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Prognostic Value of Serum Cardiac Troponin T and Nitric Oxide as Cardiac Biomarkers in Pregnancy Toxemic Goats

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Abstract: Pregnancy toxemia (PT) is a metabolic disorder occurring in goats during the late stage of gestation. The importance of cardiac troponin T (cTnT) and nitric oxide (NO) concentration as cardiac biomarkers in pregnancy toxemia has been emphasized in several diseases. However, its prognostic value in PT has not been examined. The aim of this study is to determine the prognostic value of cTnT and NO in PT goats. Twenty clinically affected goats (10 with early PT and 10 with advanced PT, along with 20 clinically healthy goats (10 pregnancies and 10 non pregnant) were used in this investigation. Goats with early toxemia had anorexia, depression, dullness, pronounce acetone odor from mouth and scanty feces. Additionally, recumbency, muscular tremors, grinding of teeth and deviated head and neck were noticed in advanced PT goats. All does with advanced PT not respond to any type of treatment. Liver function tests (ALT& AST), kidney function tests (blood urea nitrogen and creatinine), cardiac function parameters (cTnT and NO) beside the glucose and βHB showed a significant increase as the disease progressed. Elevation of cTnT and NO considered a bad prognostic marker in goats with PT, especially when reached at level 19-20 folds and 3-4 folds, respectively. The results of this study suggest that the elevation of cTnT and NO indicating cardiac insufficiency, which considered a strong prognostic marker in PT goats and can be used for monitoring animal health.

Key words: Pregnancy Toxemic Goats · Cardiac Insufficiency · Cardiac Troponin T · Nitric Oxide · Serum Biochemical Assay

INTRODUCTION

Peri-parturient losses of goats are of particular financial significance. High prevalence of metabolic disease was evident early in the lambing season. Pregnancy toxemia is considered the most common metabolic disease, therefore veterinary advice before lambing is important [1]. Pregnancy toxemia is the most dangerous metabolic disease affecting small ruminants in late pregnancy which constitutes one of the main destructive diseases threaten the productivity of sheep and goats [2, 3]. Goats that have low energy levels are more susceptible to toxemia. This low energy level is caused by sudden increase in nutritional demands as a result of rapid fetus development [2]. Early detection of pregnancy toxemia in susceptible animals is essential for successful treatment. In clinical pregnancy toxemia, the diagnosis is based on history, clinical signs of hepatic encephalopathy and the results of serum biochemical analyses [4].

Cardiac Troponin T (cTnT), is a cardiac structural regulatory protein, controls the calcium mediated interaction between actin and myosin [5]. It is used clinically as a highly specific marker of myocardial damage in the diagnosis of acute myocardial infarction and cardiovascular diseases [6]. It released into the circulation during myocardial cell damage [7, 8]. It is found in a small extent in skeletal muscle; but cardiac trobonin differ from

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the other isomers that derived from skeletal muscle [5]. Cardiac troponins T (cTnT) detection is sensitive and specific marker of cardiomyocyte necrosis. It detected in the serum by the use of monoclonal antibodies to epitopes of cTnT. These antibodies are highly specific for cardiac troponin only and negligible to skeletal muscle troponins [9]. Although a circulating troponin T levels elevated gradually in healthy condition due to the exercise [8], the association between cTnT levels and pregnancy toxemia points to its role as an indicator of pregnancy toxemic status.

Nitric oxide (NO) is an interesting free radical gas molecule involved in numerous physiological and pathological processes. It is a key molecule in whole body cells as it performs several biological functions [10]. Nitric oxide exerts a significant effects on cardiovascular function via autonomic control mechanism beside its contribution on the dysfunctional state of both sympathetic and parasympathetic nervous system in several cardiovascular diseases [11]. Nitric oxide is produced from virtually all cell types composing the myocardium and regulates cardiac function through both vascular-dependent and independent effects [12]. Production of serum nitric oxide (NO) is significantly increased in pre-eclampsia and eclampsia, so it considers compensatory mechanism able to maintain blood flow in the fetal–maternal circulations. Also, its increase is considered a diagnostic marker for the prediction of pre-eclampsia severity [13]. This study was carried out with the purpose of determining the prognostic values of cTnT and NO as cardiac biomarkers in early and advanced stages of clinical pregnancy toxemia in goats.

