Correlations among homocysteine, cardiac troponin I and cardiac enzymes in different ages of clinically healthy male dromedary camels

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Abstract:

BACKGROUND: Information regarding serum biochemical profile can reflect cardiovascular performance in animals. Although studies have evaluated the inter-relationship among cardiovascular biomarkers in animals and human beings, there are no reports of such a probable relationship in camelids. **OBJECTIVES:** The aim of the present study was to provide data on the correlations among cardiovascular biomarkers in different ages of clinically healthy male dromedary camels to provide a basis for assessing cardiac muscle healthiness in this species. **METHODS:** Thirty clinically healthy dromedary camels (*Camelus dromedarius*) were selected and divided into four age groups including 1-3 (n=7), 4-6 (n=7), 7-9 (n=8), and above 10 (n=8) years old. Blood samples were collected and sera were separated. Serum concentrations of homocysteine (Hcy), cardiac troponin I (cTnI), creatine kinase-myocardial specific isoenzymes (CK-MB), lactate dehydrogenase (LDH), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were evaluated. **RESULTS:** The results of the present study showed that there were significant correlations among cTnI and CK-MB (r=-0.853; p=0.015) and Hcy (r=0.916; p=0.004) in the 4 to 6-years-old group of clinically healthy male dromedary camels. LDH was significantly correlated with CK-MB in the 7 to 9-year-old group (r=-0.710; p=0.045). There were no significant correlations among different factors of 1-3 and above 10-year-old groups (p>0.05). **CONCLUSIONS:** The data provided here is the first report on cardiac health assessment parameters in dromedary camels. Moreover, the data is valuable in camel racing clubs, when an overall cardiac health and fitness is to be assessed. The correlation reported here might also be helpful for easier analysis of cardiac health status in dromedary camels. The data may be useful for assessing suspected cases of myocardial diseases and its changes maybe of prognostic value.

Introduction

The levels of circulating enzymes can be useful as an aid in diagnosis of certain diseases (Eldirdiri et al., 1987). Serum biochemical analysis can often provide valuable information regarding the cardiovascular healthiness and sickness in animals (Coodley, 1970). When there are damages to the myocardium, the level of Homocysteine (Hcy) (CIACCIO et al., 2008), cardiac troponin I (cTnI) (Radostits et al., 2007) and enzymes such as creatine kinase-myocardial specific isoenzymes (CK-MB) (Kaneko, 1989), lactate dehydrogenase (LDH) (Reinaldo et al., 2010), aspartate

aminotransferase (AST) and alanine aminotransferase (ALT) (Coodley, 1970) in the serum are elevated.

Hcy is a sulfur-containing amino acid, which is found in blood and produced in the metabolism of the essential amino acid methionine (Ciaccio et al., 2008). Epidemiological studies have shown that too much serum concentrations of Hcy are related to a higher risk of coronary heart disease, stroke, peripheral vascular disease, and deep venous thrombosis (Weikert et al., 2005; Kullo et al., 2006). Several studies have suggested that cTnI has especially become important in early diagnosis of myocardial damages in large animals (Radostits et al., 2007; Tunca et al., 2009). Moreover, cTnI level is a more specific marker than CK-MB level for diagnosing myocardial necrosis (Basbugan et al., 2010); however, myocardium is one of the richest sources of CK-MB. Therefore, it is the most widely used serum enzyme determination in cardiac diseases of large animals (Kaneko, 1989). LDH catalyzes the reversible oxidation of pyruvate to lactate. multiple forms of LDH enzymes in several tissues (Bergmeyer et al., 1983); however, LDH has been found to be a general indicator for the existence and severity of acute or chronic myocardial tissue damages (Reinaldo et al., 2010). LDH is not organ-specific and may be of value in order to diagnose the cardiac problems in conjunction with other enzymes such as AST and ALT (Coodley, 1970). Therefore, these diagnostic enzymes are valuable tools used in the early detection of cardiac problems as a result of ischemia, injury, or inflammation (Sacher et al., 1991).

