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THE CARDIAC BIOMARKER TROPONIN I AND OTHER HAEMATOLOGICAL AND BIOCHEMICAL VARIABLES IN DOWNER CAMELS (*Camelus dromedarius*)

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ABSTRACT

The aim of the present study was to investigate the concentration of the cardiac biomarker troponin I (cTnI) as well as other haematological and biochemical parameters in downer camels. Thirty-three downer camels were examined in addition to 25 healthy controls. A complete blood count (CBC), as well as chemistry profile was determined in healthy and diseased camels. Using a point-of-care analyser employing a two-site ensyme-linked immunosorbant assay, the serum concentrations of cTnI were determined. Clinical findings included recumbency either sternally or laterally, inappetance, weak and irregular ruminal contractions, and mucopurulent nasal discharge. Eleven of the 33 camels were cured after treatment, 18 did not and the remaining 4 had died by day 20 after treatment. Compared to control camels, results CBC showed low haematocrit, leukocytosis, neutrophilia, decreased red blood cells count, decreased haemoglobin and increased MCH. Serum chemistry profile revealed hypoalbuminemia, hypoglobulinemia, and increased the serum activities of aspartate aminotransferase, γ-glutamyl transferase, alkaline phosphatase and creatine kinase. Compared to controls (median 0.02 ng/mL; range, 0.00–0.08 ng/mL), the serum cTnI concentrations in downer camels (median 0.10 ng/mL; range, 0.01–2.20 ng/mL) differed significantly (P=0.019). The serum cTnI concentrations in downer camels were approximately 10 folds than controls.

Key words: Biomarkers, cardiac, cTnI, downer camels, troponins

A downer camel is an animal that is unable to rise to a standing position. The underlying etiology is broad ranging, and may be due to nerve damage, muscle weakness, malnutrition for long time, broken bone and severe emaciation. Recumbency is sometimes seen in camelids as an adverse reaction to pain. With bright and alert recumbent animals, a thorough examination to rule out fractures or severe joint trauma is essential. Once these are ruled out, the inability to stand may be indicative of generalised muscle paresis or paralysis. These can be of a primary nature, as it is seen with lesions within the spinal column, or secondary to mineral or electrolyte deficiencies (Al-Ani and Faye, 2004).

Biomarkers are distinctive biological indicators of processes, events, or conditions occurring within the body. They can indicate physiological processes (such as growth and aging), or pathophysiological processes that occur with disease (e.g. cardiac damage, and heart failure). Cardiac troponins are proteins that control the calcium-mediated interaction between actin and myosin, allowing contraction at the sarcomere level. Concentration of the cTn can be correlated microscopic lesion and loss of immunolabeling in myocardium damage. Troponin concentration remains elevated in blood for 1-2 wks so that wide window is available for diagnosis of myocardial damage (Undhad *et al,* 2012). In human medicine, there has been a push for the discovery of novel cardiac biomarkers to aid in the early detection, diagnosis, and prognosis of human cardiac diseases (Ginsburg and Haga, 2006).

Cardiac troponin I (cTnI) is the ''gold standard'' for the noninvasive diagnosis of myocardial injury in humans (Archer, 2003; Ladenson, 2007). The cTnI is a protein found in the myocardial cell that initiate tropomyosin contraction in the presence of calcium. Its serum concentration elevates after acute myocardial injury because of leakage from the damaged myocardial cells (O'Brien *et al,* 2006). Therefore, cTnI is now the preferred biochemical parameter in human medicine for assessing myocardial necrosis and myocyte damage, with virtually absolute myocardial tissue specificity as well

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as higher sensitivity (Alpert *et al,* 2000; Collinson *et al,* 2012). To the author's knowledge, the concentrations of the cardiac biomarker cTnI were not evaluated in downer camels. The aim of the present study was therefore to investigate the effect of dower state on the serum level of cTnI as well as other haematological and biochemical parameters in downer camels.

Materials and Methods

Camels

Thirty-three downer female camels dromedary aged 10.5±3.7 years weighted 512±114 kg, were investigated between October 2010 to March 2012. Of them 29 were non-pregnant and non-lactating and the remaining 4 were 3-5 month pregnant and lactating. In addition, 25 healthy camels were used as controls. All camels were clinically examined at Veterinary Teaching Hospital, Qassim University, Saudi Arabia. Clinical examinations of camels were carried out as previously described (Köhler-Rollefson *et al,* 2001). This included examination of the musculoskeletal system, general behaviour and condition, auscultation of the heart, lungs, rumen and intestine, measurement of heart rate, respiratory rate and rectal temperature, swinging auscultation, percussion and auscultation of both sides of the abdomen and rectal examination. Based on a 1 (very thin) to 5 (fat) scale as previously reported (Sghiri and Driancourt, 1999), the body condition score (BCS) of the camels was determined; the average was found to be 3.5±0.5.

