

Evaluation of cardiac troponin I (cTnI), creatine kinase (CK), aspartate aminotransferase (AST) and electrocardiography, in diagnosis of selenium deficiency in goat kids

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Abstract:

BACKGROUND: Cardiac Troponin I (cTnI) is known as a cardiac biomarker in determining the myocardial damage of diseases which affect the heart muscle. **OBJECTIVES:** This study aims to evaluate the serum cTnI concentration in Markhoz Breed goats suffering selenium (Se) deficiency and its correlation with electrocardiographic parameters, and activity of creatine kinase (CK) and aspartate aminotransferase (AST) enzymes, as well as to determine the diagnostic value of troponin in that disease. **METHODS:** Blood samples of 94 goat kids of Markhoz breed with the age less than one month were taken, serum analyzed for the assessment of cTnI, CK and AST, and 2 ml blood was used for determination of selenium concentration. Electrocardiography was recorded from all kids by using base-apex lead. Kids were divided into two groups based on serum selenium concentration. **RESULTS:** The concentration of cTnI was significantly higher in deficient kids compared with the control group. Results indicated significant negative correlation between cTnI and selenium concentration. There was no significant correlation between cTnI and AST, CK in deficient kids. Results show no significant difference in concentration of AST and CK enzymes for two groups of deficient kids and control group. The presence of arrhythmia is significantly higher in deficient kids than control group. **CONCLUSIONS:** The results of this study showed that the measurement of serum cTnI concentration can be useful in diagnosis of kids suffering from Se deficiency. Also, ECG can be used in the diagnosis of arrhythmia in kids with Se deficiency.

Introduction

Nutritional muscular Dystrophy (NMD) is a peracute muscular degenerative disease to sub acute of skeletal and cardiac muscles

which occurs in all farm animal species, but most commonly in young, rapidly growing calves, lambs, goat kids and foals born from dams that have been fed for long periods, usually during the winter months, on diets

low in selenium and vitamin E (Radostits et al., 2014).

Selenium and vitamin E appear to be synergistic in preventing NMD. However, on the basis of prophylaxis and response to treatment, selenium deficiency appears to be more important (Smith, 2015).

Selenium and vitamin E both are involved in enzyme responses which control oxidative process in mammalian tissues by oxidation reduction reactions (Smith and Sherman, 2009).

Lack of vitamin E and selenium in body cause accumulation of toxic peroxides and loss of cell and tissue membranes and evident myopathy.

Muscle damage is associated with the increase of the release of lysosomal enzymes and myoglobin in the cell (Mostaghni et al., 2005).

Plasma Creatine kinase (CK) is most commonly used enzyme in laboratory and in the diagnosis of NMD. The enzyme is highly specific for cardiac and skeletal muscles and is released into the blood following unaccustomed exercise and myodegeneration. AST (Aspartate Aminotransferase) activity is also an indicator of muscle damage, but is not as reliable as the CK because increased AST level may also indicate liver damage. The magnitude of the increase in AST and CK is directly proportional to the extent of muscle damage. Both are elevated initially, an elevated AST and declining CK would suggest that muscle degeneration is no longer active (Radostits et al., 2014).

Today, Troponin I and Troponin T, as selective analytes of cardiac damage have been replaced for the above mentioned enzymes (Wells and Sleeper 2008).

Troponin is the biomarker of choice for the detection of cardiac injury. The 3-unit

Troponin complex (Troponin I, T, and C) along with Tropomyosin is located on the actin filament and is essential for the calcium mediated regulation of skeletal and cardiac muscle contraction. Because the cardiac isoform of the Troponin C is shared by "slow-twitch" skeletal muscles, troponin C does not have cardiac specificity and thus is not used in assays for the diagnosis of cardiac injury. There is one cardiac Troponin I (cTnI) isoform in myocardial tissue.

Since the half-life of troponin and its complexes is about 2 hours, the prolonged window during which troponin levels are elevated allows for increased clinical detection of cardiac events and thus, functionally, greater clinical sensitivity (Babium et al., 2004, Uncogil et al. 2004, Leonardi et al. 2008).

Ataollahy et al. (2011) conducted the diagnostic value of cardiac troponin I compared with other indices of muscle damage such as aspartate aminotransferase, and creatine kinase in sheep with selenium deficiency in Khorasan Province. Tunca et al. (2008) reported that the increase of cardiac troponin I and decrease or lack of troponin I in tissues are widely associated with the severity of FMD and changes in myocardial degeneration. Mellor et al. (2006) for the first time, announced the increase of serum troponin concentrations for a period of 16 days as a diagnostic marker of severe heart muscle injury in connection with heat stress in dogs. Identifying the animals affected or in general understanding the epidemiology status of metabolic disease of selenium deficiency can be studied in any area with different ways, which include the measurement of the blood and hair selenium in animals, liver selenium in slaughterhouse animals, regional soil selenium, regional plant

Table 1. The mean \pm SE of serum selenium, cTnI, CK and AST concentration in se-deficient and non-deficient goat kids. ^{a,b} Mean with different superscript on the same row are significantly different ($p \leq 0.5$).

