotometer, Cecil, Italy).

Statistical Analysis

Statistical analysis was performed for all of the data completed during the study. The Mean \pm SEM and ANOVA analysis were carried out with SAS v9.1 (SAS Institute Inc., Cary, NC, USA). The significance level was specified at $P < 0.01$. In addition, cut-off

points of parameters were clarified and ROC analysis (Receiver Operating Characteristic) was carried out for sensitivity and specificity determination.

RESULTS

The results have been denoted in Tables 1, 2, 3, 4 and 5. In the Table 1, the significant increase ($P < 0.01$) of arginase activity and Cys c and remarkable reduction ($P < 0.01$) of GLS were obtained in babesiosis group in comparison with control ones. Moreover, the same results were observed in different parasitemia rates. In Tables 2, 3, 4 and 5 high sensitivity of arginase and Cys c was demonstrated than GLS in different parasitemia rates.

Table 1. Alterations of Arginase, GLS and Cys c in the Babesiosis and healthy group based on parasitemia rate.

Tablo 1. Babesiosisli ve sağlıklı grupta parazitemi oranlarına göre Arginaz, GLS ve CYC c'deki değişimler.

Parasitemia rate (%)	GLS (mg/L)	Arginase (U/L)	Cys-c (ng/ml)
Healthy group number (25) 0%	219.89 \pm 7.41	6.36 \pm 0.09	8.02 \pm 0.1
Babesiosis group less than 1% (5)	128.71 \pm 2.12 [†]	23.87 \pm 0.51 [†]	17.31 \pm 0.73 [†]
1% (11)	62.85 \pm 1.07 [†]	41.37 \pm 0.71 [†]	36.56 \pm 1.89 [†]
2% (6)	25.81 \pm 1.13 [†]	69.11 \pm 2.01 [†]	51.50 \pm 2.04 [†]
3% (3)	3.16 \pm 0.22 [†]	112.15 \pm 2.28 [†]	95.78 \pm 2.17 [†]

Data are expressed as mean \pm standard error of mean (SEM). Upper superscript in the column denote significant difference among groups ($P < 0.01$).

Table 2. In less than 1% parasitemia rate, Arginase and Cys c sensitivity are higher than GLS.

Tablo 2. % 1'den az parazitemi oranında Arjinaz ve Cys c sensitivitesi GLS'den daha yüksektir.

Parameter	Cut-off point	AUC	P value	Sensitivity (%)	Specificity (%)
Arginase	11.49	1	0.0001	95.5-98	100
Cys c	5.12	1	0.0001	97	100
GLS	94.63	1	0.0001	79-82.5	100

Table 3. In 1% parasitemia rate, Arginase and Cys c sensitivity are higher than GLS.

Tablo 3. %1 parazitemi oranında, Arjinaz ve Cys c sensitivitesi GLS'den daha yüksektir.

Parameter	Cut-off point	AUC	P value	Sensitivity (%)	Specificity (%)
Arginase	18.06	1	0.0001	96-97.5	100
Cys c	11.84	1	0.0001	96.5-97	100
GLS	65.12	1	0.0001	76-81	100

Table 4. In 2% parasitemia rate, arginase and Cys c sensitivity are higher than GLS.

Tablo 4. %2 parasitemi oranında, Arjinaz ve Cys c sensitivitesi GLS'den daha yüksektir.

Parameter	Cut-off point	AUC	P value	Sensitivity (%)	Specificity (%)
Arginase	32.49	1	0.0001	97-99	100
Cys c	19.28	1	0.0001	96-97.5	100
GLS	31.53	1	0.0001	80-82.5	100

Table 5. In 3% parasitemia rate, Arginase and Cys c sensitivity are higher than GLS.

Tablo 5. %3 parasitemi oranında, Arjinaz ve Cys c sensitivitesi GLS'den daha yüksektir.