Camels without bone fractures were then treated with 500 mL of 23% calcium borogluconate IV, 250 mL of 23% calcium borogluconate SC, 5000 mL of 10% dextrose IV, and 5000 mL propylene glycol orally. Each camel was assigned 1 of 2 clinical outcomes as follows: 1) cured (able to stand and return to good general health by day 10 post treatment) or 2) died (dead within the first 10 days post treatment or still recumbent and died by the 10th days post treatment).

Haematology and serum biochemistry

Hematological examinations (total and differential leukocyte count, red blood cells, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and platelet count) were carried out using an automated analyser (VetScan HM5, Abaxis, California, USA). The serum samples were used to determine the concentrations of total protein, albumin, globulin, blood urea nitrogen (BUN), calcium, magnesium and phosphorus. The serum activity of γ-glutamyl transferase (GGT), aspartate aminotransferase (AST) and creatine kinase (CK) were also measured. An automated biochemical analyser (VetScan VS2, Abaxis, California, USA) was used for measurement of the serum parameters. The concentration of lactic acid was measured in serum samples using a point-of-care analyser (GEM Premier 3000, Instrumentation Laboratory Company, Bedford, USA).

Cardiac troponin I assay

The serum samples were thawed immediately before analysis and analyzed on the same morning for cTnI using the point-of-care analyser (VetScan i-STAT® 1, Abaxis, California, USA) according to manufacturer instructions. This analyser employs a two-site enzyme-linked immunosorbant assay (ELISA). The lower limit of detection of cTnI for this assay is 0.02 ng/mL. The i-STAT cTnI test will report 0.00 to 50.00 ng/mL (µg/L). Samples above the reportable range will yield ">50.00 ng/mL" on the analyser display screen. However, the performance characteristics of the i-STAT cTnI measurement have not been established for cTnI values above 35.00 ng/mL (µg/L). Values < 0.02 ng/mL cannot be discriminated, however, the analyser provides a specific point estimate of either 0.00, 0.01 or 0.02 ng/mL.

Statistical method

The distributions of cTnI were determined as the minimum, maximum, mean, SD and median values, alongside the 25th, 75th and 95th percentiles. Haematological and other biochemical data are presented as means ± SD. The concentrations of the cardiac biomarker, cTnI, and other haematological and biochemical parameters were compared between controls and diseased camels, using Student's t test of the SPSS program (2007). Values of $p<0.05$ were considered significant.

Results

Compared to a BCS of 3.5±0.6 in control camels, the BCS in the downer camels was 3.5±0.5. During physical examination, the most prominent clinical signs in the diseased camels were the inability to stand in all camels from the downer position. The inability to get up in the diseased camels was divided into 4 classes (1 to 4) (Fig 1). Only 1 (3%) of the 33 camels had admitted in a lateral recumbency (Fig 2). None of the camels had bone fractures. Other clinical findings included inappetance in 21 (64%) camels and weak, irregular ruminal contractions in 19 (58%)

Fig 1. Clinical presentation of 4 downer camels that admitted for examination in a setting position. During lifting by a winch, camels were classified as having mild (**A**), moderate (**B**), severe (**C**) and advanced (**D**) degrees of inability to stand.

Fig 2. Clinical presentation of a downer camel that was admitted for examination in a lateral recumbency.

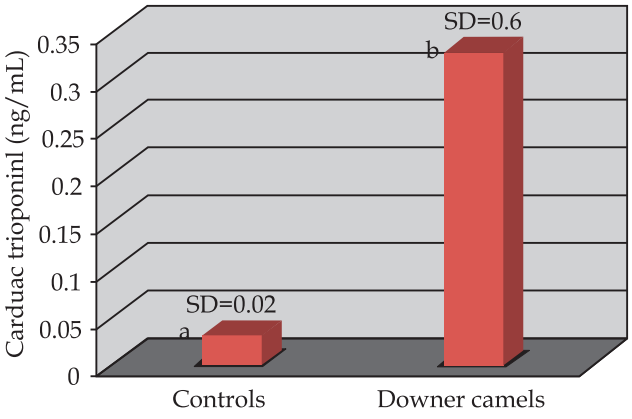

Fig 3. Cardiac troponin I values in downer camels compared to control healthy camels. [a,b] different letters indicate a significant difference (P=0.019). SD = standard deviation.

