

# Determination of Potential Biomarker among Plasma Sphingosine-1-Phosphate, Total Sialic Acid and Adenosine Deaminase in Cattle with Naturally Infected Liver Cystic Echinococcosis

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## Abstract

The purpose of this trial was to assay the alterations of some blood parameters such as sphingosine 1 phosphate (S1P), total sialic acid (TSA) and adenosine deaminase (ADA) and clarification of possible potential biomarker among them in naturally infected liver cystic echinococcosis in cattle. After observation of severe liver parasitic infestation (as cystic form) and blood sampling from parasitized and healthy ones, all selected biochemical analytes were clarified and results determined significant increase ( $P \leq 0.01$ ) of aforementioned parameters in parasitized group rather than healthy ones. In conclusion, based on high sensitivity of TSA and ADA than S1P, they could be considered as potential biomarker in CE.

**Keywords:** Biochemical parameters, Cattle, Echinococcosis

## Doğal Enfekte Karaciğer Kistik Ekinokokozisli Sığırlarda Plazma Sfingosin-1-fosfat, Total Sialik asit ve Adenozindeaminaz Arasından Potansiyel Biomarkerın Belirlenmesi

### Özet

Bu çalışmanın amacı, doğal enfekte karaciğer kistik ekinokokozisli sığırlarda sfingosin-1-fosfat (S1P), total siyalik asit (TSA) ve adenozin deaminaz (ADA) için kan değerlerinin belirlenmesi ve böylece aralarından hangisinin muhtemel potansiyel bir biyomarker olabileceğinin belirlenmesidir. Parazitli olan (şiddetli kistik formu) ve sağlıklı sığırlardan kan örnekleri alındıktan sonra, belirtilen tüm biyokimyasal analizler yapıldı ve sonuçlar karşılaştırıldığında parazitli olan grupta anlamlı bir artış ( $P \leq 0.01$ ) olduğu belirlendi. Sonuç olarak, TSA ve ADA S1P'e göre daha yüksek hassasiyetlik göstermesi sebebiyle, kistik ekinokokozisde potansiyel biyomarker olarak değerlendirilebilir.

**Anahtar sözcükler:** Biyokimyasal parametreler, Sığır, Ekinokokozis

## INTRODUCTION

*Echinococcus granulosus* contributes in the occurrence of cystic echinococcosis (CE) in animals (domestic and wild herbivores) and man. Echinococcosis is known as one of the most essential zoonotic diseases in the world and possesses important effect on human and animal health with remarkable economic detriments [1]. Carnivores play substantial effect as definitive hosts, while domestic ungulates and human are involved as the intermediate hosts. Similarly, sheep participate in disease transmission and the strain (G1) of *Echinococcus granulosus* causes CE. It is usually asymptomatic in livestock, however, during

inspection of carcass at the slaughterhouse can lead to detection of disease [2,3].

Sphingosine 1-phosphate (S1P) is known as novel bioactive lipid mediator which belongs to sphingolipids group and it participates in both cellular physiological and pathophysiological pathways [4]. Subsequent studies determined that S1P is abundantly existed in plasma and other body fluids, where it acts as autocrine or paracrine effects onto fundamental cell functions [5]. Erythrocytes and platelets store and liberate S1P into blood and they are considered as fundamental sources of S1P [6]. In addition, S1P involve as major bioactive molecule in exhausting



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of lymphocyte from the secondary lymphoid tissues into the lymph. Thus, these findings forcefully suggest that S1P has fundamental effects *in vivo* as well as potentially pathophysiological roles as a circulating paracrine mediator [5]. It should be noted that ability of S1P in the modulation of fibroblasts migration has been determined and plays substantial role in fibrosis in different tissues [7].

Sialic acid (SA) is acetylated derivative of neuraminic acid which has been broadly distributed in mammal tissues and body fluids. SA is classified in three forms namely protein-bounded sialic acid (PBSA), lipid-bounded sialic acid (LBSA) and free form and it is involved at the end chain of many acute phase proteins [8]. Thus, the detection of SA might be a momentous marker for diagnosis and prognosis of inflammatory diseases [8,9]. In many infectious diseases, SA are determined in cattle, such as keratoconjunctivitis, leptospirosis, pneumonia, theileriosis, anaplasmosis and traumatic reticulo-peritonitis [8]. It is linked to residues of the carbohydrate chains of glycoproteins and glycolipids (especially non-reducing sections) [10]. This linkage becomes it susceptible to get involved in cellular and molecular interrelationships and also participates in lipoproteins and lipid metabolism [11].