	Se non-deficient group		Se deficient group	
	Number (%)		Number (%)	
Selenium (ppm)	60(63.8%)	0.13 \pm 0.18 ^a	34(36.2%)	0.033 \pm 0.14 ^b
Troponin (μ g/L)	82(87.2%)	0.118 \pm 0.11 ^a	12(12.8%)	2.18 \pm 3.07 ^b
CK(IU/L)	35(37.2%)	145 \pm 42.8 ^a	59(62.8%)	3302 \pm 476 ^a
AST (IU/L)	73(77.7%)	142.8 \pm 122 ^a	21(22.23%)	1327 \pm 729 ^a

Table 2. relationship between changes of amount of cTnI, Se and the activities of AST and CK in goats. * Significant relationship ** No significant relationship.

	Correlation coefficient
cTnI and Se	-0.496*
cTnI and CK	0.150**
cTnI and AST	0.607**

Table 3. The type and number of arrhythmia in Se-deficient and non-deficient goat kids.

Arrhythmia	Se-deficient group	Se- non deficient
Sinus tachycardia	20	35
Atrial premature	18	12
Sinus arrhythmia	13	4
Atrial fibrillation beat	8	1
AV block II	4	1
Sinus arrest	3	0
Ventricular premature beat	1	0
AV block III	1	0
Ventricular fibrillation	1	0

selenium or the measurement of muscular damages indices such as CK and AST. The aim of this study is assessment of serum concentration of cardiac troponin I in Markhoz breed goat kids with the deficiency of selenium and its relationship with electrocardiography parameters and activity of CK and AST enzymes, as well as determining the diagnostic value of troponin in the same disease.

Materials and Methods

In this study blood samples of 94 Markhoz goats of both sexes, female (n=37) and male (n=37), weight 5-10 kg, kept in Breed-

ing Center of Markhoz Goats in Sanandaj (in the west of Iran) with the age of less than one month old were taken randomly. Electrocardiography was also done, while previously coordinating and ensuring that no injection of selenium to pregnant ewes took place.

First, electrocardiogram was recorded in standing position from all goats, using electrocardiography device (model ECG2000 (Bionet) made in Korea) on base-apex lead. The paper speed was adjusted to 25 mm/sec and calibration was 10 mm/mV. Electrocardiograms were studied with respect to the presence of the arrhythmia and its type recognition. Then 5 ml blood sample were collected from jugular vein of all goats. 3 ml blood was added into the plain tube for serum harvesting. Plain tubes were centrifuged at 3500 \times g for 10 min followed by removal serum. The activities of AST, CK enzyme were measured by commercial kits of (Pars Azmoon, Tehran, Iran); using an autoanalyzer (Auto-chemistry Analyzer, model DIRUI, CS-T240, China) and concentration of cardiac troponin I was measured also using ELISA method by commercial kit (Monobind Inc, USA) and the results were determined, according to manufacturer's instructions. Mean concentrations for AST and CK in goat kids were 513 IU/L and 219 IU/L respectively (Smith, 2015).

2 ml of the remaining blood was used as whole blood for determining the selenium concentration using atomic absorption spec-

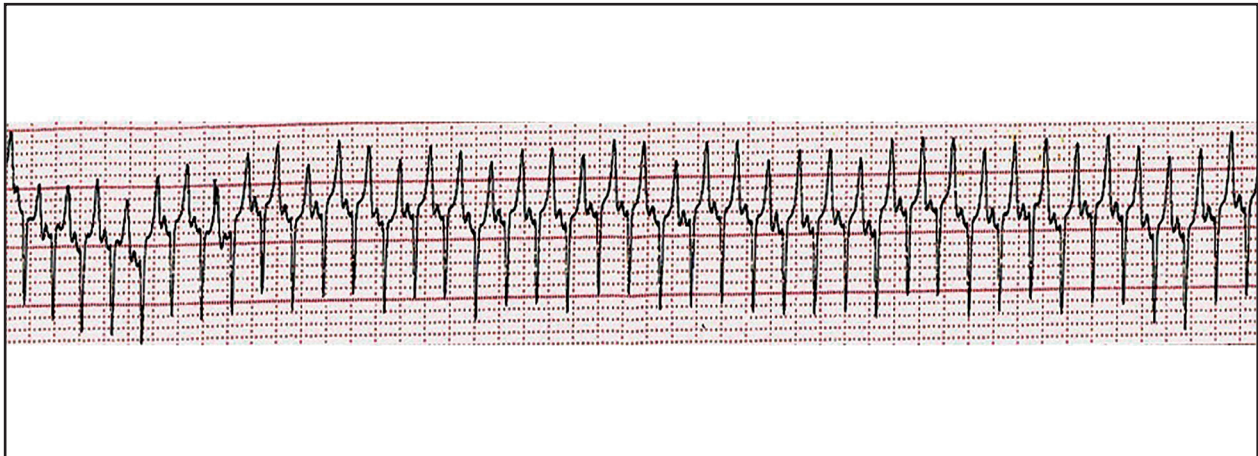


Figure 1. Sinus tachycardia in selenium deficient goat kids.