MATERIALS AND METHODS

Animals and History: Forty healthy goats of similar age were used in the present experiment. All the examined goats were from Zagazig city, Sharkia Province, Egypt. All goats under investigation aged more than four years old, polyparus and pregnant with 2-3 fetus as observed by ultrasound. Twenty healthy goats were examined at the animal farm of faculty of veterinary medicine, Zagazig University, Egypt. Out of twenty healthy goats, ten were at early pregnancy less than sixty days of pregnancy and ten were at advanced pregnancy more than 120 days of pregnancy. Twenty goats suffered from pregnancy toxemia as a clinical diseaed cases examined at the animal hospital, Faculty of Veterinary Medicine, Zagazig University, Egypt. All diseased goats were pregnant more than 120 days. All goats were classified as pregnancy toxemic after the estimation of blood glucose, serum βHB and urinary ketone bodies. Furthermore, the clinical investigation and response to treatment, PT goats were classified into early PT (10 cases) and advanced PT (10 cases).

Table 1: Chemical composition of the ration fed to healthy goats on the animal farm of the Faculty of Veterinary Medicine, Zagazig University, Egypt.

Composition	Ration %	Rice straw %
- Dry matter	88.2	90.1
- Crude protein	15.1	3.6
- Crude fiber	10.4	41.5
- Crude ash	10.5	8.7
- Calcium	0.8	0.1
- Phosphorous	0.5	0.08

Feedstuff and Feeding: Healthy goats were received circulating prophylactic treatment against internal and external parasite. They were feds on a balanced ration to face its requirement for maintenance and production. Each animal was fed on commercial concentrate mixture consists of (35% wheat bran, 32% Cotton seed cake, 30% crushed maize) and supplemented with 2% limestone and 1% common salts. Rice straw was available throughout the pregnancy period, green fodder as Egyptian clover (*Trifolium alaxandrinum*), darawa, maize fodder was offered when available, the ration was offered into two equal portions twice daily. The chemical compositions of the analyzed ration is illustrated in Table 1. Water was accessible at all times. Rational, natural pasture (Green herbage, grass and remnant of plant, barseem and darawa) was utilized when available. During the night, ewes and does were fed rice straw ad libitum. They were given salt licks and fresh water ad libitum.

Clinical Examination: Clinical examination were performed in all cases according to the methods described by Sherman and Robinson [14]. Brefily, physical examination of each individual animal and correlate it to the case history of the animal. Assesment of systemic state of each goats such as (Pulse, respirtaion, temperature), physical examination of rumen, lung and heart plus examination of mucas membrane and lymphnode of the animal.

Blood Sampling and Biochemical Assay: Blood samples were collected from jugular vein in both healthy and diseased goats. Blood was allowed to flow smoothly into a clean glass tube and left to clot for 2 hours at room temperature, then centrifuged at 3000 RPM for 15 min.

The clear supernatant serum was collected using sterile Pasteur pipettes. The collected serum was transferred to dry, sterile labeled epindorfe tubes for different biochemical assays. Regarding βHB and glucose, serum samples were used directly for the biochemical analysis. The levels of ALT, AST, BUN, creatinine, glucose and βHB were determined colorimetrically using semi-automated Photometer 5010 V5+ (RIELE GmbH & Co, Berlin, Germany). The commercial kits used in our study were provided by Biomerieux, Egypt. Serum alanine aminotransferase (ALT) activity, serum aspartate aminotransferase (AST) activity [15], BUN according to Tabacco *et al.* [16]. Serum creatinine was colometrically determined according to Heinegard and Tiderstrom [17]. Biochemical analysis carried out on the serum for estimation of nitric oxide concentration according to Griess assay [18], while cTnT was measured quantitatively using electrochemiluminescence technology 3rd generation cTnT (Roche Diagnostics, Mannheim, Germany) [19]. β-hydroxybutyrate (βHB) were estimated using nefa randox kits. Colorimeteric estimation of serum glucose accordind to Sugiura and Hirano [20].

