

Evaluation of Serum Proteins in Cattle with Liver Abscess

Javad TAJIK^{1*}, Saeed NAZIFI², Fatemeh ABBASI²

¹Department of Clinical Studies, School of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran

²Department of Clinical Studies, School of Veterinary Medicine; Shiraz University, Shiraz, Iran

*Corresponding Author: Javad TAJIK Department of Clinical Studies, School of Veterinary Medicine,
Shahid Bahonar University of Kerman, Kerman, Iran

e-mail: tajik@uk.ac.ir

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ABSTRACT

To evaluate the serum protein electrophoresis in cows with hepatic abscess and to investigate the correlation of changes in the serum proteins and the severity of hepatic involvement in affected cows, the liver of 435 randomly selected cattle were examined after slaughter. Liver abscesses were diagnosed in 20 cows. The size of hepatic abscesses (in diameter) had a significant correlation with serum concentration γ -globulin ($r=0.498$, $P=0.05$), and had marginally significant correlations with serum total protein ($r=0.487$, $P=0.056$) and total globulin ($r=0.438$, $P=0.09$). There were also significant correlations between the number of hepatic abscesses and serum concentration of β -globulin ($r=0.506$, $P=0.032$) and β -globulin percent ($r=0.463$, $P=0.05$). Also, cows with hepatic abscess on left lobe of the liver had a higher β -globulin percent than the cows with hepatic abscess on diaphragmatic surface ($P=0.013$). Cows with diffused tiny hepatic abscesses had a significantly higher β -globulin concentration than the cows with hepatic abscess on diaphragmatic surface ($P=0.036$), and a marginally significant less serum β -globulin concentration in comparison to cows with hepatic abscess in left lobe of the liver ($P=0.07$). Age revealed a significant correlation with the β -globulin percent ($r=-0.219$, $P=0.047$), and had a marginally significant correlation with the serum concentration of β -globulin ($r=-0.194$, $P=0.07$). The results of the current study showed that the serum protein electrophoresis may be useful in evaluation of the severity of hepatic involvement and abscess location in cows suffering from hepatic abscess.

Key Words: Serum protein electrophoresis, liver abscess, cattle

ÖZET

KARACİĞER APSELİ SİĞIRLARDA SERUM PROTEİNLERİNİN DEĞERLENDİRİLMESİ

Araştırmada, karaciğer apseleri bulunan ineklerin serum protein elektroforezinin ve serum proteinlerindeki değişimlerin korelasyonlarının değerlendirilmesi ve enfekte ineklerde karaciğerdeki enfeksiyon seviyesinin belirlenmesi amaçlarıyla kesimden sonra rastlantısal olarak seçilen 435 ineğin karaciğerleri incelenmiştir. İçlerinden 20 inekte karaciğer apsesi teşhis edilmiştir. Ölçülen karaciğer apselerinin çapları ile serum γ -globulin konsantrasyonları arasında oldukça önemli korelasyonlar ($r=0,498$, $P=0,05$) ve karaciğer apselerinin çapları ile serum total protein ($r=0,487$, $P=0,056$) ve total globulin ($r=0,438$, $P=0,09$) arasında ise daha düşük öneme sahip korelasyonlar bulunmuştur. Ayrıca, karaciğerde bulunan apse sayıları ile serum β -globulin konsantrasyonu ($r=0,506$, $P=0,032$) ve β -globulin oranları ($r=0,463$, $P=0,05$) arasında oldukça önemli korelasyonlar bulunmuştur. Bununla birlikte, karaciğerin sol lobunda apse bulunan ineklerin β -globulin oranları, diyaframatik yüzeyde apse bulunan hayvanlara göre daha yüksek bulunmuştur ($P=0,013$). Karaciğerin tamamına ufak apseler olarak yayılan enfeksiyona sahip hayvanların β -globulin konsantrasyonları diyaframatik yüzeyde apse bulunan hayvanlara göre daha yüksek ($P=0,036$), karaciğerin sol kısmında apse bulunan hayvanların serum β -globulin konsantrasyonlarına göre daha düşük bulunmuştur ($P=0,07$).

