Evaluation of platelet activating factor in neonatal calves with sepsis

Ramazan YILDIZ*, Mustafa BESLEK*, Yasemin BEYDİLLİ*, Merve ÖZÇELİK M*,

Özlem BİÇİCİ**

Abstract: Early diagnosis and treatment of sepsis are very important conditions in patients for their survival. Novel biomarkers such as platelet activating factor (PAF) have been found that it has an important role in sepsis process. The aim of this study is to determine the role of PAF in pathogenesis and the diagnosis importance in calves with sepsis. For this purposes, 30 neonatal calves with sepsis (sepsis group) and 10 healthy neonatal calves (control group) were used. Plasma samples were used to determine the PAF level with using bovine specific ELISA kits. Plasma concentration of PAF was found significantly higher ($P < 0.01$) in sepsis group compare to control group. We concluded that PAF has an important role in sepsis and it may have diagnostic value in calves with sepsis. Further studies are required to determine the importance of PAF as diagnostic and prognostic biomarker in calves with sepsis and evaluate the concentrations of these biomarker also in other disease states.

Keywords: Calves, platelet-activating factor, sepsis

Sepsis şüphesi bulunan neonatal buzağılarda platelet aktive edici faktörün değerlendirilmesi

Öz: Sepsisin erken tanısı ve tedavisi, hastaların hayatta kalmaları için çok büyük önem arzeder. Sepsis sürecinde önemli rol oynayan trombosit aktive edici faktör (PAF) gibi yeni biyolojik belirteçler bulunmuştur. Bu çalışmanın amacı sepsisli buzağılarda PAF'ın patogenezdeki rolünü ve diagnostik önemini belirlemektir. Bu amaçla sepsisli 30 yeni doğan buzağı ve 10 sağlıklı neonatal buzağı kullanıldı. Buzağılardan alınan plazma örneklerinde sığır spesifik ELISA kiti kullanılarak PAF plazma konsantrasyonu belirlendi. PAF plazma konsantrasyonu sepsis grubunda kontrol grubuna göre anlamlı derecede yüksekti ($P < 0.01$). PAF'ın sepsisde önemli bir rolü olduğu ve sepsisli buzağılarda tanısal değeri olabileceği sonucuna varıldı. Öte yandan, sepsisli buzağılarda PAF'ın tanısal ve prognostik önemini belirlemek için diğer biyolojik biyomarkırlarla birlikte değerlendirildiği ileri çalışmalara ihtiyaç olduğu görüldü.

Anahtar sözcükler: Buzağı, platelet aktive edici faktör, sepsis

Introduction

Sepsis is a condition characterized by the development of a systemic inflammatory response syndrome (SIRS) as a result of confirmed or suspected infection and may be accompanied by systemic organ failure often leading to death

* *Faculty of Veterinary Medicine, University of Mehmet Akif Ersoy, Burdur, Turkey*
** *Faculty of Veterinary Medicine, University of Siirt, Siirt, Turkey*

(32, 37). Sepsis and diarrhea is generally the most common cause of morbidity and mortality in neonatal calves related to the failure of the colostral transfer (4,5). Sepsis can cause serious economic loss in farm animals (5,15). Therefore, the markers that can be used in the early diagnosis of sepsis have importance in the veterinary field (5).

Platelet activating factor (PAF) defined as a phospholipid-derived mediator released by phospholipases in various cell types during the early stages of inflammation. There is strong evidence that platelet activating factor (PAF) plays an important role in the release of inflammatory mediators in the sepsis (25, 36). PAF has the potential to increase the toxic effect of TNF (27) and plays a role in the induction of nitric oxide synthase (38).

The aim of this study is to determine the role of PAF in the sepsis of neonatal calves.

Materials and methods

Animals: The animals used in this study were 30 neonatal calves with sepsis and 10 healthy calves that served as the control group. All calves were 1 to 21 days of age. All procedures were performed with the study protocol approved by the Ethics Committee of Veterinary Faculty, Mehmet Akif Ersoy University (Ethics Committee Certificate no: 2016/225).

The inclusion criteria in the sepsis group required the calves to have an absence or lack of suction reflex, lateral recumbency and depression with at least two or more recorded SIRS signs (temperature > 39.5 ℃ or < 37 ℃, Heart rate < 100 or > 160 pulse/min (minute), respiratory rate > 45/min, total leukocyte value > $12 \times 10^3/\mu L$ or < $4 \times 10^3/\mu L$), plus infection or suspected infection (15, 16). Calves with positive for sepsis criteria were included in the study.

