



Acute-phase proteins, oxidative stress biomarkers, proinflammatory cytokines, and cardiac troponin in Arabian mares affected with pyometra



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ABSTRACT

New biomarkers are essential for diagnosis of pyometra in mares. In this context, 12 sub-fertile Arabian mares suffered from pyometra were admitted to the Veterinary Teaching Hospital. The basis for diagnosis of pyometra was positive findings of clinical examination and rectal palpation. Blood samples were collected from diseased animals and from five Arabian healthy mares, which were considered as control group. Acute-phase proteins (APP), oxidative stress biomarkers, proinflammatory cytokines, and cardiac troponin I were estimated in the harvested sera of both groups. Clinical examination revealed purulent yellowish fluid discharged from vagina of affected animals and rectal palpation of the reproductive tract revealed uterine distention. The biochemical analysis of the serum revealed significant increase in cardiac troponin I, creatin kinase, alkaline phosphatase, malondialdehyde, tumor necrosis factor α , interleukins 6, prostaglandin F₂ α , haptoglobin, and serum amyloid A and significant decrease in reduced glutathione, superoxide dismutase (SOD), total antioxidant capacity, and nitric oxide (NO) of mares affected with pyometra compare to control. Cardiac troponin I was positively correlated with aspartate aminotransferase, creatin kinase, malondialdehyde, alkaline phosphatase, tumor necrosis factor α , interleukins 6, prostaglandin F₂ α , haptoglobin and serum amyloid A and negatively correlated with glutathione, superoxide dismutase, total antioxidant capacity and nitric oxide in serum of mares affected with pyometra. Moreover, there was high positive correlation between proinflammatory cytokines and APP in serum of mares affected with pyometra. The present study suggests cardiac troponin I together with APP, proinflammatory cytokines, and oxidative stress parameters as biomarkers for pyometra in Arabian mares.

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1. Introduction

Infertility in mares defined as the absence of the ability to conceive, and it results in a significant economic loss in horse industry per year [1]. Pyometra is one of the most

important causes of infertility in mares [2], characterized by an accumulation of purulent exudates in the uterus [3]. Equine pyometra diagnosed wrongly as pseudo pregnancy leading to high economic loss. Poor anus-vulvular conformation, cervical adhesion, and irregular cervix were reported as common factors enhanced pyometra in mares [4]. In a pyometra, infection augmented because the uterus is closed and cannot clear the infectious agents and subsequently fills with pus [5]. Besides immune system, there are

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three natural mechanisms to avoid pyometra in mares, namely vulva seal, vestibulovagina seal, and closure of the cervix [6]. Any defect on one of these mechanisms leads to reproductive abnormality and subsequent pyometra [6]. Confirmation of clinical examination by estimation of certain biochemical parameter in the blood of the diseased animals is essential tool for accurate diagnosis. Deviation of certain blood parameters from their normal limits used as an indication for diagnosis of pyometra in domestic animals [7,8]. However, searching for novel diagnostic biomarkers for pyometra in mares may be of interest to indicate prognosis of the disease. Acute-phase proteins (APP) constitute negative (albumin and transferrin) and positive (haptoglobin [Hp] and serum amyloid A [SAA]) proteins. Positive AAP in contrast to negative one are increased as a result of stimuli [9]. Serum amyloid A was a major APP in horses, whereas Hp acts as moderate APPs [10]. Hepatic biosynthesis of APP is mediated by proinflammatory cytokines such as interleukins 6 (IL-6) and tumor necrosis factor- α (TNF- α), which are released mainly by macrophages. Inflammation, infection, and tissue damage trigger cytokine release by defense-oriented cells, thereby inducing APP synthesis. Serum amyloid A concentration and other APP were increased in blood of horses suffering from bacterial or viral infection [11]. Malondialdehyde (MDA) levels in blood and tissues are mirror to status of lipid peroxidation [12]. Superoxide dismutase (SOD) is one of the most important antioxidant enzymes in biological system. It converts superoxide anion into hydrogen peroxide and subsequent catalysis by catalase, and glutathione peroxidase transforms hydrogen peroxide into water [13]. Cardiac troponin I (cTnI), is a highly sensitive biomarker for diagnosis of myocardial injury in animals [14,15] and humans [16]. Significant increase in cTnI was reported in canine pyometra [17]. Successfully, AAP, proinflammatory cytokines, and oxidative stress parameters were presented to scientific community by our team as useful biomarkers in the diagnosis of rhabdomyolysis in equine [18,19], bronchopneumonia and transportation stress in water buffalo [20,21], and paratuberculosis in dromedary camels [22]. In addition, cTnI was reported as diagnostic biomarker in camels infected with *trypanosoma evansi* [23]. There are few publications regarding the estimation of the oxidative stress status in mares affected with pyometra. In this sense, additional studies are required. In addition, data concerning APPs, proinflammatory cytokines, and cardiac troponin in mares affected with pyometra are scarce yet. Therefore, the present study aimed to determine the role of cardiac troponin, oxidative stress biomarkers, acute-phase proteins, and proinflammatory cytokines as biomarkers for pyometra in mares. The aim of the present study has been extended to identify the correlation between all estimated biomarkers.