Although studies have evaluated the interrelationship among Hcy, cTnI, CK-MB, LDH, ALT and AST in animals (Kozat et al., 2011) and human beings (Polena et al., 2005), there are no reports of such a probable relationship in camelids. Thus, the present study was carried out to provide data on the correlations among Hcy, cTnI, and cardiac enzymes (CK-MB, LDH, ALT and AST) in different ages of clinically healthy male dromedary camels to provide a basis for assessing cardiac muscle healthiness in this species.

Materials and Methods

The present study was carried out in November 2010 on 30 male clinically healthy dromedary camels (*Camelus dromedarius*) from several farms in Yazd province (latitude 31°54'N and longitude 054°24'E, 1203 m above sea level), located in the central part of Iran. Animals were maintained in open-shed barns with free access to water and shade. The ration included mainly alfalfa hay, wheat straw, corn, and barley. Camels were examined prior to the sampling and proved to be clinically healthy. Animals were assigned into four age groups, including 1-3 (n=7), 4-6 (n=7), 7-9 (n=8), and above 10 (n=8) years old. Blood samples were collected by jugular venipuncture in sterile silicone-coated vacutainers, allowed to clot, and then separated after centrifugation for 10 min at 3000×g. Serum samples were stored at -20°C until analysis.

Values of serum AST, ALT, CK-MB, and LDH were measured with Integra 800 auto-analyzer (Roche-Cobes, Switzerland). Levels of serum cTnI were determined by ELISA equipment (ELISA Reader®-DAS Italy) and calculated with commercial test kit as instructed by the manufacturer (Troponin I kit-DRG Diagnostic). The measurement of serum Hcy was performed using the enzymatic assay kit (Diazyme Com, USA).

Data were expressed as mean ± standard deviation (SD). Correlations among Hcy, cTnI, CK-MB, LDH, ALT and AST in each age group of dromedary camels were assigned. In the present study, the correlation coefficient greater than 0.8 was considered as strong, whereas, a correlation smaller than 0.5 described as weak. Statistical analyses were performed by SPSS software (SPSS for Windows, version 11.5, SPSS Inc, Chicago, Illinois). The level of significance was set at $p<0.05$.

Results

Normal serum concentrations of Hcy, cTnI, CK-MB, LDH, ALT and AST in different ages of clinically healthy male dromedary camels are presented in Table 1. Tables 2 to 5 show the correlations among serum concentrations of these factors in 1-3, 4-6, 7-9 and above 10 years old groups of dromedary camels. There were no significant correlations among different factors of 1-3 and greater than 10 years old groups ($p>0.05$; Tables 2 and 5). There were weak and no significant correlations

Table 1. Mean ± SD of serum concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase isoenzyme MB (CK-MB), lactate dehydrogenase (LDH), homocysteine (Hcy) and troponin I (cTnI) in 4 age groups of clinically healthy male dromedary camels.

Age Groups	AST (U/L)	ALT (U/L)	CK-MB (U/L)	LDH (U/L)	Hcy (µmol/L)	cTnI (ng/mL)
1-3 years old	101.57±14.38	113.42±14.38	225.06±109.40	2833.49±748.05	7.48±0.56	0.52±0.18
4-6 years old	72.42±24.37	92.14±48.27	250.16±62.61	1714.44±887.57	7.37±0.75	0.61±0.19
7-9 years old	89.37±25.44	117.37±49.28	196.76±68.90	2331.69±1083.00	7.18±0.68	0.65±0.19
>10 years old	98.00±29.17	125.62±28.67	238.98±97.90	2302.97±1017.39	7.07±0.72	0.55±0.22

Table 2. Correlation coefficients (r) among serum concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase isoenzyme MB (CK-MB), lactate dehydrogenase (LDH), homocysteine (Hcy) and troponin I (cTnI) in 1-3 years old group of clinically healthy male dromedary camels (n=7).

	AST (U/L)	ALT (U/L)	CK-MB (U/L)	LDH (U/L)	Hcy (µmol/L)
ALT (U/L)	-0.529				
CK-MB (U/L)	-0.292	-0.272			
LDH (U/L)	0.494	0.003	-0.423		
Hcy (µmol/L)	0.199	-0.340	-0.525	0.481	
cTnI (ng/mL)	0.438	0.131	0.051	-0.357	0.128

Table 3. Correlation coefficients (r) among serum concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase isoenzyme MB (CK-MB), lactate dehydrogenase (LDH), homocysteine (Hcy) and troponin I (cTnI) in 4-6 years old group of clinically healthy male dromedary camels (n=7). Significant correlations are indicated by star ($p<0.05$).

