

Novel biomarkers for Pregnancy Toxemia in Ewes: Acute Phase Proteins and Pro-inflammatory Cytokines

Wael M. EL-Deeb*

Department of Veterinary Medicine, infectious diseases and fish diseases, Faculty of Veterinary Medicine, Mansoura University, Egypt

Abstract

The present study aimed to investigate the Acute Phase Proteins (APPs), pro-inflammatory cytokines and traditional biomarkers in healthy and pregnancy toxemia affected ewes at the same gestation period. The affected ewes (n=25) exhibited clinical signs of pregnancy toxemia one to three weeks pre-lambing. Clinically healthy ewes (n=20) with the same age and gestation period were used as control. Results indicated that there was a significant ($P \leq 0.05$) increase in the levels of Haptoglobin (HP), Serum Amyloid A (SAA), Fibrinogen, interleukins (IL-1 α , IL-1 β , IL-6), TNF- α , and interferon gamma (IFN- γ) in ewes with pregnancy toxemia when compared to corresponding control. Moreover, there was significant ($P \leq 0.05$) increase in the levels of β -Hydroxybutyrate (BHBA), Non esterified free fatty acids (NEFA), triglyceride, LDL-c, AST, ALT and Uric acid in ewes with pregnancy toxemia compared to control. Furthermore, a significant ($P \leq 0.05$) decrease in the levels of glucose, HDL-c, cholesterol and BUN were detected in diseased ewes compared to control. Conclusively APPs and pro-inflammatory cytokines could be used as a novel biomarkers for pregnancy toxemia diagnosis in ewes.

Keywords: HP; SAA; IL-1 α ; IL-1 β ; TNF- α ; IL-6; IFN- γ ; ewe; pregnancy

Introduction

Pregnancy toxemia is a metabolic disease caused by the sudden extra demand for energy in the fast-growing kids or lambs during the last few weeks of pregnancy [1,2]. Economic losses because of the disease have been considerable and it is the most commonly occurring metabolic disease of sheep and goats [3]. Short starvation period during late pregnancy in ewes can produce liver microvesicular degeneration, which could affect the whole liver acinus and can be spontaneously reversed. Severity of liver damage was found to be associated with an increased activity of AST [4]. Acute phase proteins (APPs) have been proposed as sensitive and rapid indicators of inflammatory disturbances in ruminants [2,5]. However, the APPs in small ruminants is poorly described. The different APPs may play a similar role both in sheep and goat but some differences have been reported [6]. Haptoglobin (Hp) and Serum Amyloid A (SAA) are considered as major APPs in both ovine and caprine. Nonetheless, Fibrinogen (Fb) participates as a minor APP in sheep but as a moderate APP in goat [6,7]. Most of the studies performed on sheep are focused on the role of APPs after several inflammatory stimuli, being carried out few studies concerning specific bacterial, viral or parasitic infections. Some studies are focused on the expression of APPs against lentiviral infections, being observed a local expression of SAA [8] but no serum enhancement of Hp or Fb concentrations [9]. It was reported by Hiss et al., [10] that Hp levels were increased in cows around parturition. It has been postulated to be related to negative energy balance, since cows with high milk Hp also showed high non-esterified fatty acid (NEFA) concentration in serum. A significant correlation has been also reported between Hp and BHBA in lactating goats [11]. Previous studies in ruminants suggested a relationship between selected APPs and lipid mobilization [2]. Cytokines are associated with immune responses and are implicated in the pathogenesis of pregnancy toxemia owing to their roles in endothelial damage [12-15]. An intense systemic inflammatory response and augmented production of inflammatory cytokines occur in pregnancy toxemia, for which trophoblast cell defects and destruction of placental debris are considered to be important inducing factors [16,17]. Hypoxia in the placenta due to reduced blood perfusion, in turn, induces placental production of

inflammatory cytokines IL-1 α and TNF- α [18]. According to author best of knowledge there is limited data on concentrations of HP, SAA, Fb, IL-1 β , IL-6 and IFN- γ in ewes with pregnancy toxemia. The present work aimed to determine the concentration of selected APPs and pro-inflammatory cytokines in ewes with pregnancy toxemia. Moreover, evaluation of these parameters and the possible practical applications of APPs and proinflammatory cytokines in the diagnosis and prediction of pregnancy toxemia in ewes.

