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Research Article

Salivary and Serum Levels of Serum Amyloid A, Haptoglobin, Ceruloplasmin and Albumin in Neonatal Calves with Diarrhoea [1]

Ekin Emre ERKILIÇ [1,a] Oğuz MERHAN [2,b] Ali Haydar KIRMIZIGÜL [1,c]
Metin ÖĞÜN [2,d] Enes AKYÜZ [1,e] Mehmet ÇİTİL [3,f]

[1] Kafkas University, Faculty of Veterinary Medicine, Department of Internal Medicine, TR-36100 Kars - TURKEY
[2] Kafkas University, Faculty of Veterinary Medicine, Department of Biochemistry, TR-36100 Kars - TURKEY
[3] Erciyes University, Faculty of Veterinary Medicine, Department of Internal Medicine, TR-38039 Kayseri - TURKEY
[a] ORCID: 0000-0003-2461-5598; [b] ORCID: 0000-0002-3399-0667; [c] ORCID: 0000-0002-6660-2149; [d] ORCID: 0000-0002-2599-8589
[e] ORCID: 0000-0002-3288-2058; [f] ORCID: 0000-0001-9839-7533

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Abstract

This study was aimed at the determination of the serum levels of serum amyloid A (SAA), haptoglobin (Hp), ceruloplasmin (Cp) and albumin (Alb), and the salivary levels of Hp and Cp in neonatal calves with diarrhoea. Male and female neonatal Simmental calves, 15 of which were sick and 10 of which were healthy, constituted the study material. Five mL blood samples from the jugular vein and saliva samples absorbed with swabs were collected once from all animals. While serum SAA ($P<0.001$), Hp ($P<0.001$) and Cp ($P<0.001$) levels were found to be significantly higher in the sick calves, compared to the healthy calves, no significant difference was detected for the serum Alb levels ($P>0.05$). Furthermore, the salivary Hp ($P<0.05$) and Cp ($P<0.001$) levels of the sick animals were higher than those of the healthy animals. In result, it was ascertained that serum Hp, SAA and Cp levels and salivary Hp and Cp levels significantly altered in diarrhoeic animals. Thus, it is suggested that in studies involving the measurement of Hp and Cp levels, saliva samples could be collected non-invasively as an alternative to blood samples.

Keywords: *Calf, Serum amyloid A, Haptoglobin, Ceruloplasmin, Saliva*

İshalli Neonatal Buzağılarda Salya ve Serumda Serum Amiloid A, Haptoglobin, Seruloplazmin ve Albumin Seviyeleri

Öz

Bu çalışma ishalli neonatal buzağılarda serumda serum amiloid A (SAA), haptoglobin (Hp), seruloplazmin (Cp) ve albumin (Alb), salyada ise Hp ve Cp seviyelerinin belirlenmesi amacıyla yapılmıştır. Çalışmanın materyalini neonatal dönemdeki farklı cinsiyette 15 hasta, 10 sağlıklı simental ırkı buzağı oluşturdu. Çalışmaya dahil edilen buzağıların *vena jugularis*'lerinden bir kez 5 mL kan ve swaplar yardımı ile salya örnekleri alındı. Serum SAA ($P<0.001$), Hp ($P<0.001$) ve Cp ($P<0.001$) seviyeleri hastalarda sağlıklılara göre istatistiksel olarak önemli seviyede yüksek belirlenirken serum Alb ($P>0.05$) seviyesinde önemli bir değişiklik olmadığı görüldü. Hasta hayvanlarda salya Hp ($P<0.05$) ve Cp ($P<0.001$) seviyeleri sağlıklılara göre yüksek olduğu belirlendi. Sonuç olarak ishalli hayvanlarda serum Hp, SAA ve Cp seviyeleri ile salyada Hp ve Cp seviyelerinde önemli değişikliklerin olduğu belirlendi. Hp ve Cp'nin değerlendirileceği çalışmalarda serum alınmasına alternatif olarak non-invaziv bir yöntem olan salya alınmasıyla da ölçülebileceği kanısını uyandırdı.

Anahtar sözcükler: *Buzağı, Serum amiloid A, Haptoglobin, Seruloplazmin, Salya*

INTRODUCTION

Diarrhoea in neonatal calves is a major cause of economic loss [1]. The risk of diarrhoea is highest during the first month of life and decreases with the advance of age. Diarrhoea may result from the single infection of various agents or from mixed infections caused by multiple infectious agents. The severity and prognosis of the disease are influenced by several factors. These include, among others, nutrition, management and environmental factors [2].

İletişim (Correspondence)
+90 474 242 6836/5235
ekin_emre_24@hotmail.com

The levels of acute-phase proteins, which are synthesized in the liver, are very low in healthy animals and alter very rapidly in the event of inflammation [3]. Acute-phase proteins are classified as either negative or positive. Those, the levels of which rapidly decrease after infection, are classified as negative acute-phase proteins [such as albumin (Alb)], whilst those, the levels of which rapidly increase after infection, are classified as positive acute-phase proteins [such as serum amyloid A (SAA), ceruloplasmin (Cp), and haptoglobin (Hp)] [4].