	AST (U/L)	ALT (U/L)	CK-MB (U/L)	LDH (U/L)	Hcy (µmol/L)
ALT (U/L)	-0.438				
CK-MB (U/L)	-0.222	0.271			
LDH (U/L)	0.475	-0.394	-0.712		
Hcy (µmol/L)	-0.272	0.273	-0.618	0.426	
cTnI (ng/mL)	0.004	0.086	-0.853*	0.569	0.916*

among cTnI and other factors in 1-3, 7-9 and greater than 10 years old groups ($p>0.05$; Tables 2, 4 and 5). The results of the present study showed that there were significant correlations between cTnI and CK-MB ($r=-0.853$; $p=0.015$) and Hcy ($r=0.916$; $p=0.004$) in 4-6 years old group of clinically healthy male dromedary camels (Table 3). AST and ALT had weak and no significant correlations with other studied factors in 4-6 years old group ($p>0.05$; Table 3). LDH was significantly correlated with CK-MB in 7-9 years old group ($r=-0.710$; $p=0.045$; Table 4). ALT had weak and no significant correlations with other studied factors in older than 10 years old group ($p>0.05$; Table 5).

Discussion

Cardiac troponin is a myofibrillar protein with two diagnostically-relevant forms (cTnI and cTnT) that regulate contraction of the heart. cTnI binds to actin and inhibits interactions between actin and myosin. Cardiac troponin is released from injured myocardiocytes into the circulation within hours (Polena et al., 2005). In recent years, the development of cardiac troponins as the gold standard, sensitive and specific biochemical markers of myocardial injuries have aided the diagnosis and management of myocardial injuries (Wells and sleeper, 2008). In the present study, serum concentrations of cTnI were significantly correlated with Hcy and CK-MB in 4-6 years old group. Tharwat (2012) reported that serum cTnI in downer camels were significantly higher than normal camels. He mentioned that myocardial injuries could increase serum cTnI in downer camels. Tharwat et al. (2013a) also mentioned that transportation could induce myocardial problems in camels, and increase of the serum concentrations of cTnI can be detected. Furthermore, serum cTnI in clinically healthy racing camels is higher than non-racing camels (Tharwat et al., 2013b). The serum concentrations of cardiac troponin correlate well with histopathological changes in the myocardium, extent of cardiac injury, clinical signs, and prognosis (Wells and Sleeper, 2008). Assay of troponins constitutes the preferred biochemical marker for acute myocardial infarction (Polena et al., 2005). Increases in cTnI correlate with a wide range of animal cardiac diseases including dilated cardio-

Table 4. Correlation coefficients (r) among serum concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase isoenzyme MB (CK-MB), lactate dehydrogenase (LDH), homocysteine (Hcy) and troponin I (cTnI) in 7-9 years old group of clinically healthy male dromedary camels (n=8). Significant correlation is indicated by star ($p<0.05$).

	AST (U/L)	**ALT (U/L)**	**CK-MB (U/L)**	**LDH (U/L)**	**Hcy (µmol/L)**
ALT (U/L)	0.625				
CK-MB (U/L)	-0.567	-0.620			
LDH (U/L)	0.280	0.607	-0.710*		
Hcy (µmol/L)	0.652	0.433	-0.511	-0.065	
cTnI (ng/mL)	0.184	-0.202	-0.080	-0.186	0.014

Table 5. Correlation coefficients (r) among serum concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase isoenzyme MB (CK-MB), lactate dehydrogenase (LDH), homocysteine (Hcy) and troponin I (cTnI) in greater than 10 years old group of clinically healthy male dromedary camels (n=8).