Urine Sampling and Analysis: Urinalysis reagent strips were used according to the directions of the manufacturer (Roche combur urine strips®, Boehringer Monnheim, Germany). Fresh and well-mixed urine samples were collected from each animal (About 5ml was collected in a clean and dry plastic container). Urine was collected through spontaneous urination and or using a small ruminant catheter. Hold strip in a horizontal position and dip it manually into each individual urine sample, remove immediately after short time. The strip is then compared to chart colors to read the results. This test has been used to test the ketone bodies in the collected urine samples.

Medical Approach: In order to save the affected goats, they received 0.5-1 liter glucose 25% and 50 ml calcium borogluconate (24 mg calcium/ml Cal bor mag® Adwia Co.) administered by intravenous route and 15 to 30 ml propylene glycol administered orally every 12 hours (Ketol® Bayer Co.). Chemically inducing parturition was done by administering 2.5 to 10 mg of prostaglandin F2 α (lutalyse® Pfizer Co.) according to Currie and Thorburn [21]. Furthermore, Cesarean sections were applied in 12 cases which were not respond to the previous medication.

Statistical Analysis: The resulted data were collected and subjected to analysis using ANOVA [22] for significant differences between the groups using Sigma Stat v.3.1 software (SPSS, Inc., Chicago, IL, USA). Data are expressed as mean value ± standard deviation (SD). The differences in means were considered statistically significant at $P \leq 0.05$.

RESULTS

A total forty goats of similar age, more than four years old from Sharkia governoment in Egypt was used in this study. No detected abnormal clinical signs in all healthy goats. Physical examination and clinical findings of goats with PT are tabulated in Table 2. Goats with early PT appeared with altered appetite, depression, scanty feces and acetone odor from the mouth. On the other hand, advanced PT does have the same previous symptoms in addition to recumbancy and nervous manifestation such as: champs jaw, apparently blindness and deviated head and neck (Figure 1).

Table 2: Clinical findings and examination of pregnancy toxemic goats (early and advanced PT)

	Early PT (N0= 10)		Advanced PT (N0= 10)	
Clinical parameters	Number	Percent	Number	Percent
Appetite				
Normal	0	0	0	0
Decreased	8	80	0	0
Anorexia	2	20	10	100
Depression and Lethargy	10	100	10	100
Recumbency	0	0	10	100
Acetone odor from mouth	10	100	10	100
Muscular tremors	4	40	9	90
Champing jaw	0	0	10	100
Apparently blindness	0	0	8	80
Grinding of teeth	0	0	6	60
Deviated head and neck	0	0	9	90
Mild tympani	2	20	4	40
Heart rate*				
Normal	4	40	0	0
Increased	6	60	2	20
Decreased	0	0	8	80
Respiratory rate*				
Normal	4	40	0	0
Increased	6	60	2	20
Decreased	0	0	8	80
Scanty hard feces	10	100	8	80
Scanty soft feces	0	0	2	20

* Heart rate and respiratory rate increased at a level more than 90 beats/minute and 30/minute, respectively. Decreased at a level less than 70 beats/minute and 15/minute, respectively [14].

Table 3: Serum biochemical analysis in healthy pregnant and pregnancy toxemic goats. One way analysis of variance is used. Values are represented by means ± SD. Different superscripts in the same row indicate a significant difference at ($P \leq 0.05$)