Table 1. Haematological and biochemical variables in healthy and in downer camels.

	Healthy camels (n=25)	Downer camels (n=33)	P value
White blood cells ($\times10^9$/L)	16.9 ± 2.7[a]	23.5 ± 10.5[b]	0.01
Lymphocytes ($\times10^9$/L)	5.9 ± 2.4[a]	8.0 ± 3.3[a]	0.06
Monocytes ($\times10^9$/L)	0.9 ± 0.6[a]	0.6 ± 0.5[a]	0.06
Neutrophils($\times10^9$/L)	9.8 ± 3.0[a]	16.5 ± 8.4[b]	0.002
Red blood cells ($\times10^{12}$/L)	11.3 ± 1.4[a]	9.0 ± 1.5[b]	0.01
Haemoglobin (g/dL)	16.0 ± 2.3[a]	14.2 ± 2.5[b]	0.018
Haematocrit (%)	28.9 ± 2.7[a]	22.2 ± 3.5[b]	0.01
Mean Corpuscular Volume (fl)	25.5 ± 1.5[a]	24.7 ± 1.7[a]	0.15
Mean Corpuscular Haemoglobin (pg)	14.7 ± 2.4[a]	21.9 ± 13.9[b]	0.02
Mean Corpuscular Haemoglobin Concentration (g/dL)	57.6 ± 9.0[a]	59.8 ± 13.6[a]	0.5
Platelet count ($\times10^9$/L)	163 ± 90[a]	172 ± 86[a]	0.7
Albumin (g/L)	60.9 ± 3.0[a]	47.02 ± 12.6[b]	0.001
Alkaline phosphatase (U/L)	7 ± 3[a]	72 ± 35[b]	0.0001
Aspartate aminotransferase (U/L)	80 ± 17[a]	252 ± 139[b]	0.0001
Calcium (mmol/L)	2.4 ± 0.1[a]	2.2 ± 0.5[a]	0.07
Gamma glutamyl transferase(U/L)	12 ± 5[a]	27.4 ± 22.4[b]	0.01
Total protein (g/L)	67.3 ± 4.3[a]	68.5 ± 11.9[a]	0.7
Globulin (g/L)	7.0 ± 3.8[a]	21.6 ± 6.01[b]	0.0001
Blood urea nitrogen (mmol/L)	6.4 ± 1.1[a]	7.02 ± 1.79[a]	0.2
Creatine kinase (U/L)	139 ± 22[a]	1350 ± 1554[b]	0.003
Phosphorus (mmol/L)	2.6 ± 0.4[a]	2.4 ± 0.6a	0.07
Magnesium (mmol/L)	0.26 ± 0.03[a]	0.31 ± 0.14[a]	0.09
Lactic acid (mmol/L)	4.4 ± 3.3[a]	4.7 ± 3.8[a]	0.8

Values are expressed as means ± SD. Values with different letters in the same row differ significantly.

Table 2. Serum cardiac troponin I (cTnI) concentrations (ng/mL) in healthy and in downer camels.

	Mean	SD	Minimum	25th percentile	Median	75th percentile	95th percentile	Maximum
Healthy camels (n=25)	0.03a	0.02	0.00	0.02	0.02	0.05	0.07	0.08
Downer camels (n=33)	0.33b	0.60	0.01	0.05	0.10	0.42	1.30	2.20

Values with different letters in the same column differ significantly (P = 0.019).

camels and mucopurulent nasal discharge in 1(3%) camel. Eleven of the 33 camels (33%) were cured within the 10 days after treatment, 18 did not recover and the remaining 4 camels had died by day 20 after treatment.

Haematological and biochemical findings are summarised in Table 1. Compared to controls camels, results of complete blood count (CBC) obtained from diseased camels showed low haematocrit (P=0.01), leukocytosis (P=0.01), neutrophilia (P=0.002), decreased RBCs count (P=0.01), decreased haemoglobin (P=0.018) and increased MCH (P=0.02). Other CBC parameters including lymphocyte and monocyte counts, MCV, MCHC and platelets count did not differ significantly when compared to controls (P>0.05). Serum chemistry profile showed hypoalbuminemia (P=0.001), hypoglobulinemia (P=0.0001), and increased the serum activities of AST (P=0.0001), GGT (P=0.01), ALP (P=0.0001) and CK (P=0.003). Other chemistries including calcium, phosphorus, magnesium, BUN and lactic acid did not differ significantly when compared to controls (P>0.05).