2. Material and methods

2.1. Animals and sampling protocol

A total of subfertile 12 Arabian mares (4–6 years old) were admitted to the Veterinary Teaching Hospital of King Faisal University, Saudi Arabia with signs of pyometra. Five

Arabian healthy mares belonging to Veterinary Research station, King Faisal University, Saudi Arabia were considered as control group. Animal were subjected to breeding soundness examination. The investigation involved the breeding history (interval since last foaling, number of services without conception, male fertility, and herd fertility). Rectal palpation of the reproductive tract revealed uterine distention. All mares had a diagnosis of pyometra. Blood samples were collected from jugular vein of healthy and diseased animals at the time of admission in plain vacutainers. After centrifugation ($1500 \times g/15$ minutes) of the collected blood samples, the harvested sera were used for estimation of cTnI, creatin kinase (CK), aspartate aminotransferase (AST), alkaline phosphatase (ALP), MDA, glutathione (GSH), SOD, total antioxidant capacity (TAC), nitric oxide (NO), TNF- α , IL-6, prostaglandin F 2α (PF 2α), Hp, and SAA.

2.2. Samples analysis

Aspartate aminotransferase, CK, and ALP were measured on a Beckman CX-7 autoanalyzer using commercial kits (Sigma Chemical Co. Ltd., Poole, Dorset, UK). Serum Hp was measured with a commercial ELISA kit (Phase SAA kit, Tridelta Ltd., Ireland). Serum amyloid A was measured with a commercial ELISA kit (Phase SAA kit, Tridelta Ltd., Ireland), according to the manufacturer's instructions. Levels of MDA, PF 2α , GSH activity, SOD activity, and TAC were determined using commercial ELISA Kits (Cayman, USA). Interleukins 6 and TNF- α levels were determined from undiluted serum samples using commercial ELISA Kits (BioSource, Belgium). Nitric oxide was assayed using commercial available kits (Bio-diagnostic, Kit number NO2532). Cardiac troponin I was analyzed in serum samples using a point-of-care analyzer (VetScan i-STAT 1, Abaxis, CA, USA) according to the manufacturer's instructions.

2.3. Statistical analysis

Because of the small size of the control group and nonnormally distributed (Kolmogorov–Smirnov test) markers in mares affected with pyometra, differences in selected biomarkers between mares were compared using nonparametric analysis (Wilcoxon Mann–Whitney) at P value less than 0.05. Spearman's correlation coefficient was analyzed between different biomarkers only in mares affected with pyometra. All analyses were done using Stata version 13 (Stata Corp, College Station TX, USA).

3. Results

The clinical examination of mares affected with pyometra revealed purulent yellowish fluid discharged from vagina of affected animals and rectal palpation of the reproductive tract revealed uterine distention. The descriptive findings of cardiac and oxidative stress biomarkers in control and pyometra-affected mares are presented in Table 1. The data presented in this table revealed a significant ($P < 0.01$) increase of cTnI, CK, ALP, and MDA values in mares affected with pyometra compare with control healthy mares. However, data presented in the same table revealed a significant

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