	AST (U/L)	**ALT (U/L)**	**CK-MB (U/L)**	**LDH (U/L)**	**Hcy (µmol/L)**
ALT (U/L)	0.005				
CK-MB (U/L)	-0.565	-0.272			
LDH (U/L)	0.625	0.003	-0.423		
Hcy (µmol/L)	0.682	-0.340	-0.525	0.481	
cTnI (ng/mL)	0.214	0.131	0.051	-0.357	0.128

myopathy, endocardiosis, endocarditis, and congestive heart failure, as well as with various forms of severe respiratory disease (Serra et al., 2010).

Hcy is a highly reactive amino acid derived from methionine metabolism and is known to produce endothelial cell injury in experimental animals (Harker et al., 1983) and cell culture (Wall et al., 1980). Elevated total serum Hcy has been stated as an independent risk factor for peripheral vascular, cerebrovascular and coronary artery diseases (Nygard et al., 1997). Studies showed that increased plasma and heart tissue Hcy concentrations could be considered as a risk factor in myocardium damage in conditions associated with oxidative stress (Rezaei and Dalir-Naghadeh, 2009). In the current study, correlations between Hcy and CK-MB were either negative or not significant in all of the studied groups. Several meta-analyses have shown an association between total plasma Hcy concentration and cardiovascular diseases (Hankey and Weikelboom, 1999).

Serum CK-MB in downer camels is significantly higher than normal camels during myocardial injuries (Tharwat, 2012). CK-MB during transportation increases in dromedary camels (Tharwat et al., 2013a). Furthermore, serum CK-MB in clinically healthy racing camels is higher than non-racing ones (Tharwat et al., 2013b).

CK-MB and LDH are cytoplasmic enzymes with a high activity in heart, skeletal muscle, liver, kidney, and red blood cells. These enzymes are indicators of a higher level of cellular damage, and their increased activity is a consequence of their increased release from the damaged cells and a reflection of metabolic changes in the inflamed tissues especially in the heart (Graeber et al., 1990). The damage to the skeletal or heart musculature results in a considerable increase in the level of serum CK-MB and LDH due to the fact that the bulk of the vessels throughout the body could be considered as an ample reservoir of enzymes liable to be released and detected during pathological situations. Hence, any damages to the vasculature could result in leakage of the enzymes, and could thus be considered as a valuable tool in early diagnosis of pathological conditions (Graeber et al., 1990).

LDH activity rises slowly after myocardial infarction and becomes maximal after CK-MB elevations (Ohman et al., 1982). Determinations of LDH activity have been used diagnostically to determine whether acute myocardial infarction occurred in the days before a patient was evaluated (Adams et al., 1993). Measurement of cTnI is clearly more sensitive than the LDH cutoff value for retrospective diagnoses of acute myocardial injuries. Resolution of this problem has been advanced by the development of techniques that separate CK into its three isoenzymes containing MM, MB, and BB (Van Der Veen and Willebrands, 1966). Separation and quantification of MB isoenzyme, which is found almost exclusively in heart muscle, provides a more specific indicator of acute myocardial infarction than total CK alone. CK-MB as a cardio specific enzyme has been introduced as a sensitive marker of myocardial injury (Roe et al., 1972). Recent studies report that although the sensitivity of cTnI is comparable to that of CK-MB, its specificity seems to be higher (Adams et al., 1994). In the diagnosis of

acute myocardial infarction, the measurement of elevated levels of CK-MB and LDH are well known (Jaffe et al., 1984).

Measurements of AST and ALT activities are common laboratory tests, requested usually as an aid to diagnosis and surveillance of cardiac problems and both AST and ALT activities rise after an acute myocardial infarction (Ohman et al., 1982). AST lacks organ specificity but is present in skeletal muscle, cardiac muscle and liver of large animals; the pathological changes in these organs elevate the activity of AST in the blood (Kaneko, 1989). AST is also an intracellular enzyme involved in amino acid and carbohydrate metabolism, and its elevated levels show the damage in the organ whose cells are rich in this enzyme such as the heart (Rubina and Tabassum, 2008). In contrast, elevation in ALT levels is widely viewed as a specific indicator of liver necrosis and cardiac injuries. Elevated ALT activity is associated with the high risk of chronic heart disease. Both AST and ALT activities rise after an acute myocardial infarction (Ohman et al., 1982). Serum concentrations of AST and ALT in downer camels are significantly higher than normal camels, due to myocardial problems during recumbency (Tharwat, 2012). The results of the present study showed that there were no significant correlations among ALT and AST and other studied factors in all age groups ($p>0.05$; Tables 2 to 5).