Adenosine deaminase (ADA) is involved in degradation of adenosine and deoxyadenosine into inosine and deoxyinosine. ADA, as an essential enzyme, contributes to the maturation and differentiation of T lymphocytes and its activity is higher in T cells than B cells [12]. The ADA regulates the cellular mechanisms associated with blood flow, vasodilatation, angiogenesis and proliferation [13]. It has been indicated that serum ADA activity is higher in diseases associated with immune response stimulation such as liver cirrhosis, chronic hepatitis and hepatocellular carcinoma. Generally, serum ADA level has been referred in human and animals as an important indicator for detection of liver diseases [14].

To our knowledge, no research has been conducted to determine potential biomarker among TSA, ADA and S1P in cattle echinococcosis. Thus, the present study aimed to investigate the above-mentioned issue in cattle echinococcosis.

## MATERIAL and METHODS

This study was conducted in Urmia city (West Azerbaijan province), Iran. Ten milliliters of blood were collected via the jugular vein of cattle (20-22 months) that had been admitted for slaughtering at the abattoir of Urmia and blood samples transferred equally to EDTA-contained and non-EDTA contained tubes. After slaughtering, animals were surveyed based on observation of severe hepatic CE in liver. Eighty animals were infected to hepatic CE and same number were also selected as control group (healthy animals). Microscopic examination of blood smears staining

in the immersion objective (X100) revealed no parasite in infected and healthy sheep.

All samples were centrifuged 6.000 g for 10 min at room temperature for plasma and serum preparation and were kept frozen (-25°C) until the analysis. The plasma S1P level was determined using a RA1000 in accordance with the ELISA method by (East Biopharm Co, Hangzhou, China). TSA was measured by Sydow method [15] in serum (spectrophotometer, model Spekol 1500, Germany). Total bilirubin and unconjugated bilirubin were measured colorimetrically by (Pars Azmoon Co. kits Tehran, Iran) in serum. Finally, ADA activity was determined by the electrochemiluminescence method (Roche Co. Elecsys 2010).

Statistical analysis was accomplished in all analyses. The Mean  $\pm$  SD and the determination of variation between the data results were carried out with Student's *t*-test through SAS v9.1 (SAS Institute Inc., Cary, NC, USA). The significance level was specified at ( $P < 0.01$ ). Moreover, determination of cut-off point among with ROC analysis were carried out in all parameters for sensitivity and specificity detection.

## RESULTS

All of the altered parameters are shown in *Table 1*. Significant increase ( $P < 0.01$ ) in S1P, TSA, ADA, total bilirubin and unconjugated bilirubin levels were revealed in the patient group compared to the healthy ones. In the respect of *Table 2*, based on cut-off point, AUC and ROC curve statistical analysis, all parameters possesses different sensitivity and specificity percentage and among them TSA and ADA sensitivity are better than S1P sensitivity. In the *Fig. 1*, significant increase of TSA (42.84 mg/dl) in the patient group has been noted compared with control ones (14.07 mg/dl). The *Fig. 2* illustrate ADA alterations between two groups. Considerable elevation (42.17 U/L) in patient group versus (15.92 U/L) in control ones. Regarding S1P as bioactive mediator (*Fig. 3*), considerable increase (271.77ng/L) was demonstrated compared control group (89.33 ng/L). In connection with bilirubin metabolism (*Fig. 4*), total and unconjugated form have been severely increased