Materials and Methods

Animals

Twenty five pregnant native Saudi Arabia ewes (3.7 ± 0.4 years old), were referred to the Veterinary Teaching Hospital, King Faisal University, Saudi Arabia, showing clinical signs of pregnancy toxemia, one to three weeks pre-lambing. In addition, 20 more healthy pregnant ewes of similar age and gestation period were used as a control group. All ewes were examined clinically.

Sampling

Blood samples were collected from pregnant uncomplicated ewes and ewes with clinical signs of pregnancy toxemia. Two separate blood samples were collected from jugular vein; one sample was taken in vacutainers tubes for hematological studies. The second blood samples were taken in plain centrifuge tube for serobiochemical analysis. The samples were put in an inclined position for 20 minutes at room

*Corresponding author: Wael M. EL-Deeb, Department of Veterinary Medicine, infectious diseases and fish diseases, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt; Tel: 0020 50-2372592; Fax: 0020-50-2379952; E-mail: drwaeldeeb@yahoo.com

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temperature, and, kept in refrigerator to avoid glycolysis and for clot retraction. The samples were centrifuged at 3000 rpm for 10 minutes and the clear serum was separated carefully and stored at -20°C until estimation of serum biochemistry.

Methodology

Determination of Acute Phase Proteins and Pro-Inflammatory Cytokines

Serum Hp and SAA were measured with a commercially available ELISA kit (Phase HP and Phase SAA kit, Tridelta Ltd., Ireland), according to the manufacturer's instructions. Fibrinogen (Fb) concentration in plasma was determined according to the method described by [19]. IL-1 β , TNF- α , IL-6 and IFN- γ levels were determined from undiluted serum samples using commercially available ELISA Kits (Biosource International, California, USA). The plates were read at 450 nm on a computerized automated micro plate ELISA reader (Bio TEC, ELX800G, USA). All measurements were made in duplicate.

Serum Biochemical Analysis

The levels of glucose, triglyceride, cholesterol, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and blood urea nitrogen (BUN), as well as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined in serum samples on a Beckman CX-7 autoanalyser using the corresponding kits (Sigma Chemical Co. Ltd., Poole, Dorset, UK). Serum β -Hydroxybutyrate (BHBA) was determined by a kinetic enzymatic method using a commercially available kit (Ranbut D-3-hydroxybutyrate, Randox, Crumlin Co., Antrim, UK) according to the methods described by [20]. The assay is based on the reversible reaction between 3-hydroxybutyrate and NAD⁺ catalyzed by 3-hydroxybutyrate dehydrogenase, and the change in NADH concentration was measured by changes in the absorbance at 340 nm. Serum concentration of Non esterified free fatty acids (NEFA) was carried out using commercially available test kits supplied by Randox laboratories Ltd. Uric acid was determined by uricase-POD enzymatic colorimetric method by using kits provided by Spinreact, Spain according to [21].

Statistical Analysis

The obtained data was analyzed using Student's t-test to compare between means of different groups according to the method described by [22]. All tests were performed using computer package of the statistical analysis system [23].