Hayvanların yaşları ile β -globulin oranları ($r=-0,219$, $P=0,047$) arasındaki önemli korelasyonlar ve hayvanların yaşları ile serum β -globulin konsantrasyonları ($r=-0,194$, $P=0,07$) arasındaki daha düşük öneme sahip korelasyonlar ortaya konmuştur. Çalışmanın sonucunda, karaciğerinde apse bulunan ineklerin serum protein elektroforezi yardımı ile karaciğerdeki enfeksiyonun seviyesinin ve apselerin konumlarının belirlenebileceği ortaya konmuştur.

Anahtar Kelimeler: Serum protein elektroforezi, karaciğer apseleri, sığır

Introduction

Liver abscesses are pus-filled capsules with variable size, from a small pinpoint to over 15 cm in diameter, and variable wall thickness that can occur in all ages of cattle (Nagaraja and Chebgappa, 1998). Bacteria can reach the liver through the portal vein, hepatic artery, umbilical vein, and the biliary duct and cause a focal infection. Acidosis-rumenitis-liver abscess complex has been introduced as the etiopathogenesis of liver abscesses in cattle fed diets containing high fermentable carbohydrates and low roughage (Dore et al., 2007). Liver abscesses have no breed or sex predilection and occur in both dairy and feedlot cattle. The prevalence of hepatic abscesses has been reported as up to 23.4% in culled Holstein cattle and up to 50% in feedlot cattle (Nagaraja et al., 1996; O'Sullivan, 1999).

The economic effect of cattle liver abscesses has not been completely investigated, however, sudden death, decreased feed intake, decreased growth rate, reduced feed efficacy, carcass yield decrement and carcass condemnation are the known causes of economic loss (Dore et al., 2007; Roberts, 1982). Although high prevalence of bovine hepatic abscesses has been reported worldwide, cattle suffering from liver abscesses have mild or undetectable clinical signs and are usually not diagnosed until slaughter (Dore et al., 2007).

Alterations in serum protein concentrations are among the most common biochemical markers measured routinely as screening tests for detecting underlying disease or for monitoring disease activity, and serum protein levels are the most important laboratory abnormality in a small number of diseases (Nazifi et al., 2009; Putignano et al., 2000). Changes in serum proteins may not be specific, but they may be helpful in the diagnosis of liver abscess affected cows. Although there are a few

previous studies regarding the comparison of serum protein electrophoresis between affected and non-affected cows, to the best of our knowledge, there is no previous study about the correlation of changes in the serum proteins and the severity of hepatic involvement in cows suffering from hepatic abscess. This study was undertaken to compare the serum protein electrophoresis between cows suffering from hepatic abscess and healthy cows, and to investigate the correlation of changes in the serum proteins and the severity of hepatic involvement in affected cows.

Materials and Methods

The investigation was carried out on cows which were slaughtered in Shiraz abattoir, south Iran, from September 2011 to March 2012. A total 435 cattle consisting of Holstein and Holstein cross bred animals was examined. After clinical examination and before slaughter, jugular blood samples in plane tubes, free from anticoagulant, were collected. Immediately after slaughter, the liver of the animals were removed and inspected. Multiple incisions were made in the liver to detect the subcapsular lesions (O'Sullivan, 1999), and size, number, and location of hepatic abscesses were recorded. The most frequent type of lesion was referred to as the main lesion. The cows were of both sexes with different ages, and were selected randomly. The age of the animals was estimated using dental characteristics. The blood serum was separated after centrifugation at 1800 g for 10 min and the serum samples stored at -20°C until analysis.

For comparison between affected and non-affected cows, two sex- and age-matched healthy cows were selected randomly as control for each affected cow.

Serum total protein was measured by Biuret method (Latimer et al., 2003). Electrophoresis

separation of serum proteins was carried out using cellulose acetate strips (voltage of 180V and about 15 mA for 15 minutes, apparatus: Elphor, Germany). The strips were subsequently evaluated using densitometry.

Statistical analysis was performed using SPSS18 (Illinois, Chicago). The correlation of serum proteins with the number and size of hepatic abscesses and age was analyzed by Pearson's correlation tests. Two sample t-tests were used to compare between the affected and non-affected cows. Analysis of variance (ANOVA) tests were used to compare the serum proteins between cows with different

abscess locations in the liver. Differences were considered significant at $P \leq 0.05$.