Biochemical parameters	Early pregnant healthy goats	Advanced pregnant healthy goats	Early pregnant toxemic goats	Advanced pregnant toxemic goats
Glucose mg/dL	62.39 ± 2.54^a	54.98 ± 6.32^b	30.64 ± 1.32^c	27.98 ± 2.25^c
βHB mmol/L	0.39 ± 0.18^a	0.45 ± 1.6^a	4.64 ± 1.6^b	5.81 ± 1.5^c
ALT U/L	22.06 ± 2.7^a	22.86 ± 2.3^a	29.33 ± 3.6^b	36.19 ± 3.5^c
AST U/L	80.59 ± 3.8^a	81.43 ± 2.7^a	97.27 ± 6.9^b	111.04 ± 8.8^c
BUN mg/dL	20.37 ± 2.3^a	23.59 ± 2.4^b	27.8 ± 1.5^b	29.7 ± 1.6^c
Creatinine mg/dL	0.72 ± 0.13^a	0.79 ± 0.1^a	1.75 ± 0.34^b	2.26 ± 0.59^c
cTnT ng/mL	0.02 ± 0.008^a	0.027 ± 0.01^a	0.17 ± 0.15^b	0.39 ± 0.22^c
NO µM	5.96 ± 0.36^a	6.47 ± 0.69^a	10.59 ± 1.2^b	16.46 ± 1.58^c

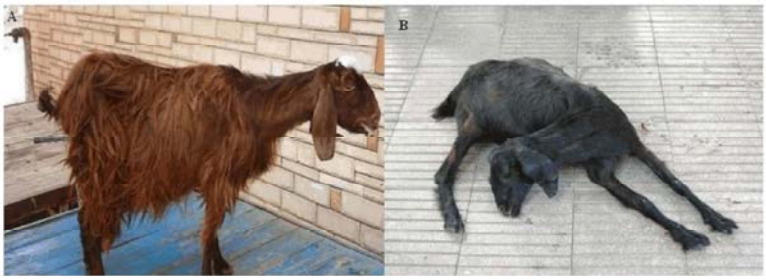

Fig. 1: Goat with pregnancy toxemia, showing the difference between the early and late PT. A) Early PT goat with depression and dullness. B) Advanced or late PT goat with recumbency and nervous manifestation such as: champs jaw, apparently blindness and deviated head and neck

The serum has been analyzed for evaluation of liver, kidney and cardiac function, plus glucose and βHB in control and diseased goats. Results are tabulated in Table 3. Gradual significant increase in the of ALT and AST (Liver marker enzymes), BUN and creatinine (Kidney marker enzymes), cardiac troponin T and Nitric oxide(cTnT and NO as cardiac markers), glucose and βHB in goats with pregnancy toxemia ($P \leq 0.05$) compared with the control has been reported as the disease progressed.

The collected urine sample was tested for ketone bodies using Roche combur urine strips®. ketone bodies were negative in the urine of all healthy pregnant does, while it was positive in the urine of pregnacy toxiemic does. The ratio of the keton bodies was different, strip detect a keton bodies ratio of (+/++) in the urine of early PT does and (++/+++) in urine of advanced PT does.

The medical approach was an important step to save affected animals. Out of 10 goats with early PT, 8 cases were responding to treatment by medicinal treatment and 2 cases with cesarean section. In contrast, all 10 goats with advanced PT aren't responding to any type of treatment and die after arriving to the clinic by 2-6 hours.

DISCUSSION

All goats with pregnancy toxemia had altered appetite, depression, dullness and pronounce acetone odor from mouth. These signs were typically described in goats and ewes had pregnancy toxemia [2]. In the present study, nervous manifestation including champing of jaw, muscular tremors, deviated head and neck, apparent blindness and grinding of teeth were recorded in goats with advanced PT. The most acceptable explanation for nervous manifestations is that the impairment of glucose utilization is the real cause of the nervous signs because the hypoglycemic encephalopathy resulted from the inability of the brain to use glucose due to hormonal changes and irreversible hypoglycemic encephalopathy is the point at which the animals don't respond to the treatment [2, 23]. Occurrence of nervous manifestation thought to be caused by the production of iso-propyle alcohol which produced from the breakdown of acetoacetic acid in the rumen [24]. The heart and respiratory rates increased in 60% of goats with early PT while decreasing in 80% in goats with advanced PT. An elevation in both heart and respiratory rates indicate hyperdynamic stages of toxemia while decreases in both heart and respiratory rates indicate the hypodynamic stage of toxemia which accompanied with peripheral circulatory failure and death [1, 2].