The data provided here is the first report on cardiac health assessment parameters in dromedary camels. Moreover, the data is valuable in camel racing clubs, when an overall cardiac health and fitness is to be assessed. The correlation reported here might also be helpful for easier analysis of cardiac health status in dromedary camels. The data may be useful for assessing suspected cases of myocardial diseases and its changes may be of prognostic value.

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همبستگی بین هوموسیستئین، تروپونین قلبی I و آنزیم های قلبی در سنین مختلف شترهای نر به ظاهر سالم یک کوهانه

مهرداد پورجعفر * علی اصغر چالمه خلیل بدیعی سعید نظیفی سمانه کشاورز مجتبی نقیب

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چکیده

زمینه مطالعه: آگاهی از الگوی بیوشیمیایی سرم می تواند منعکس کننده سلامت و بیماری دستگاه قلبی، عروقی در حیوانات باشد. هرچند که مطالعاتی، همبستگی بین بیومارکرهای قلبی، عروقی را در حیوانات و انسان مورد ارزیابی قرار داده اند اما گزارشاتی از همبستگی های احتمالی بین این فراسنجه ها در شترسانان به چشم نمی خورد. **هدف:** هدف از انجام این مطالعه، فراهم کردن اطلاعاتی در زمینه همبستگی بین بیومارکرهای قلبی، عروقی در سنین مختلف شترهای نر به ظاهر سالم یک کوهانه به منظور ارزیابی سلامت لایه عظلانی قلب در این گونه حیوانی بود. **روش کار:** تعداد ۳۰ نفر شتر نر به ظاهر سالم یک کوهانه (*Camelus dromedarius*) در ۴ گروه سنی شامل ۳-۱ (۷ نفر)، ۶-۴ (۷ نفر)، ۹-۷ (۸ نفر) و بالای ۱۰ سال (۸ نفر) قرار گرفتند. خونگیری انجام شد و جداسازی سرم ها صورت پذیرفت. غلظت های سرمی هوموسیستئین (Hcy)، تروپونین قلبی I (cTnI)، ایزوآنزیم قلبی کراتین کیناز (CK-MB)، لاکتات دهیدروژناز (LDH)، آلانین آمینوترانسفراز (ALT) و آسپارتات آمینوترانسفراز (AST) مورد سنجش واقع شد. **نتایج:** نتایج این مطالعه همبستگی معنی داری را بین cTnI و CK-MB ($r=-۰/۸۵۳$؛ $p=۰/۰۱۵$) و Hcy ($r=۰/۹۱۶$؛ $p=۰/۰۰۴$) در شترهای ۶-۴ ساله نشان داد. همبستگی معنی داری نیز بین LDH و CK-MB در شترهای گروه سنی ۹-۷ سال مشاهده شد ($r=-۰/۷۱۰$؛ $p=۰/۰۴۵$). همبستگی معنی داری بین فراسنجه های مورد ارزیابی در گروه های سنی ۳-۱ سال و بالای ۱۰ سال مشاهده نشد ($p>۰/۰۵$). **نتیجه گیری نهایی:** این مطالعه برای اولین بار فراسنجه هایی را به منظور ارزیابی سلامت دستگاه قلبی، عروقی شترهای یک کوهانه ارائه نمود. همچنین در مواردی که ارزیابی سلامت دستگاه قلبی، عروقی شترها در مسابقات و باشگاه های شترسواری مورد نیاز است، می توان از این داده ها بهره برد. همبستگی های گزارش شده در این مطالعه می تواند به سهولتِ ارزیابی سلامت قلبی شترهای یک کوهانه کمک کند. این داده ها می تواند در ارزیابی بیماران مشکوک به مشکلات لایه عضلانی قلب مورد استفاده قرار گرفته و تغییرات آنها از ارزش پیش آگهی دهنده ای برخوردار باشد.

واژه های کلیدی: فراسنجه های ارزیابی سلامت قلبی، همبستگی، شترهای نر به ظاهر سالم یک کوهانه

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