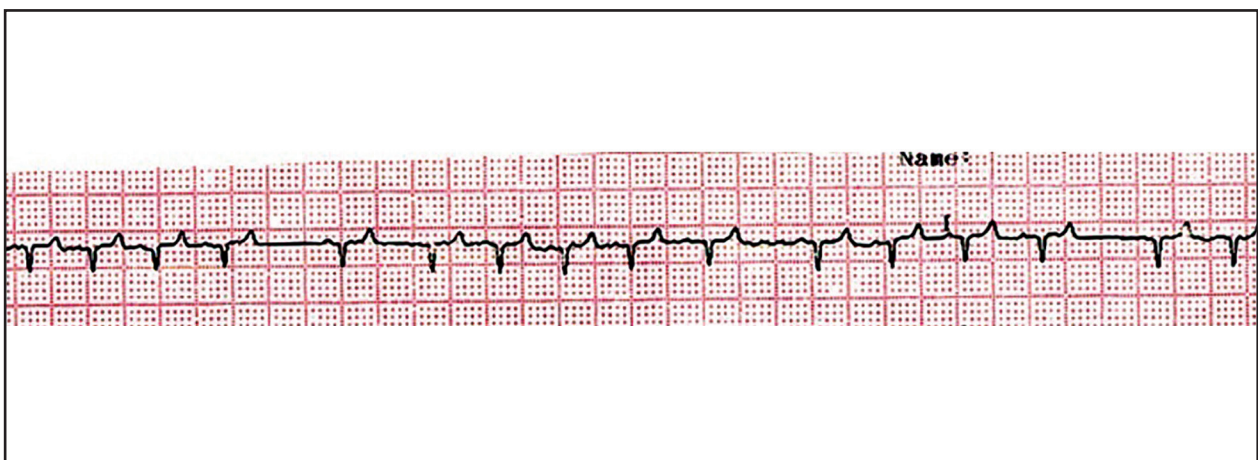


Figure 2. Sinus arrhythmia in selenium deficient goat kids.

trophotometry (Perkin-Elmer 2380, Britain, Model) with a wavelength of 196 nm.

Kid goats were divided into two groups based on serum selenium concentration: deficient kids with Se concentration of ≤ 0.05 ppm (n=34) and control kids (n=60) with Se concentration of higher value (Smith, 2015).

There are no reports indicating the normal value of cardiac troponin I (cTnI) in Markhoz breed goats and for the kids less than one month, hence, for determining this value, the level of serum (cTnI) concentration of 100 Markhoz breed goats with the age of less than one month and apparently healthy was measured by a method similar to that mentioned above during the present study. All sampled kids underwent clinical

exam and 2 kids had WMD based on clinical signs, which was confirmed by necropsy, pathology, and serum Se concentration. The statistical analysis was conducted using SPSS for windows (release 16, SPSS Inc, Chicago, USA). Based on Kolmogorov-Smirnov normality test, parametric t-test, non-parametric Mann-Whitney U test, and correlation coefficient test were used to investigate significant difference between groups for measured analyses. For all comparisons $p \leq 0.05$ was considered as significant (Ataollahy et al., 2013).

Results

The results of selenium concentration for 94 kids with the age of less than one month