In the present study, serum hepatic enzymes (ALT and AST) showed a significant increase as pregnancy toxemia progressed. The altered liver function is associated with pregnancy toxemia. In case of energy

deficiency, the body uses its fatty tissue reserves as a source of energy which in turn increases the response of circulating free fatty acids that reach the liver and subsequent induce fatty infiltration [25].

In the present study, kidney function parameters (BUN and creatinine) in pregnancy toxemia goats, showed a significant increase as pregnancy toxemia progressed. These results were coincided with those previously reported by Ismail *et al.* [26]. The increase in BUN and creatinine levels attributed to severe kidney dysfunction, accompanied with acidosis due to the shift of the ketogenic group (Acetic acid and Butyric acid) from the entrance into the tricarboxylic acid cycle for energy production and lipogenesis to enter into ketogenesis under the effect of glucose deficiency and oxaloacetate insufficiency, with the subsequent production of excessive amount of ketone bodies. The elevated values of serum urea in ketotic goats might be attributed to fatty infiltration in the tubular epithelium of the kidney [25].

In the present study, cardiac function parameters (cTnT and NO) showed a gradual increase as pregnancy toxemia progressed. Cardiac tropoonin T indicated bad prognostic marker when reached 0.39 ± 0.22 ng/ml about 18-20 folds of those healthy early pregnant one (0.02±0.008 ng/ml). An elevation of cTnT considered a reflection of myocardial damage and cardiac insufficiency [5]. Where cTnT and NO indicated bad prognostic marker when reached 16.46 ± 1.58 µM about 3-4 folds of those healthy early pregnant one (5.96 ± 0.36 µM). A compensatory effect to increase efficiency of circulation in which the nitric oxide has vasodilator activity [27]. Nitric oxide (NO) is a primary determinant of blood vessel tone and thrombogenicity. Applied to heart tissue, these functions alone largely justify the growing interest for NO as a regulator of cardiac function [12].

The mean value of serum glucose was significantly decreased in all goats with pregnancy toxemia as well as in advanced healthy pregnant goats. These results were in agreement with those previously reported by Hefnawy *et al*. [28]. The importance of glucose in the pregnant goats as the major source of energy to the fetus is well known. So, pregnant goats are at high risk of developing pregnancy toxemia due to the rapid fetal growth. The energy requirements of the pregnant goats increase by a factor of 1.5 when they carries one fetus and by a factor of two when they carries two fetuses [29]. A blood glucose level in pregnant goats is generally low because of fetal demand. The last six weeks of gestation in goats are a critical period for the pregnant animal because approximately 80% of the fetal growth occurs during this period.

The mean value of serum βHB in PT goats, showed a marked elevation compared with clinically healthy pregnant ones. This increase in the serum ßHB level of pregnancy toxemic goats was previously reported [28]. The increases in the serum ßHB level could attributed to the lipolysis of tissue and the release of long chain fatty acids which were converted by the liver into ketones in goat. Moreover, this increase could be attributed to disturbance in carbohydrate and fat metabolism leading to hypoglycemia and mobilization of fat stores which lead to hepatic ketogenesis [2, 28].

Ketonuria has been detected in pregnancy toxemic goat [30]. The level of ketone bodies in urine was comparable to the degree of illness, urine diagnostic strip is a rapid and useful approach for securing field cases. Presence of ketone bodies in urine explained in accordance with the fact that when fetal needs for glucose exceeds the dietary supply leading to an increasing of lipolysis and incomplete oxidation of the 2-carbon radical due to a relative lack of oxaloacetate in the tricarboxylic acid cycle, which lead to build up acetoacetyl CoA. Hydrogenation of acetyl CoA results in the formation of acetone by decaboxylation which in return, descend in urine and milk.

In conclusion, elevation of cTnT and NO considered a bad prognostic marker in goats with PT, especially when reached at level 19-20 folds and 3-4 folds, respectively in-comparison with healthy early pregnant one. This drastic elevation could be explained as an indicator to involve the heart in the pathogenesis of pregnancy toxemia and considered the main cause of treatment failure in PT goats.

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