Beside the means, medians, minimum and maximum values, Table 2 summarises the 25th, 75th and 95th percentiles for cTnI in the downer camels compared to the controls. Compared to controls (median 0.02 ng/mL; range, 0.00–0.08 ng/mL),

the serum cTnI concentrations (median 0.10 ng/mL; range, 0.01–2.20 ng/mL) differed significantly (P=0.019). The serum cTnI concentrations in downer camels were approximately 10 folds than controls (0.33 in downer vs. 0.03 ng/mL in controls) (Fig 3).

Discussion

It is apparent from this study that the serum cTnI concentrations in downer camels were increased to approximately 10 folds than control ones. We have recently reported the reference values of cTnI concentrations in healthy camels, and the influence of long road transportation on these concentrations (Tharwat *et al,* 2013a). We have also reported recently the influence of racing on the cardiac biomarker cTnI (Tharwat *et al,* 2013b). It was found that the concentrations of cTnI increase significantly in healthy camels as a response to road transportation of 500 km or as result of a 7 km race. The present study is the first to investigate the influence of recumbent state on the serum concentrations of cTnI in downer dromedary camels.

The reasons behind the increases found in cTnI concentrations after racing are not clear. Cardiac troponin I is reported to have a very high specificity for the myocardium. It is therefore likely that the increases in cTnI seen in the downer camels are related to myocardial damage. One theory explaining the increase in cTnI levels is that cardiac hypoxia could cause a change in the permeability of the myocardium, resulting in a leakage of macromolecules into the blood (Nostell and Haggstrom, 2008). This idea is supported by the fact that a previous study in rats has shown that short periods of hypoxia can induce release of cTnI without cell death (Piper *et al,* 1984). However, despite this significant increase, it is not clear if this can be clinically relevant.

In this study, concerning the biochemical variables the most important changes were the elevations of the serum enzymes, AST and CK. In downer cows with increased AST activity, concurrent analysis of serum CK activity helps to identify the origin of AST (muscle or liver) (Kalaitzakis *et al,* 2010). In the present study, increases in AST were likely due to muscle damage, because serum CK activity was high. A portion of the AST elevation may have been liver-derived, as evidenced in the marked serum elevations of the other liver-derived enzymes, ALP and GGT. Consequently, as the liver-derived portion of serum AST activity cannot be distinguished, the diagnostic value of AST in downer camels suspected for liver dysfunction is diminished. It is interesting from the results of this study that the calcium, phosphorus and magnesium levels were normal. In downer cows, normal serum calcium, phosphorus and magnesium levels were also found in cows with the downer syndrome (Fatur, 1998).

Although the i- STAT® 1 cTnI assay was initially designed for use in humans, there is good reason to anticipate cross reactivity based on the highly conserved nature of the cardiac troponins across mammalian species (Jin *et al,* 2001). In this study, we thought that the i- STAT® 1 cTnI assay would detect camel cTnI, because, despite the lack of a known camel cTnI amino-acid sequence, the sequence of cTnI is highly conserved among all species for which the sequence is known, and the analyser has been validated against reference analysers in other species (Leszyk *et al,* 1988; Mittmann *et al,* 1990; Sleeper *et al,* 2001; Apple *et al,* 2004; Rishniw and Simpson, 2005; Wells and Sleeper, 2008; Kraus *et al,* 2010). Additionally, the same methodology of this study was used in another study published recently (Blass *et al,* 2011) documenting cTnI values in healthy alpacas. In addition, the high homology of amino-acid sequences between mammals within the sequence regions detected by cTnI analysers allows veterinarians to measure cTnI concentrations in many animal species with the same equipment as has been developed for humans, rather than necessitating the development of species-specific technology (Blass *et al,* 2011).

In this study, marked elevations of cTnI in the downer camels could be considered as a strong indication of myocardial damage and can be used as a prognostic indicator. One of the limitations of this study was the inability to carry out histopathological examination of cardiac muscle; therefore additional studies are required for confirmation of correlation between cardiac pathologies and cTnI serum elevations. In addition, further studies are required to evaluate the serum cTnI concentrations in camels with signs of cardiac disease in order to establish the correlation between elevated concentrations of cTnI and pathological findings.

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