Blood sampling and laboratory analysis: Blood samples were collected through a catheter placed in right jugular vein into tubes with heparin for plasma to determine the PAF levels and with K_3EDTA to establish the hematological parameters [white blood cell (WBC), granulocyte (GRA), erythrocytes (RBC), hematocrit (Hct) and platelet (PLT)] at 0. hour (pre-treatment). The hematological parameters were measured on an Abacus Junior Vet analyzer (Diatron MI Ltd. Hungary) device within 15–30 min of sample collection. Samples were centrifuged (10 min at 4000 rpm) and the plasma stored at -20 °C until assayed. The plasma concentrations of PAF were determined using bovine specific enzyme-linked immunosorbent assay ELISA kits according to the manufacturer's instructions (Sunred Biological Technology, China). Absorbant was recorded using a microplate reader (ELx800™ Absorbance Microplate Reader, USA).

Statistical analysis: All data were presented as mean values and included standard error of the mean (mean ± SEM). Normality distribution of the data was examined using Kolmogorov-Smirnov test. Student's t-test was used for statistical analysis of parameters. SPSS software for Windows, version 14.01 was employed for the statistical analysis. *P*-value of < 0.05 was accepted as the level of statistical significance.

Results

Hypothermia, poor suction reflex, weakness, tachypnea, depression and hyperemic mucosa are the most common clinical signs of sepsis observed in calves (Table 1). Body temperature ($P < 0.01$), capillary refill time (CRT) ($P < 0.001$), respiration rate ($P < 0.001$), heart rate ($P < 0.001$) and oxygen saturation with pulse oximeter (SpO_2) ($P < 0.001$) showed significant differences between sepsis and control group (Table 1). The level of WBC was found to be significantly higher ($P < 0.01$) in the sepsis group than in control group (Table 1).

Table 1: Hemogram, clinical and monitoring findings of sepsis and control groups (mean ± SEM)
Tablo 1: Sepsis ve kontrol gruplarının hemogram, klinik ve monitorizasyon bulguları (mean ± SEM)

Parameters	Control (n:10)	Sepsis (n:30)	P value
WBC (×10³/µL)	10.4 ± 0.64	17.5 ± 1.70	<0.001
GRA(×10³/µL)	5.12 ± 0.47	10.4 ± 1.33	0.001
RBC (×10⁶/µL)	6.73 ± 0.24	7.30 ± 0.31	0.156
HCT (%)	22.6 ± 1.11	27.1 ± 1.39	0.233
PLT (×10³/µL)	565 ± 71.2	532 ± 48.4	0.699
Temperature (°C)	38.5 ± 0.88	37.3 ± 0.20	<0.001
MAP (mmHg)	93.6 ± 2.40	85.6 ± 2.88	0.041
Respiration (min)	25.1 ± 1.43	56.6 ± 4.49	<0.001
SpO₂ (%)	93.0 ± 1.06	79.8 ± 1.39	<0.001
CRT (sn)	1.6 ± 0.16	6.00 ± 0.37	<0.001
Heart rate (min)	108 ± 1.94	93.8 ± 4.07	0.003

WBC = White blood cell, GRA = Granulocyte, RBC = Erythrocyte, HCT = Hematocrit, PLT = Platelet, MAP = Mean arterial pressure, SpO2 = pulse oximeter-oxygen saturation, CRT = Capillary refill time
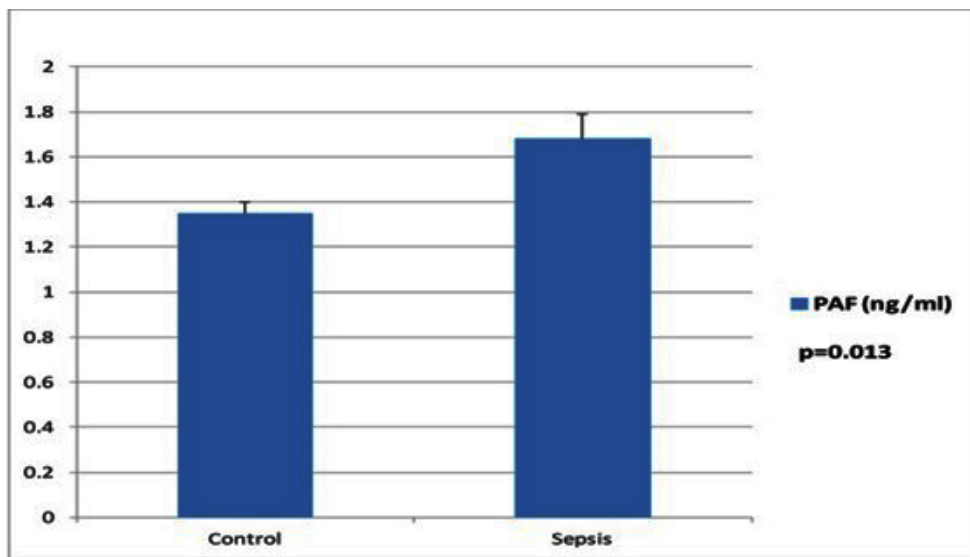
PAF=Platelet activating factor

Figure 1: PAF values of sepsis and control groups (mean ± SEM)
Şekil 1: Sepsis ve kontrol gruplarının PAF değerleri (mean ± SEM)

The plasma level of PAF in sepsis group was statistically higher than control group ($P < 0.05$) (Figure 1).