Results

Overall, 435 cows (242 males and 193 females) between 6 months and 5 years old were examined and hepatic abscesses were found in 20 livers, which consisted of 16 males and 4 females. Five cows had 1 abscess, 10 cows had 2-10 abscesses, and 5 cows had numerous tiny abscesses. The results of the measured serum proteins in affected and non-affected cows are shown in Table 1.

Table 1. The concentrations (Mean \pm SEM) of serum proteins in affected and non-affected cows.

Table 1. Hastalıktan etkilenen ve etkilenmeyen ineklere ait serum protein konsantrasyonları (Ortalama \pm SEM).

	All evaluated cows	Affected cows	Non-affected cows
Number of cows	60	20	40
Total protein (g/dL)	7.04 \pm 0.13	7.15 \pm 0.23	6.99 \pm 0.16
Albumin (g/dL)	3.36 \pm 0.13	3.23 \pm 0.29	3.43 \pm 0.17
Albumin (%)	49.92 \pm 2.68	47.01 \pm 4.12	51.38 \pm 3.46
Total globulin (g/dL)	3.67 \pm 0.25	3.91 \pm 0.41	3.55 \pm 0.31
α -globulin (g/dL)	0.67 \pm 0.065	0.59 \pm 0.075	0.72 \pm 0.09
α -globulin (%)	9.42 \pm 0.87	8.27 \pm 1.04	9.99 \pm 1.2
β -globulin (g/dL)	0.85 \pm 0.06	0.97 \pm 0.12	0.79 \pm 0.07
β -globulin (%)	12.03 \pm 0.81	13.63 \pm 1.5	11.24 \pm 0.92
γ -globulin (g/dL)	2.14 \pm 0.2	2.35 \pm 0.37	2.04 \pm 0.24
γ -globulin (%)	28.52 \pm 2.14	31.02 \pm 3.8	27.27 \pm 2.6
A/G ratio	1.43 \pm 0.18	1.09 \pm 0.17	1.6 \pm 0.26

Comparison between 20 affected and 40 non-affected cows showed that affected cows had higher serum concentrations of total protein, total globulin, β -globulin and γ -globulin, and less albumin, α -globulin, and albumin/globulin (A/G) ratio, however, the differences were not statistically significant ($P > 0.05$).

The size of hepatic abscess (in diameter) showed a significant correlation with serum concentration of γ -globulin ($r = 0.498$, $P = 0.05$), and had marginally significant correlations with serum total protein ($r = 0.487$, $P = 0.056$) and total globulin ($r = 0.438$, $P = 0.09$).

There were also significant correlations between the number of hepatic abscesses and the serum concentration of β -globulin ($r = 0.506$, $P = 0.032$) and β -globulin percent ($r = 0.463$, $P = 0.05$).

Comparison of the measured serum factors between cows with different abscess locations in the liver, consisting of diffused tiny abscesses, diaphragmatic surface, and right, left, caudal and apical lobes, revealed no significant difference in measured parameters except β -globulin percent and serum concentration of β -globulin. Because of the unequal variances, the Kruskal-Wallis test and Mann-Whitney U tests were used to

compare the β -globulin concentration between the different abscess locations. Cows with hepatic abscess on left lobe of the liver had a significantly higher β -globulin percent in comparison to cows with hepatic abscess on the diaphragmatic surface of the liver ($P=0.013$). Additionally, cows with diffused tiny hepatic abscesses had a significantly higher β -globulin concentration than the cows with hepatic abscess on diaphragmatic surface of the liver ($P=0.036$), and a marginally significant less serum β -globulin concentration in comparison to cows with hepatic abscess in left lobe of the liver ($P=0.07$).

Age revealed a significant correlation with the β -globulin percent ($r=-0.219$, $P=0.046$), and also had a marginally significant correlation with the serum concentration of β -globulin ($r=-0.194$, $P=0.07$).