Saliva, which is a continuously produced secretion, is being used for analyses in human medicine [5]. In veterinary medicine, saliva samples have been used for the measurement of total sialic acid levels and oxidative stress parameters (malondialdehyde (MDA), glutathione, nitric oxide (NO)) in cattle [6], SAA [7,8], Hp [8-10], cortisol [8], interleukin-18 (IL-18) [11], and C-reactive protein (CRP) [9,10,12] levels in pigs and CRP levels in dogs [5]. However, veterinary research in this field is scarce. To our knowledge, no previous study has been conducted in diarrhoeic calves using saliva samples. This study was aimed at the determination of the serum levels of SAA, Hp, Cp and Alb, and the salivary levels of Hp and Cp in neonatal calves with diarrhoea.

MATERIAL and METHODS

Animals

This study was conducted pursuant to the approval of the Local Ethics Board for Animal Experiments of Kafkas University (KAU-HADYEK, Research Code: KAU-HADYEK/2017-021). Male and female neonatal Simmental calves, 15 of which suffered from diarrhoea and 10 of which were healthy, constituted the study material. The sick animals included in the study showed clinical signs such as diarrhoea, decrease or absence of the sucking reflex, lateral recumbency, the inability to stand up, dehydration and coldness of the extremities. Five mL blood samples were taken once from the jugular vein of each animal, and were centrifuged at 3000 rpm for 10 min for the seperated of sera. The serum samples were transferred into eppendorf tubes and stored at -20ºC until being analysed.

Each animal was also sampled for saliva. For this purpose; cotton-tipped swabs were brushed mouth mucosa until the cotton tip became wet with saliva. This sampling method was repeated until a sufficient amount of saliva (approximately 0.3 mL) was collected. Once collected, the saliva samples were centrifuged at 3000 rpm for 10 min, transferred into eppendorf tubes and stored at -20°C until being analysed. SAA and Hp levels in serum samples determined with a enzyme-linked immunosorbent assay kit (Tridelta®, Ireland). Serum Alb levels were determined colorimetricly (Biolabo®, France) in accordance with the manufacturers' instructions. Serum Cp levels were determined as described by Colombo and Richterich [13]. The colorimetric measurement of salivary Hp and Cp levels was performed using the methods described by Skinner et al.[14] and Colombo and Richterich [13], respectively.

Statistical Analysis

The results obtained in the present study were applied a normality test using the SPSS 18 software package, and it was determined that the data showed a normal distribution. The results were compared using the t-test. All results were given as mean ± standard deviation.

RESULTS

All of the sick and healthy animals included in this study were in the neonatal stage of life (0-28 days). Clinically, the sick animals manifested diarrhoea, decrease or absence of the sucking reflex, dehydration, enophthalmos, coldness of the extremities, lateral recumbency, and the inability to stand up.

The serum SAA, Hp and Cp levels of the sick animals were determined to be 42.23±6.14 µg/mL, 0.30±0.02 g/L, and 19.40±2.70 mg/dL, respectively, whilst the same parameters were 14.43±3.29 µg/mL, 0.068±0.014 g/L, and 13.25±2.71 mg/dL, respectively, in the healthy animals *(Table 1)*. Accordingly, it was observed that the serum levels of these parameters were significantly higher in the sick animals, compared to the healthy animals (P<0.001). The serum Alb levels of the healthy and sick animals were measured as 2.95±0.25 g/dL and 2.85±0.25 g/dL, respectively *(Table 1)*. The serum Alb levels of the sick animals were lower than those measured in the healthy animals, but this difference was statistically insignificant (P>0.05).

The salivary Hp and Cp levels of the sick animals were determined as 7.90±2.65 µg/mL and 2.38±0.41 mg/dL, respectively. Furthermore, the salivary Hp and Cp levels of the healthy animals were measured as 5.28±1.76 µg/mL and 1.66±0.36 mg/dL, respectively *(Table 2)*. The

Table 1. *The serum Hp, SAA, Cp and Alb levels of the sick and healthy animals*

Parameter	Control Animals	Sick Animals	P Value
SAA (µg/mL)	14.43±3.29	42.23±6.14	P<0.001
Hp (g/L)	0.068±0.014	0.30±0.02	P<0.001
Cp (mg/dL)	13.25±2.71	19.40±2.70	P<0.001
Alb (g/dL)	2.95±0.25	2.85±0.25	P>0.05
P<0.05 is statistically significant			

Table 2. *The salivary Hp and Cp levels of the sick and healthy animals*

Parameter	Control Animals	Sick Animals	P Value
Hp (µg/mL)	5.28±1.76	7.90±2.65	P<0.05
Cp (mg/dL)	1.66±0.36	2.38±0.41	P<0.001
P<0.05 is statistically significant			

salivary Hp (P<0.05) and Cp (P<0.001) levels of the sick animals were found to be significantly higher than those of the healthy animals. Moreover, it was observed that the salivary levels were rather low in comparison to the serum levels.