Results

The diseased ewes with pregnancy toxemia showed dull attitude, reluctance to move or even recumbent, teeth gnashing and some neurological signs such as, blindness, lapping, head pressing, tremors and convulsions. Concerning the acute phase response, there was significant ($P \leq 0.05$) increase in the levels of HP, SAA, Fb, IL-1 α , IL-1 β , TNF- α , IL-6 and IFN- γ (1.2 \pm 0.11 mg/l, 29.4 mg/l, 3.64 g/l, 22.32 pg/ml, 32.83 pg/ml, 19.36 pg/ml, 16.32 pg/ml, 16.34 pg/ml) in ewes with pregnancy toxemia when compared to healthy ewes (Table 1)

In the present study there was significant ($P \leq 0.05$) increase in the levels of BHBA, NEFA, triglyceride, LDL-c, AST, ALT and uric acid with significant ($P \leq 0.05$) decrease in the levels of glucose, HDL-c, cholesterol and BUN in ewes with pregnancy toxemia when compared to healthy ones (Table 2).

Variable	Healthy ewes	Ewes with pregnancy toxemia
Hp (mg/l)	0.06 \pm 0.001	1.2 \pm 0.11*
SAA (mg/l)	4.36 \pm 1.6	29.4 \pm 4.42*
Fibrinogen (g/l)	2.32 \pm 0.23	3.64 \pm 0.31*
IL-1 α (pg/ml)	13.25 \pm 3.32	22.32 \pm 4.31*
IL-1 β (pg/ml)	18.74 \pm 2.43	32.83 \pm 3.3*
TNF- α (pg/ml)	8.54 \pm 2.23	19.36 \pm 4.65*
IL-6 (pg/ml)	11.44 \pm 3.02	16.32 \pm 3.23*
IFN- γ (pg/ml)	10.23 \pm 2.32	16.34 \pm 3.3*

*Means are significantly different at the level ($p \leq 0.05$)

Table 1: The acute phase proteins and pro-inflammatory cytokines in clinically healthy pregnant ewes and those with pregnancy toxemia.

Discussion

The present results revealed significant ($P \leq 0.05$) increase in the levels of HP, SAA, Fb in ewes with pregnancy toxemia when compared to healthy pregnant ewes (Table 1). These results are in agreement with findings of [24]. These authors documented relationships between inflammatory mediators and metabolic disorders. The present results are partially in line with Yoshino et al., [25]. The authors reported increases in Hp levels after starvation of cows.

The significant increases in APPs found in ewes with pregnancy toxemia could be related to the changes in lipid metabolism that occurs in this process [26] Moreover, [27] reported that when a fasted period of 3 days was applied to a group of calves, NEFA mobilization was observed on the first fasting day. In the same study, Hp was also observed to be increased but lagged behind the rise in NEFA and was not increased in most calves after 2–3 days of re-feeding. There are some evidences of a relationship between Hp and lipids.

From another point of view [2] reported that Hp does not seem to be a good marker of pregnancy toxemia because other markers such as plasma NEFA and ketone bodies in urine showed significant changes at 24 hr. In the same concern [28] reported increased concentrations of BHBA and normal Hp concentrations in cows with subclinical ketosis.

Native Saudi Arabia ewes with pregnancy toxemia showed elevated levels of circulating pro-inflammatory cytokines IL-1 α , IL-1 β , TNF- α , IL-6 and IFN- γ compared to control ewes (Table 1). The elevated levels of cytokines suggesting that pregnancy toxemia in ewes was coupled with systemic inflammation. Cytokines including IL-1 and TNF- α , either produced locally or originating from the circulation, influence the maternal-placental immune interactions, trophoblast invasion and differentiation and the metabolic and endocrine regulation of placenta [13,15,29].

Abnormal placental cytokine production in pregnancy disorders such as pregnancy toxemia has been implicated in the aetiopathogenesis of local and systemic inflammatory responses in such conditions [15,29] in human. Endothelial cell activation has also been concerned as a potential mediator of the inflammatory response in pregnancy toxemia [30] in bovines.

