



## RESEARCH ARTICLE

### Evaluation of serum concentration of cardiac troponin I in normal racing thoroughbred horses

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Received: 15.05.2013, Accepted: 08.06.2013

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#### Özet

**Tajik J, Sazmand A, Hekmatimoghaddam SH, Shirazi MR.** Safkan yarış atlarında serum kardiyak troponin I düzeylerinin değerlendirilmesi. **Eurasian J Vet Sci, 2013, 29, 4, 176-179**

**Amaç:** Safkan yarış atlarında serum kardiyak troponin I düzeylerini belirlemektir.

**Gereç ve Yöntem:** Klinik olarak sağlıklı 49 adet safkan yarış atından elde edilen serumlardan troponin I düzeyi ticari olarak satılan ELISA kiti ile belirlendi.

**Bulgular:** Serum troponin I düzeyi ortalaması  $0.504 \pm 0.083$  ng/mL ( $<0.2-1.7$  ng/mL) ve medianı  $<0.2$  ng/mL olarak belirlendi. Median olarak bulunan değer kitin belirleme limitinin altında olarak değerlendirildi.

**Öneri:** İran'da atlarda ölçülen serum troponin I düzeyleri diğer araştırmalardan kısmen farklı belirlendi.

**Anahtar kelimeler:** Serum, kardiyak troponin I, safkan at

#### Abstract

**Tajik J, Sazmand A, Hekmatimoghaddam SH, Shirazi MR.** Evaluation of serum concentration of cardiac troponin I in normal racing thoroughbred horses. **Eurasian J Vet Sci, 2013, 29, 4, 176-179**

**Aim:** Evaluation of serum concentration of cardiac troponin I in racing thoroughbred horses.

**Materials and Methods:** Serum concentration of troponin I was measured in 49 clinically healthy thoroughbred horses using commercial ELISA detection kits.

**Results:** The mean serum concentrations of troponin I was  $0.504 \pm 0.083$  ng/mL ( $<0.2-1.7$  ng/mL), and the median of serum cTnI was  $<0.2$  ng/mL, which was the lower limit of detection.

**Conclusions:** The serum concentration of troponin I measured in horse in Iran was partially different from those of other studies.

**Keywords:** Serum, cardiac troponin I, thoroughbred horse





## Introduction

Myocardial disease in large animals remains challenging with regard to their diagnosis and prognosis (Reef and McGuirk 2008, Varga et al 2009a). Various biochemical parameters called cardiac biomarkers can be used in the diagnosis of heart disease. The myocardial bound creatine kinase (CK-MB) and lactate dehydrogenase type 1 (LDH1) are specific cardiac isoenzymes which increase in various heart disorders. However, there is a lack of sensitivity and specificity of these markers in large animals (Begg et al 2006, Buczinski and Belanger 2010).

Cardiac troponin I (cTnI) is a small myofibrillar protein associated with the thin filaments of sarcomeres and individually expressed in the myocardium (Varga 2008). Troponin is not found, to any significant extent, in other tissues (O'Brien et al 2006) and measurement of circulating cTnI has been accepted as the gold standard biomarker for the non-invasive diagnosis of cardiac damage in some mammalian species. Measurement of circulating cTnI has a high sensitivity and nearly absolute specificity in diagnosis of myocardial damage, and the duration and amplitude of the blood cTnI increment are correlated with the severity of myocardium histopathological damage. Therefore, measurement of circulating cTnI is used not only for identification of myocardial injury and it has been shown that cTnI measurement can be used in evaluation of disease severity and prognosis (O'Brien 2008).

It has been shown that cTnI concentration in equine myocardial tissue is equal to that in human cardiac muscle, and cTnI activity in equine skeletal muscle is only 0.05 to 0.1% of the cardiac reactivity level. Therefore, cTnI can be a useful test in diagnosis of cardiac muscle damages in the horse (Begg et al 2006). There is a growing interest in using cTnI in veterinary medicine (O'Brien et al 2006) and elevated serum concentration of cTnI may be useful in the detection of cardiac damages in the horse (Begg et al 2006). However, there is little information regarding the normal serum concentration of cTnI in different mammalian including in healthy horses.