Discussion

This is the first study to evaluate the levels of PAF in calves with sepsis. In the present study, we demonstrated that the plasma concentration of PAF is increased in calves with sepsis. This result suggests that PAF may be a biomarker for early diagnosis of sepsis.

Clinical findings in the early stages of sepsis are not very specific but still important for the sepsis scoring and response to the therapy (3, 5, 32, 34). The symptoms such as hypothermia, prolonged capillary refill time, dehydration and depression have been reported in calves diagnosed with sepsis (16, 34). Clinical symptoms of the calves were similar to the clinical findings of sepsis as expressed in the previous investigations (16, 5, 3). Significant changes occur in hematological parameters in septic calves. Changes in hematologic parameters may vary according to the severity of the disease. An increase in the number of leukocytes and a shift to the left are expected in sepsis (16, 21, 30).

In this study, it was determined that the level of WBC was significantly higher ($P < 0.001$) than in control group. The results of the our study also support that WBC level is important finding for diagnosis of sepsis in neonatal calves (3, 35, 40). The currently available tests for identifying of sepsis in patients are not specific, often indirect and presumptive. The diagnosis of sepsis is still based on physical and laboratory findings.

Traditional markers such as WBC counts, body temperature, and CRP expression levels are not reliable for assessing the diagnosis of sepsis (5, 14). The studies continue for to understand the pathogenesis of sepsis and the possibility for early diagnosis. The pathogenesis of sepsis, defined as a systemic inflammation in response to infection, continues to be an area of active research. Advances in our understanding of molecular signaling and cellular interactions have focused many studies of sepsis on inflammatory and anti-inflammatory pathways. One of that parameter is PAF, that is defined as an important mediator in myocardial depression (20), pulmonary dysfunction and DIC (disseminated intravascular coagulation) in sepsis (33). Intravascular administration of PAF to animals in experimental studies causes capillary leak, shock, neutropenia, thrombocytopenia, bronchoconstriction and pulmonary hypertension (19). It has been reported that the level of PAF increased in human sepsis (33). Pre-clinical animal data on PAF receptor antagonism has provided promising support for PAF inhibitors as a therapeutic intervention for sepsis (17, 23). It has been reported that experimentally administered lipopolysaccharide (LPS) affects the expression of both PAF and its receptor. Serum PAF levels have been reported to be elevated in rat, pig, cat and mouse models after systemic LPS administration (9, 29). Local production of PAF was detected in gastrointestinal and pulmonary tissue specimens from septic animals (9, 11). Many mediators have been implicated in intestinal damage associated with ischemia-reperfusion, but it is known that platelet

activating factor and free oxy-oxygen radicals have an important effect in this process (18, 42). Studies in mice have shown hypotension, neutropenia, hemoconcentration, and small intestinal necrosis after PAF given to the aorta (7). It was observed that intestinal damage decreased when PAF antagonist was given to mice (26). Caplan et al. (8) reported that PAF and TNF (tumor necrosis factor) were elevated in patients with enterocolitis. PAF stimulates polymorphonuclear leukocytes (PMNs) directly to produce chemotaxis, degranulation, and superoxide radicals, as well as responsible for the aggregation and degranulation of the active platelets. This mediator also facilitates endothelial damage of elastase, which is predisposed to clot formation (41). PAF is thought to have important roles in experimental gram-negative sepsis, and there are reports that it's adverse effects can be corrected by PAF receptor antagonists (13).

In the present study conducted, PAF concentration in calves with sepsis was significantly higher than control group ($P < 0.05$) (Figure 1), indicating that it can be used in the diagnosis of sepsis and plays an important role in the pathogenesis of sepsis. The limitation of this study is that it can not be evaluated together with other sepsis biomarkers.

In conclusion, we find that PAF may be a diagnostic value for sepsis in neonatal calves. The increase in PAF concentration may indicate an important role in sepsis and when assessed with other sepsis markers that it may provide more reliable information on the pathogenesis of sepsis in neonatal calves.

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Corresponding author:

Dr. Ramazan YILDIZ

Department of Internal Medicine, Faculty of Veterinary Medicine,

University of Mehmet Akif Ersoy, Campus Ortulu, 15030, Burdur, Turkey.

e-mail: ramazanyildiz@mehmetakif.edu.tr