**Table 1.** Alterations of plasma S1P, ADA and TSA levels in the control and patient groups

**Tablo 1.** Kontrol ve hasta gruplarında plasmadaki S1P, ADA ve TSA seviyelerindeki değişiklikler

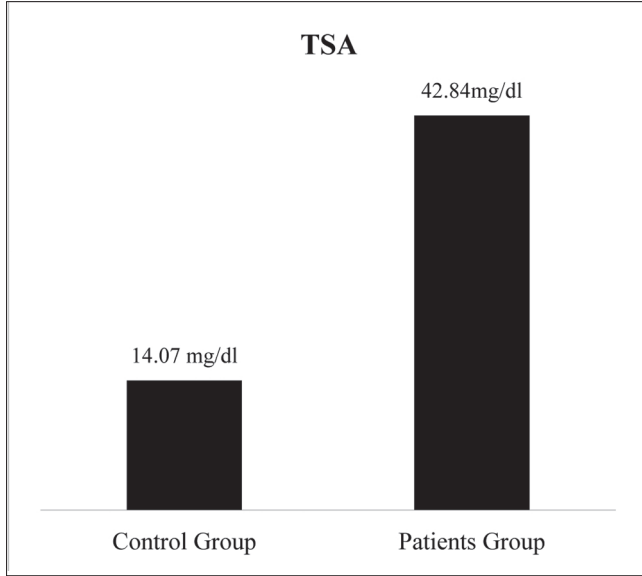
Parameters	Control Group	Patient Group
TSA (mg/dl)	14.07 $\pm$ 2.85	42.84 $\pm$ 8.56 <sup>†</sup>
ADA (U/L)	15.92 $\pm$ 3.15	42.17 $\pm$ 10.02 <sup>†</sup>
S1P (ng/l)	89.33 $\pm$ 20.48	271.77 $\pm$ 82.57 <sup>†</sup>
Total bilirubin (mg/dl)	0.34 $\pm$ 0.012	1.49 $\pm$ 0.02 <sup>†</sup>
Unconjugated bilirubin (mg/dl)	0.07 $\pm$ 0.001	0.82 $\pm$ 0.06 <sup>†</sup>

Data are expressed as mean  $\pm$  standard deviation. <sup>†</sup> Significantly different from the control group ( $P < 0.01$ )

**Table 2.** According to table, as area under the curve for each parameter is equal to 1. Hence all parameters have good specificity, but TSA and ADA sensitivity are higher than S1P

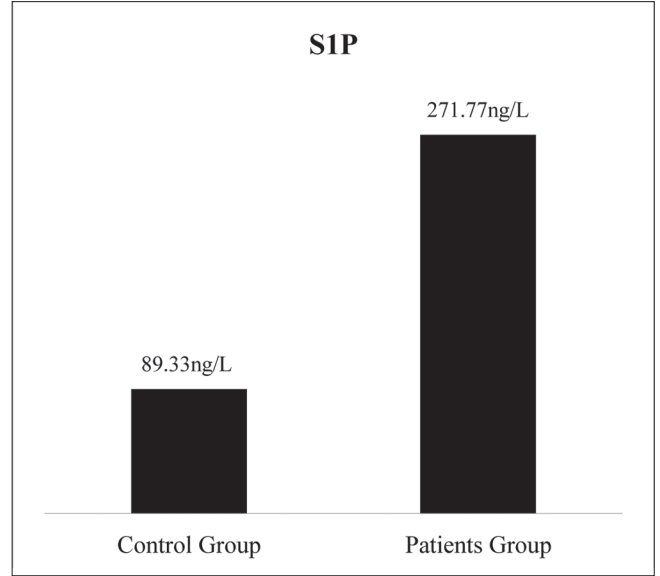
**Tablo 2.** Tablo ya göre, her bir parametre için eğrinin altında kalan alan olarak 1'e eşittir. Dolayısıyla tüm parametrelerin iyi özgüllüğü vardır, fakat TSA ve ADA hassasiyeti S1P göre daha yüksektir

Parameter	Cut-off Point	AUC	P Value	Sensitivity (%)	Specificity (%)
S1P	55/180	1	0001/0	5/87-85	100
TSA	69/28	1	0001/0	100- 5/97	100
ADA	81/28	1	0001/0	95	100