Therefore, this study was undertaken to evaluate the serum concentrations of cTnI in normal thoroughbreds in Iran.

## Materials and Methods

The investigation was carried out on clinically healthy 50 female racing thoroughbreds horses (2-3 years) kept by athletes in Gonbad region, north of Iran. After clinical examination, blood samples were taken from jugular vein in tubes without anticoagulant. The blood serum was separated after centrifugation at 850 g for 10 min, and the serum samples were stored at -20 C until analysis. One of the samples was failed because of tube leakage. Serum troponin I was measured using commercial ELISA detection kits (cTnI ELISA, Delaware Biotech Inc., USA). The analytical sensitivity of this

test in serum has been determined as 0.2 ng/mL by the manufacturer and the presence of hemoglobin, CK-MB, lipemia and bilirubin had no effect on the assay precision.

Descriptive statistics including mean, SEM, minimum, maximum and mean were calculated for serum troponin in the sampled horses. Normal distribution of data was evaluated using the Kolmogorov-Smirnov test (SPSS 12.0, Illinois, Chicago). Differences were considered significant at  $p < 0.05$ .

## Results

The health of all sampled horses was confirmed by clinical examination and training performance. The mean  $\pm$  SEM serum concentration of troponin I was  $0.504 \pm 0.083$  ng/mL ( $< 0.2$ -1.7 ng/mL), and the median of serum cTnI was  $< 0.2$  ng/mL, which was the lower limit of detection. Twenty one horses had serum cTnI concentrations lesser than 0.2 ng/mL, and the rest of the measured serum concentrations of cTnI had normal distribution. The 95% central reference intervals of serum concentration of cTnI was 0-1.646 ng/mL.

## Discussion

In recent years, measurement of circulating troponin as an important marker in diagnosis of myocardial damages in veterinary medicine has been attended. The range of serum cTnI in healthy animals has been investigated in some domestic species, such as cattle, dogs, cats and sheep (Varga 2008). However, serum concentration of cTnI in healthy horses has not been detailed evaluated. There are numerous studies of application of human immunoassays in other mammalian (O'Brien et al 2006). High amino acid sequence homology ( $> 96$  %) of cTnI in people and other mammalian species has been reported. Hence, immunoassays cTnI kit used in the diagnosis of human myocardial damage can be evaluated in the determination of cTnI levels in some animal species (Varga 2008). Cardiac troponin I may be a valuable cardiac index especially in skeletal muscle myopathic patients (Varga 2008). Lack of a generally available diagnostic test for diagnosis of myocardial damages in large animal suggests that a simple blood test, such as the measurement of cTnI, could be used in early and rapid detection of myocardial damage and probably in detection of the prognosis. Additionally, cTnI has been proposed as a marker of cardiac function in horses with poor performance (Jesty 2012) and in diagnosis of myocardial damages in horses affected by colic, endotoxemia and sepsis (Nostell et al 2012).

Interaction of cTnI with tropomyosin is crucial in the regulation of muscle contraction (Peek et al 2008, Varga 2008). Following myocardial damage, cardiac troponin leaks from the myocyte and appears in blood (Karapinar et al 2010). cTnI does not exist or is negligible in the blood of healthy animals (Varga et al 2009b, Basbugan et al 2010). The serum ranges of cTnI in healthy horses in the current study was  $< 0.2$ -1.7