DISCUSSION

Diarrhoea cases of various aetiology are one of the main causes of morbidity and mortality in animal holdings. The retarded growth of diarrhoeic animals, treatment costs, human labour and mortality all cause economic loss [15]. The sick animals included in the present study showed clinical signs of diarrhoea, decrease or absence of the sucking reflex, dehydration, enophthalmos, coldness of the extremities, lateral recumbency and the inability to stand up. The clinical signs observed in this study are similar to those observed and reported by Özkan and Akgül [16].

The levels of acute-phase proteins alter in the event of inflammation, trauma, stress and infection [17]. Serum Alb levels decrease with acute phase reactions [18]. In previous research carried out in calves with diarrhoea [19] and pneumonia [20,21], the Alb levels of the sick animals were found to be lower than the levels measured in the control animals. Similarly, in the present study, it was determined that serum Alb levels were lower in the sick animals, when compared to the healthy animals, yet this difference was statistically insignificant (P>0.05).

Haptoglobin and SAA are acute-phase proteins, which are significant for cattle [22]. In healthy cattle, Hp is found at very low levels [23], but its blood levels increase with inflammation, trauma and infection [3]. As this increase in Hp levels occurs before clinical symptoms appear, it is suggested that Hp levels could be measured for the purpose of early diagnosis [24]. In a study carried out in calves, it was observed that SAA levels increased with stress [25]. Similarly, in studies performed in calves with pneumonia, the SAA levels of the sick animals were found to be higher than those of the control animals [20,26]. Reports indicate that, in comparison to healthy animals, diarrhoeic calves presented with significantly increased Hp levels [2,19,27] and SAA levels [2,27]. Likewise, calves infected with *Cryptosporidium* have also been reported to have significantly higher levels of Hp and SAA, in comparison to healthy animals [28]. In agreement with previous research, in the present study it was determined that diarrhoeic calves had serum Hp (P<0.001) and SAA (P<0.001) levels higher than those of healthy animals.

Ceruloplasmin is used less frequently for diagnostic purposes in comparison to other acute-phase proteins [4]. It protects cells against oxidative damage and has cytoprotective activity [17]. In previous research, it was demonstrated that the Cp levels of diarrhoeic calves were significantly higher than those measured in control animals [1,19]. Similarly, the present study showed that the serum Cp levels of the diarrhoeic calves were significantly higher than those of the healthy control animals (P<0.001).

Blood sampling is an invasive method, which causes pain and stress and makes it difficult to collect samples when repeated sampling is required [7]. Gómez-Laguna et al.[10] suggested that, for the measurement of acute-phase protein levels, saliva and meat juice could be used as an alternative to serum samples. Saliva is a biological fluid and can be collected non-invasively [5]. Uzlu et al.[6] measured some oxidative stress parameters (MDA, glutathione and NO) and total sialic acid levels in serum and saliva samples taken from bulls infected with foot and mouth disease, and determined that significant alterations had occurred in both the salivary and serum levels of these parameters. In a previous medical study performed in patients with oral squamous cell carcinoma, the measurement of salivary interleukin-1 alpha, interleukin-6, interleukin-8 and granulocyte macrophage-colony stimulating factor levels demonstrated that these parameters significantly differed between the patients and the control group [29]. In another study carried out in humans with urticaria, the salivary C-reactive protein (CRP) levels of the patients were found to be higher than those of the healthy subjects, and this difference was statistically significant [30]. While research on the evaluation of stress in pigs showed that the salivary SAA level was a potential biomarker [8], in another study, salivary and plasma cortisol levels were determined to display parallel alterations, and were found to be correlated with each other (r=0.60) [31]. Parra et al.[5] ascertained that the salivary CRP levels of healthy dogs and sick dogs with various diseases significantly differed from each other. In another study carried out in pigs, the comparison of the salivary and serum CRP concentrations of healthy and sick animals revealed a statistically significant positive correlation, and the salivary CRP levels of the sick animals were observed to be higher than the levels measured in the healthy animals [12]. In the present study, the salivary levels of the acute-phase proteins Hp (P<0.05) and Cp (P<0.001) were found to be significantly higher in the diarrhoeic animals, in comparison to the healthy calves.

In conclusion, significant alterations were determined to have occurred in the serum Hp, SAA and Cp levels and the salivary Hp and Cp levels of diarrhoeic neonatal calves. The Hp and Cp levels measured in the saliva were lower than those measured in the serum samples, yet the alterations observed in the salivary levels were statistically significant like alterations detected in the serum levels. Furthermore, in view of salivary sampling being a non-invasive method and Hp and Cp levels being able to be measured in saliva samples, it is considered that in future research, saliva samples could be used as an alternative to serum samples for the measurement of these parameters.

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