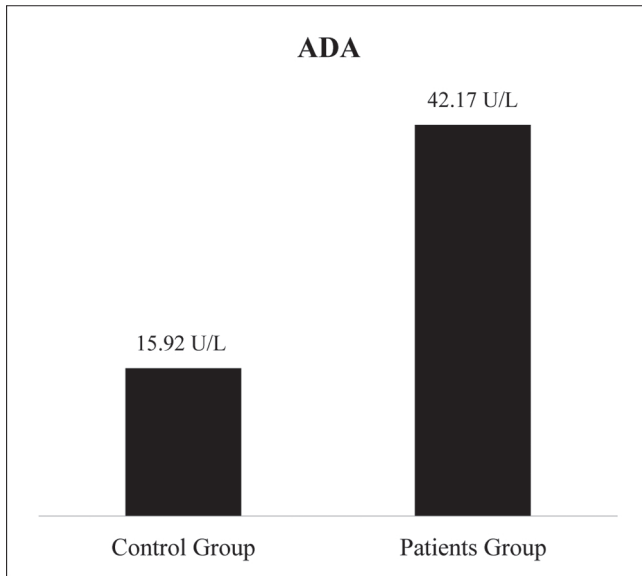
**Fig 1.** Alterations of TSA levels in infected group compared with control ones

**Şekil 1.** Kontrol grubuyla karşılaştırıldığında enfekte grupta TSA düzeylerindeki değişimler



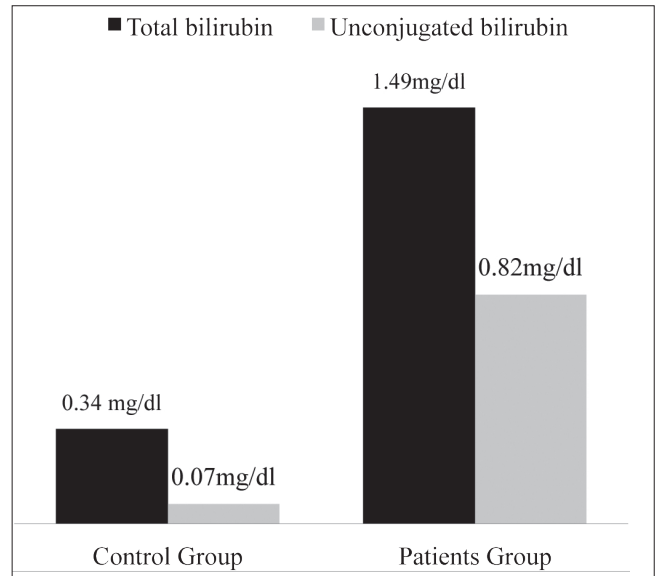
**Fig 3.** Alterations of S1P levels in infected group compared with control ones

**Şekil 3.** Kontrol grubuyla karşılaştırıldığında enfekte grupta S1P düzeylerindeki değişimler



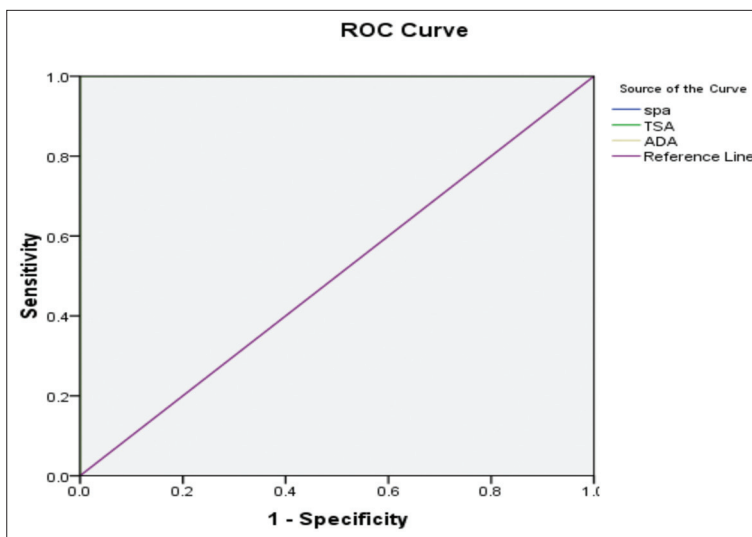
**Fig 2.** Alterations of ADA levels in infected group compared with control ones

**Şekil 2.** Kontrol grubuyla karşılaştırıldığında enfekte grupta ADA düzeylerindeki değişimler



**Fig 4.** Alterations of total bilirubin and unconjugated bilirubin levels in infected group compared with control ones

**Şekil 4.** Kontrol grubuyla karşılaştırıldığında enfekte grupta total bilirubin and konjuge olmayan bilirubin düzeylerindeki değişimler



**Fig 5.** ROC Curve for three parameters

**Şekil 5.** Üç parametre için ROC eğrisi

in patient group (1.49 and 0.82 mg/dl) rather than healthy group (0.34 and 0.07 mg/dl) respectively. *Fig. 5*, illustrates ROC (Receiver Operating Characteristic) among three parameters along with AUC (Area under Curve) for determination of sensitivity and specificity.