ng/mL and was somewhat different from the reported ranges for the horse in previous studies. Mean cTnI concentration in 10 pastured and race-training thoroughbred horses has been reported as  $0.047 \pm 0.085$  ng/mL (Phillips et al 2003) and no significant difference between race-training and pastured horses was found. Begg et al (2006) reported serum cTnI in 23 clinically healthy thoroughbred horses in Australia to be  $\leq 0.015$  ng/mL. A study on 83 clinically normal horses showed the range for plasma cTnI concentration to be 0.0 to 0.06 ng/mL (Kraus et al 2010). A study on 34 healthy thoroughbred race horses showed resting plasma cTnI concentration as  $<0.022$  mcg/L (Nostell and Haggstrom 2008). According to the results of the current study, the cTnI concentration in healthy thoroughbred horses was generally less than 2 ng/mL, which was according to the proposed reference value of cTnI in large animal species as 0-2 ng/mL (Basbugan et al 2010). However, Radostis et al (2007) believe that healthy horses have cTnI concentrations below 0.11 ng/mL. In human, an augmented level of cTnI is defined as a concentration that exceeds the 99% of a reference population or the concentration at which the assay achieves a 10% coefficient of discrepancy if that exceeds the percentile (Varga 2008). However, reference ranges of cTnI in animals have not been determined. There are numerous case reports of horses with cardiac damage and high concentrations of cTnI (Jesty 2012). A high serum cTnI concentration, 404 ng/mL, has been reported in a horse with severe myocardial coagulation necrosis (Schwarzwald et al 2003) and increased serum cTnI levels ranging from 4.3 to 5.9 ng/mL have been reported in a horse with ruptured ventricular outflow tract and ventricular tachycardia (Cornelisse et al 2000). Nath et al (2012) found that serum cTnI concentration in horses with myocardial disease was significantly higher than healthy horses and serum cTnI concentration in non-survivor horses was also significantly higher than that of survivor horses.

According to our results, the median of serum cTnI in horses was  $<0.2$  ng/mL, which was the minimum detectable serum concentration. Similar to our results, the lower limits of detection for the assays have been reported as the median of serum cTnI in healthy dogs, cats, dromedary camels, water buffaloes and horses (Phillips et al 2003, Adin et al 2005, Hekmatimoghaddam et al 2011). The results of the mentioned studies confirm the opinion that cTnI does not exist or is negligible in the blood of the majority of healthy animals.

Cardiac troponin I has been introduced as the best biomarker for myocardial damage because of its almost complete cardiac tissue specificity and higher sensitivity than creatine kinase (CK-MB), lactate dehydrogenase isoenzymes (LDH1) and myoglobin (Karapinar et al 2010). Effective use of CK-MB and LDH1 for detection of myocardial damage in animals is also limited due to the lack of tissue specificity and sensitivity. Myoglobin is also increased with both cardiac and skeletal muscle damages and its use as a biomarker of myocardium damage is limited (Karapinar et al 2010). The improved

specificity of cTnI compared with CK-MB and LDH1 is consistent with differences in their developmental biology. During ontogeny, suppression of CK-MB and LDH1 encoding genes in skeletal muscle occurs, so that adult skeletal muscle contains only small quantities of these isoenzymes. After skeletal muscle injury, there is an increased synthesis of these isoenzymes by skeletal muscle due to re-expression of the previously suppressed genes and an increase in plasma levels of CK-MB and LDH1 occurs following increase in skeletal muscle, though cTnI does not express in skeletal muscle. Hence, cTnI is not raised in the plasma of chronic muscle diseased patients (Adams et al 1993). On the other hand, capture antibodies used in marketable cTnI kits have unimportant cross reactivity with skeletal muscle troponin, and severity of cardiac cell damage has high correlation with blood troponin I levels (Varga et al 2009b). Therefore, increase in the blood cTnI can be used as a very sensitive detector of minor amounts of myocardial necrosis (Lucia et al 2001).

## Conclusions

The findings of the present study can be used in diagnosis and prognosis of myocardial damages in thoroughbred horses, however, since this study was performed on healthy animals, investigating the serum concentration of cTnI in various conditions accompanied by myocardial damages is recommended.

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