The higher levels of IL-1 α , IL-1 β , TNF- α , IL-6 and IFN- γ and their positive correlation with well-established parameters of pregnancy toxemia including serum uric acid, BHBA and NEFA in the present study even suggest that such cytokines are also mediators of placental inflammation and subsequent systemic inflammatory reactions in ewe pregnancy toxemia, as they have been previously been implicated in pregnancy toxemia in women and laboratory animals [31,32]. In the same concern [14] and [16] reported that the key role for plasma cytokines has been hypothesized in pathogenesis because a generalized

Variable	Healthy ewes	Ewes with pregnancy toxemia
Glucose (mg/dl)	61.4 ± 0.29	31.1 ± 0.39*
β-Hydroxybutyrate (mmol/l)	0.92 ± 0.12	8.24 ± 1.2*
NEFA (mmol/l)	0.54 ± 0.12	1.62 ± 0.21*
Triglyceride (mg/dl)	55.9 ± 0.15	102.09 ± 0.60*
Cholesterol (mg/dl)	78.2 ± 0.66	44.8 ± 0.25*
LDL-c (mg/dl)	27.37 ± 0.17	29.33 ± 0.20*
HDL-c (mg/dl)	49.6 ± 0.22	11.92 ± 0.72*
AST (IU/L)	47.5 ± 0.63	209.5 ± 2.51*
ALT (IU/L)	17.13 ± 0.23	57.02 ± 0.55*
BUN (mg/dl)	25.5 ± 0.54	9.92 ± 0.1*
Uric acid (mg/dl)	0.11 ± 0.01	0.21 ± 0.02*

*Means are significantly different at the level ($P \leq 0.05$)

Table 2: Selected biochemical parameters in clinically healthy pregnant ewes and those with pregnancy toxemia.

inflammatory response occurs with activation of leukocytes and elevation of cytokine production along with abnormal activation of clotting system in pregnancy toxemia.

The increased levels of IFN- γ in ewes with pregnancy toxemia when compared to healthy pregnant ones may need future research effort to clarify the role of such cytokines in this critical period of pregnancy toxemia in ewes. Interferon gamma (IFN- γ) is a proinflammatory cytokine secreted in the uterus during early pregnancy. It is abundantly produced by uterine natural killer cells in maternal endometrium but also by trophoblasts in some species. In normal pregnancies of mice, IFN- γ plays critical roles that include initiation of endometrial vasculature remodeling, angiogenesis at implantation sites, and maintenance of the maternal component of the placenta. In livestock and in humans, deviations in these processes are thought to contribute to serious gestational complications, such as fetal loss or preeclampsia [33].

Concerning the biochemical findings, there was significant ($P \leq 0.05$) decrease in glucose levels in ewes with Pregnancy toxemia when compared to healthy pregnant ones (Table 2) which appears to occur when the animal cannot meet the glucose demands of the fetal-placental unit and hypoglycemia develops. The relationship between the severity of neurological symptoms and depth of hypoglycemia provides additional clinical evidence of hypoglycemic encephalopathy [34]. Ischemia, hypoglycemia and epilepsy affects energy metabolism by arresting or impeding cellular energy production or pathologically enhancing energy consumption [35]. The present findings are in agreement with [26, 36]. The authors stated that plasma glucose concentrations of spontaneous ovine pregnancy toxemia cases were significantly lower compared to anorexic ewes and healthy ewes at a similar stage of gestation.

Moreover, there was a significant ($P \leq 0.05$) increase in the triglyceride in ewes with pregnancy toxemia when compared to healthy pregnant ewes (Table 2). Moreover, serum concentrations of cholesterol in the diseased ewes was significantly ($P \leq 0.05$) lower than in healthy ones (Table 2). It has been reported previously that serum triglyceride concentrations increase in ketosis [34, 37] which is confirmed in this study. Serum cholesterol levels significantly ($P \leq 0.05$) decrease in hepatic insufficiency [37]. Lower HDL-c, lower BUN and higher AST and ALT activities in the diseased ewes (Table 2) under investigations may have been due to liver damage [37].

These results are in consistent with those reported by Peneva and Goranov, [38]. The authors stated that serum AST and ALT activities

were significantly higher and correlated positively with the rise of ketonemia and ketonuria in cows with subclinical ketosis.