Discussion

It is believed that acidosis-rumenitis-liver abscess complex makes the liver abscesses a major problem in both dairy and beef cattle fed high concentrate diets (Dore et al., 2007; Liberg and Jonsson, 1993; Nagaraja and Chebgappa, 1998). Besides the high economic loss and the relatively high prevalence of liver abscesses in dairy and feedlot cattle, the affected cows show no specific clinical sign and are usually undiagnosed until slaughter. Electrophoretic evaluation of serum proteins as a common biochemical marker is routinely used as a screening test for detecting underlying diseases or for monitoring diseases activity (Nazifi et al., 2009; Putignano et al., 2000).

Chronic active inflammation, which appears as high serum globulin concentration and high plasma fibrinogen has been reported as the clinicopathologic finding in cows with liver abscess (Dore et al., 2007). In the current study, not statistically significant difference was found in the serum concentrations of total protein, albumin, total globulin, α -globulin, β -globulin, γ -globulin, and A/G ratio between the affected and non-affected cows. The same results had been reported by Jonsson and Liberg (1974). They found no significant change in serial

measurement of serum protein due to liver abscess affection in intensively fed cattle. In another study, Liberg and Jonsson (1993) found significantly less A/G ratio and γ -globulin, and higher α_1 -globulin and β_1 -globulin in beef cattle with hepatic abscess than in healthy cattle. However, the variations were slight and had no diagnostic value (Liberg and Jonsson, 1993). Although, change in the blood serum proteins following tissue destruction and inflammation is expected (Saleh et al., 2008), high variation between cattle with hepatic abscess in serum biochemical parameters, which may be due to different stages of lesions development (West, 1997), may decrease the diagnostic value of serum protein electrophoresis in diagnosis of hepatic abscess. In human, change in serum albumin, α_1 -globulin, α_2 -globulin, γ -globulin and A/G ratio have been found in different studies on people suffering from hepatic abscess (El-zayadi et al., 1976; Hashimoto-Yanez et al., 1981). Similar to differences in the serum proteins concentrations and distribution between different species and breeds (Abdo et al., 1987), changes in the serum proteins in similar situations may be different.

It has been shown that during inflammation, the synthesis of positive acute phase proteins in liver increases, and increased total serum protein concentration occurs as a result of hyperglobulinaemia (Eckersall, 2008; Saleh et al., 2008). γ -globulin is considered as a chronic phase protein, which increases in chronic inflammatory disease, chronic infections and abscesses, and its increment denotes a chronic inflammation. (Latimer et al., 2003; Saleh et al., 2008). On the other hand, it is believed that increase of β -globulin occurs as a part of acute phase response (Saleh et al., 2008). The positive correlation between the number of liver abscesses and the γ -globulin, total protein and total globulin, besides the correlations of the number of hepatic abscess and serum β -globulin in the current study may be due to the higher tissue destruction and inflammatory reactions in liver. There is no previous study about the relationship between the change in serum proteins and the severity of hepatic involvement, and the current study showed that serum protein

electrophoresis can be used in diagnosis of extent of the hepatic involvement in cows suffering from hepatic abscess.

Based on our results, serum β -globulin revealed significant difference between different abscess locations and may be usable in prediction of abscess location in the liver. Differences in blood supply and encouragement of immune system between distinctive lobes and surfaces of the liver can be proposed as the probable causes.

In accordance with our results, age had a negative correlation with serum β -globulin. The same results regarding the correlation of age and β -globulin have been reported in cattle (Alberghina et al., 2011). However, there are some controversial reports regarding the differences of other measured parameters between some age groups of cattle (Alberghina et al., 2011; Tothova et al., 2013). Tothova et al. (2013) believed that age should be considered in the interpretation of results of the serum protein electrophoresis in cattle.

Measurement of the serum proteins concentrations can often be helpful in narrowing the list of diseases to be considered and in some cases in confirmation of disease diagnosis in different species of domestic animals (Nazifi et al., 2009). Although more work on a larger number of animals is required before these findings can be used in the practice, our findings suggest that the serum protein electrophoresis may be useful in diagnosis of the severity of hepatic involvement and probable location of the abscesses in affected cattle.