## DISCUSSION

Sialic acid, plasma proteins, glycoproteins and lipids are mostly synthesized in the liver <sup>[11]</sup> and sialic acid is linked to non-reducing residues of the carbohydrate chains of glycoproteins and glycolipids <sup>[16]</sup>. High levels of TSA were determined in echinococcosis group compared with the healthy ones. We could not clarify any information about sialic acid alterations in cattle CE. Stefenelli et al.<sup>[17]</sup> reported a significant decrease of TSA in chronic liver diseases such as cirrhosis than control ones. Yurtseven et al.<sup>[10]</sup> revealed low concentration of TSA in cattle with theileriosis. Chrostek et al.<sup>[11]</sup> demonstrated decrease of lipid-bound sialic acid in non-alcoholic cirrhosis and attributed it to liver diseases that impressed serum level of lipids and lipoproteins and also the level of sialic acid bounded with these compounds which are not in accordance with our study. It worth mentioning that many studies have revealed significant increase of sialic acid in various diseases such as cancer, inflammatory disorders, cardiovascular diseases and diabetes mellitus and even sialic acid has been demonstrated as one of the inflammatory markers <sup>[9]</sup>. Since, sialic acid is considered to be inflammatory marker. It is likely, significant increase in plasma TSA in cattle with hepatic CE can be attributed to disease-mediated TSA synthesis and production followed by infection in liver.

The substantial effects of S1P has been determined on the function of T and B lymphocytes which is included in the maturation and migration of them. It plays especially as an substantial effect onto activation of T cell <sup>[18,19]</sup>. It has been demonstrated that during the T and B lymphocytes

activation and stimulation, subsequently, S1P synthesis is reduced <sup>[20,21]</sup> which is not in accordance with our evidence. One of the reasons of S1P increase may be attributed to high concentration of HDL. The HDL is known to be the major bio-molecule which possesses S1P. The HDL participates in remove of some blood parasites and may play a substantial role in the increase of S1P in CE. The significant increase of S1P revealed in this study. We could not find any evidence which related to S1P alterations in hepatic CE. Li et al.<sup>[22]</sup> showed that S1P concentration to be increased in CCL<sub>4</sub>-mediated hepatic fibrosis in rat which is in accordance with our study. Ikeda et al.<sup>[23]</sup> showed S1P reduction in liver fibrosis patients that is not consistent with our evidence. Platelets are known to be one of the main sources of S1P in plasma during sphingosine phosphorylation by sphingosine kinase <sup>[24,25]</sup>. In line with this, platelets are recognized to reserve S1P plentifully and after activation, release it into the plasma <sup>[26]</sup>. In the respect of platelets, Togill et al.<sup>[26]</sup> suggested arising of platelets activation followed by chronic hepatic diseases. Moreover, CE is generally associated with long time hepatic damage in cattle. Consequently, platelets might be activated in cattle with liver CE which could cause S1P elevation. Furthermore, it is possible that plasma S1P may be raised due to unknown source(s) which need further clarified. The ADA activity was significantly raised in the hepatic CE group than healthy ones. Isik et al.<sup>[27]</sup> showed low levels of ADA activity in surgically treated hydatid patients and ascribed it to amelioration of damaged tissue or cease of lymphocyte proliferation during parasite elimination. Moreover, in another study, the ADA activity decrease during experimental infection of mice with a secondary hydatid disease was attributed to immunosuppression associated with the disease <sup>[28]</sup> that are not consistent with our finding. Umaramani et al.<sup>[29]</sup> reported significant increase of ADA activity followed by viral hepatitis which is in accordance with our study. This may have been the case in our study as liver echinococcosis causes cellular-immunity stimulation related to ADA activity increase.

In conclusion, on the basis of our findings, the results denote increase of all plasma parameters. Moreover, due to high sensitivity of TSA and ADA than S1P, they could be utilized as potential biomarker in cattle CE.

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