In conclusion, APPs and pro-inflammatory cytokines could be used as additional diagnostic biomarkers for pregnancy toxemia in ewes. The increased levels of IFN- γ in ewes with pregnancy toxemia compared to healthy pregnant ones may need further research to clarify the role of such cytokines in this critical period of pregnancy toxemia in ewes.

References

- Rook JS (2000) Pregnancy toxemia of ewes, does, and beef cows. *Vet Clin North Am Food Anim Pract* 16: 293-317.
- González FH, Hernández F, Madrid J, Martínez-Subiela S, Tvarijonavičiute A, et al. (2011) Acute phase proteins in experimentally induced pregnancy toxemia in goats. *J Vet Diagn Invest* 23: 57-62.
- Marteniuk JV, Herdt TH (1988) Pregnancy toxemia and Ketosis of ewes and does. *Vet Clin North Am Food Anim Pract* 4: 307-315.
- Cal L, Borteiro1 C, Benech1 A, Rodas E, Abreu1 MN, et al. (2009) Histological changes of the liver and metabolic correlates in ewes with pregnancy toxemia [Alterações histológicas do fígado e correlações metabólicas em ovelhas com toxemia da prenhez] González Montaña2. *Arq. Bras. Med. Vet. Zootec* 61: 306-312.
- Eckersall PD (2000) Recent advances and future prospects for the use of acute phase proteins as markers of disease in animals. *Revue. Med. Vet.* 151: 577-584.
- González FH, Tecles F, Martí'nez-Subiela S, Tvarijonavičiute A, Soler L, et al. (2008) Acute phase protein response in goats. *J Vet Diagn Invest* 20: 580-584.
- Murata H, Shimada N, Yoshioka M (2004) Current research on acute phase proteins in veterinary diagnosis: an overview. *Vet J* 168: 28-40.
- Sack GH, Zink MC (1992) Serum amyloid A gene transcription in synovial cells during retroviral arthritis. *Am J Pathol* 141: 525-529.
- de la Concha-Bermejillo A, Singh B, Whitney MS, Bazer FW (2000) Acute-phase proteins and hematologic values in ovine lentivirus-infected lambs treated with recombinant ovine IFN-tau. *J Interferon Cytokine Res* 20: 41-53.
- Hiss S, Weinkauff C, Hachenberg S, Sauerwein H (2009) Relationship between metabolic status and the milk concentrations of haptoglobin and lactoferrin in dairy cows during early lactation. *J Dairy Sci* 92: 4439-4443.
- Trevisi E, D'Angelo A, Gaviraghi A, Noé L, Bertoni G, et al. (2005) Blood inflammatory indices in goats around kidding. *Ital J Anim Sci* 4: 404.
- Akoum A, Lemay A, Brunet C, Hebert J (1995) Cytokine-induced secretion of monocyte chemotactic protein -1 by human endometriotic cells in culture. The Groupe d'Investigation en Gynecologie. *Am J Obstet Gynecol* 172: 594-600.
- Conrad KP, Benyo DF (1997) Placental cytokines and the pathogenesis of preeclampsia. *American Journal of Reproductive Immunology* 37: 240-249.
- Conrad KP, Miles, TM, Benyo DF (1998) Circulating levels of immunoreactive cytokines in women with preeclampsia. *Am J Reprod Immunol* 40: 102-111.
- Redman CW, Sargent IL (2005) Latest advances in understanding preeclampsia. *Science* 308: 1592-1594.
- Sacks G, Sargent I, Redman C (1999) An innate view of human pregnancy. *Immunology Today* 20: 114-118.
- Sargent IL, Germain SJ, Sacks GP, Kumar S, Redman CW (2003) Trophoblast deportation and the maternal inflammatory response in pre-eclampsia. *J Reprod Immunol* 59: 153-160.
- Benyo DF, Miles TM, and Conrad KP (1997) Hypoxia stimulates cytokine production by villous explants from the human placenta. *J Clin Endocrinol and Metab* 82: 1582-1588.
- Becker U, Bartl K, Wahlefed AW (1984) A functional photometric assay for plasma fibrinogen. *Thromb Res* 35: 475-484.
- Ismail ZA, Al-Majali AM, Amireh F, Al-Rawashdeh OF (2008) Metabolic profiles in goat does in late pregnancy with and without subclinical pregnancy toxemia. *Vet Clin Pathol* 37: 434-437.
- Young, DS (2001) Effect of Disease on Clinical Lab. Test. (4th Edn) AACC.