REFERENCES

- Abdo, M.S., Hassanien, N.M., Manna, M.E., Hamed, M., 1987. Electrophoretic pattern of serum proteins in the Arabian camel. *Indian Veterinary Journal* 64, 841-864.
- Alberghina, D., Giannetto, C., Vazzana, I., Ferrantelli, V., Piccione, G., 2011. Reference intervals for total protein concentration, serum protein fractions, and albumin/globulin ratios in clinically healthy dairy cows. *Journal of Veterinary Diagnostic Investigation* 23, 111-114.
- Dore, E., Fedteau, G., Helie, P., Francoz, D., 2007. Liver abscesses in Holstein dairy cattle: 18 cases (1992–2003). *Journal of Veterinary Internal Medicine* 21, 853-856.
- Eckersall, P.D., 2008. Proteins, Proteomics, and the Dysproteinemias. In: *Clinical Biochemistry of Domestic Animals*, 6th Edn., Kaneko JJ, Harvey JW, Bruss ML (Eds.). Academic Press Inc., New York. pp. 1181-155.
- El-Zayadi, A.M., Hartmann, M.G., Mohr, W., 1976. Liver function tests and electrophoretic pattern of serum protein in hepatic amoebiasis. *Journal of Tropical Medicine and Hygiene* 79, 120-122.
- Hashimoto-Yanez, B., Perrillat, A.M., Carrillo, J., Garcia-Tamayo, F., 1981. Protein electrophoresis and immunoglobulin concentration in the serum of children with amebic liver abscess. *Boletin Medico del Hospital Infantil de Mexico* 38, 757-762.
- Jonsson, G., Liberg, P., 1974. Liver abscesses in intensively fed cattle. Serial investigations of serum proteins. *Acta Veterinaria Scandinavia* 15, 264-273.
- Latimer, K.S., Mahaffey, E.A., Prasse, K.W., 2003. *Duncan and Prasse's Veterinary Laboratory Medicine*. 4th Ed., Blackwell Publishing, Iowa State Press, pp. 3-45.
- Liberg, P., Jonsson, G., 1993. Ultrasonography and determination of proteins and enzymes in blood for the diagnosis of liver abscesses in intensively fed beef cattle. *Acta Veterinaria Scandinavia* 34, 21-28.
- Nagaraja, T.G., Chengappa, M.M., 1998. Liver abscesses in feedlot cattle: A review. *Journal of Animal Science* 76, 287-298.
- Nagaraja, T.G., Laudert, S.B., Parrot, J.C., 1996. Liver abscesses in feedlot cattle, part II: incidence, economic importance and prevention. *Compendium, Continuing Education for Veterinarians* 18, S264-S273.
- Nazifi, S., Razavi, S.M., Esmailnejad, Z., 2009. Evaluation of serum proteins in cows naturally infected with *Theileria annulata*. *Journal of Applied Animal Research* 36, 81-83.
- O'Sullivan, E.N., 1999. Two-year study of bovine hepatic abscessation in 10 abattoirs in County Cork, Ireland. *Veterinary Record* 145, 389-393.
- Putignano, P., Kaltsas, G.A., Korbonits, M., Jenkins, P.J., Monson, J.P., Besser, G.M., Grossman, A.B., 2000. Alterations in serum protein levels in patients with Cushing's syndrome before and after successful treatment.

- Journal of Clinical Endocrinology and Metabolism 85, 3309-3312.
- Roberts, J.L., 1982.** The prevalence and economic significance of liver disorders and contamination in grain-fed and grass-fed cattle. Australian Veterinary Journal 59, 129-132.
- Saleh, M.A., Rateb, H.Z., Misk, N.A., 2008.** Comparison of blood serum proteins in water buffaloes with traumatic reticuloperitonitis and sequelae. Research in Veterinary Science 85, 208-213.
- Tothova, C., Nagy, O., Seidel, H., Kovac, G., 2013.** Serum protein electrophoretic pattern in clinically healthy calves and cows determined by agarose gel electrophoresis. Comparative Clinical Pathology 22, 15-20.
- West, H.J., 1997.** Clinical and pathological studies in cattle with hepatic disease. Veterinary Research Communication 21, 169-185.