Parameter	Cut-off point	AUC	P value	Sensitivity (%)	Specificity (%)
Arginase	53.89	1	0.0001	98	100
Cys c	28.63	1	0.0001	97.5-99	100
GLS	14.38	1	0.0001	78-83.5	100

DISCUSSION and CONCLUSION

In babesiosis group arginase activity was high compared with healthy ones. Various factors play fundamental role in high arginase activity, such as high production of reactive oxygen species (ROS) (oxidative stress), high levels of inflammatory cytokines and hypoxia (11). In a study, oxidative stress occurrence was referred in ovine babesiosis (24) and hypoxia occurrence has been determined in sheep babesiosis (1). In addition, high concentration of pro-inflammatory cytokines has been determined in canine babesiosis (25). According to foregoing, most probably owing to babesiosis-mediated hypoxia and oxidative stress, high activity of arginase was occurred in this study and leads to high production of L-ornithine which is known as one of the main substrates of *Babesia ovis* proliferation. One of the other possible reasons on this increase can be attributed to liver damage, due to existence of high amount of arginase therein and as well as during occurrence of babesiosis in sheep, liver damage is an inevitable symptom (26). Meanwhile, high activity of arginase may ascribe to hyperactive macrophage, as essential source of arginase in blood (27). Following trypanosome-infected mice, macrophages reveal a greater increase in arginase expression than those from resistant animals (28). Moreover, high plasma arginase has been reported in acute hepatitis B,

resulting suppression of T cell function, in several cancers (29-32). According to forenamed reasons, high activity of plasma arginase can play tremendous role in parasite proliferation and/or immune response perturbation.

Gelsolin (GLS) is known as actin binding protein which eliminates and minimizes blood actin which is subsequently produced during various diseases induced tissue damage especially hematologic ones, either directly (affects the blood cells) or indirectly (involvement in organs involved in hematopoiesis). Excessive release of actin into the blood, leads to a remarkable reduction of GLS which is not appropriate prognosis for amelioration of disease. So we can say that plasma GLS is associated with the disease severity and prognosis and therefore, it is under consideration as a prognostic indicator in acute diseases. Also, in patients with pneumonia, fever and seizure GLS value decreases, which indicates that some factors other than hemolysis can also reduce the GLS concentration (33,34). In this study, plasma GLS decreased in babesiosis group (especially in high parasitemia rate group, more than 4 percent) than healthy ones. In a study, GLS reduction was observed in hepatitis B patients and they reported it as possible diagnostic biomarker for detection of severity of hepatic injury (33). Furthermore, GLS reduction demonstrated during brain hemorrhage (34). Since GLS reduction in plasma occurred following actin increase in the blood, thus most likely it can be said that during babesiosis infection, cell damage occurred in various tissues, especially in the kidneys and finally, this mechanism increases the synthesis and secretion of GLS into blood to inhibit actin which led to a reduction in the plasma GLS in babesiosis. These findings correspond with the results of our research.

By assessing the serum Cys c levels in healthy and diseased groups based on parasitemia rates, we found that the mean serum level of this parameter in the sheep was higher than healthy group and the severity of parasitemia had an incremental effect.

There is no study on this parameter in sheep babesiosis, but in one report in dogs with leishmaniasis, Cys c level was increased in comparison to the healthy group (35). In another study, lack of significant changes of Cys c was reported in dogs with dirofilariasis (36). Same author in 2015 reported a significant increase in Cys c in dog babesiosis and leishmaniasis, and even demonstrated Cys c as a new biomarker in early detection of renal injury in dogs with these two diseases (37) and finally Azimzadeh in 2017 (38) reported high Cys c level in sheep theileriosis with different parasitemia rates. Since blood parasites such as Leishmania, causes tissue damage by forming immune-complexes (immunopathologic mechanisms), especially in the kidney, it is likely that sheep babesiosis causes kidney damage by forming immune complexes (especially kidney glomeruli) and eventually leads to an increase in Cys c in serum. As a result, babesiosis mediated kidney damage causes high level of Cys c and can influence on reduction of GLS (for the removal of actin released from damaged cells). It also should be noted that with increasing of parasitemia severity, serum changes in the above parameters have been tightened and high activity of arginase can participate in impairment of immune system (especially T cells) and induction of parasite proliferation. Finally, high sensitivity of arginase and Cys c than GLS can be considered as potential diagnostic biomarker in sheep babesiosis.

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