22. Snedecor GW, Cochran PW (1989) *Statistical Methods*, eighth ed. Iowa State University, Press, Ames, IA.
23. SAS (2002) *Statistical Analysis System. User's Guide*. SAS Institute Inc. Cary, NC, USA.
24. Ametaj BN (2005) A new understanding of the causes of fatty liver in dairy cows. *Advances in Dairy Technology* 17: 97–112.
25. Yoshino K, Katoh N, Takahashi K, Yuasa A (1993) Possible involvement of protein kinase C with induction of haptoglobin in cows by treatment with dexamethasone and by starvation. *Am J Vet Res* 54: 689-694.
26. Scott PR, Sargison ND, Penny CD (1998) Evaluation of recombinant bovine somatotropin in the treatment of ovine pregnancy toxemia. *Vet J* 155: 197-199.
27. Katoh N, Oikawa S, Oohashi T, Takahashi Y, Itoh F (2002) Decreases of apolipoprotein B-100 and A-I concentrations and induction of haptoglobin and serum amyloid A in nonfed calves. *J Vet Med Sci* 64: 51-55.
28. Skinner JG, Brown RA, Roberts L (1991) Bovine haptoglobin response in clinically defined field conditions. *Vet Rec* 128: 147-149.
29. Bowen RS, Gu Y, Zhang Y, Lewis DF, Wang Y (2005) Hypoxia promotes interleukin -6 and -8 but reduces interleukin -10 production by placental trophoblast cells from preeclamptic pregnancies. *J Soc Gynecol Investig* 6: 428-432.
30. Roberts JM, Taylor RN, Musci TJ, Rodgers GM, Hubel CA et al. (1989) Preeclampsia: an endothelial cell disorder. *Am J Obstet Gynecol* 161: 1200-1204.
31. Wang Y, Walsh SW (1996) TNF alpha concentrations and mRNA expression are increased in preeclamptic placentas. *J Reprod Immunol* 32: 157-169.
32. Faas MM, Broekema M, Moes H, van der Schaaf G, Heineman MJ, et al. (2004) Altered monocyte function in experimental preeclampsia in the rat. *Am J Obstet Gynecol* 191: 1192-1198.
33. Murphy SP, Tayade C, Ashkar AA, Hatta K, Zhang J, et al. (2009) Interferon Gamma in Successful Pregnancies. *Biol Reprod* 80: 848-859.
34. Marteniuk JV, Herdt TH (1988) Pregnancy toxemia and ketosis of ewes and does. *Vet Clin North Am Food Anim Pract* 4: 307-315.
35. Auer RN, Siesjo BK (1988) Biological differences between ischemia, hypoglycemia, and epilepsy. *Ann Neurol* 24: 699-707.
36. Henze P, Bickhardt K, Fuhrman H (1994) The contributions of the hormones insulin, cortisol, somatotropin and total estragen to the pathogenesis of sheep ketosis. *Dtsch Tierarztl Wochenschr* 101: 61-65.
37. Kaneko JJ, Harvey JW, Bruss ML (1997) *Clinical biochemistry of domestic animals*, (5thedn). Academic Press, Inc, California, USA.
38. Peneva I, Goranov KH, (1984) [Changes in the serum enzymes and clinical and clinico-biochemical indices of cows with subclinical ketosis]. *Vet Med Nauki*, 21: 28-36.