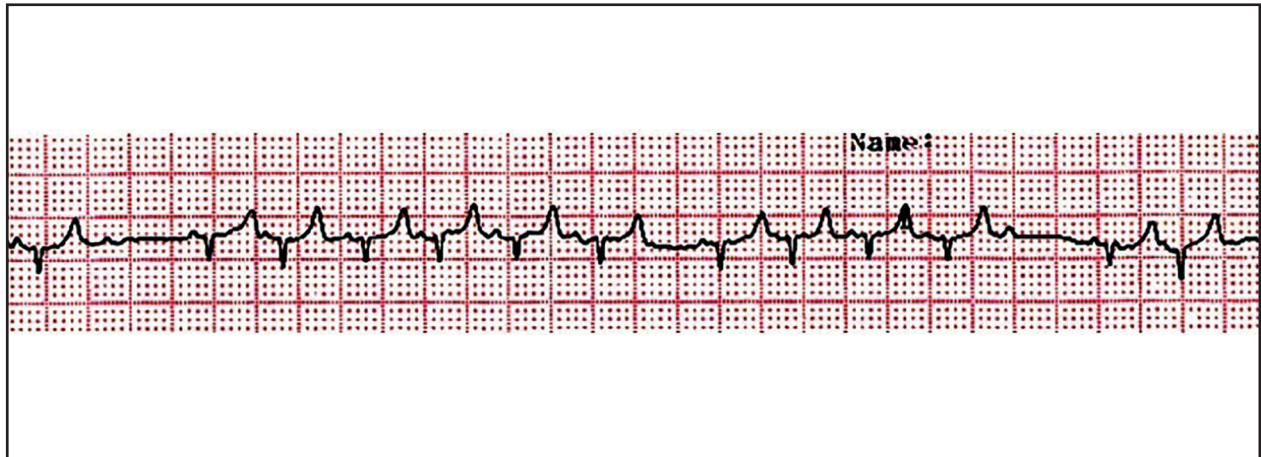


Figure 3. Second- degree AV block in selenium deficient goat kids.

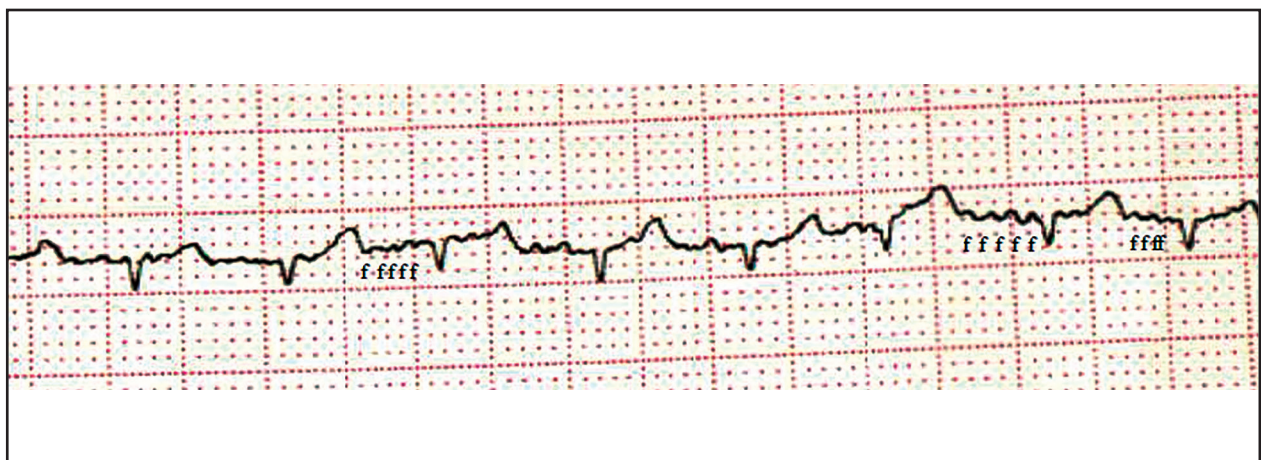


Figure 4. Atrial fibrillation in selenium deficient goat kids.

in both sexes are shown in Table 1. Accordingly, 94 goats were divided into two groups, one group with selenium deficiency (34 goats) and control group with normal selenium (60 goats).

As mentioned, due to the absence of report indicating the reference value of cTnI in goat kids aged less than one month of Markhoz breed, cTnI of 100 apparently healthy goats less than one month was measured and range of 0.0_ 0.47 $\mu\text{g/l}$ was considered as reference value in the goats under study. Similar action was done in the study of Basbugan et al. (2010).

The results of cTnI concentration for 94 goats are revealed in Table 1.

The results show that serum concentration of cTnI in the group with selenium de-

ficiency was significantly higher than that of control groups ($p \leq 0.05$) and using the correlation coefficient statistical test reveals that there is a significant negative correlation between changes of amount of cardiac troponin I and selenium deficiency in Markhoz goats, $p \leq 0.05$.

The results of the AST and CK activities in 94 goats are shown in Table 1.

Using the correlation coefficient statistical test reveals that there is no significant relationship between changes in amount of cTnI and the activities of AST and CK in goats, ($p > 0.05$) (Table 2). Also, the changes of the AST and CK activities in two groups of goats did not show significant differences ($p > 0.05$).

The results obtained from the electrocar-

diography indicate that there is one or more types of arrhythmia in 94 goats. Table 3 shows the different types of arrhythmia.

Since a large number of sampled kids had more than one type of arrhythmia, therefore, for evaluating the diagnostic efficiency of arrhythmia in two groups, only the presence of arrhythmia without considering its type has been analyzed using correlation coefficient statistical test, and a significant relationship between the selenium deficiency and the presence of arrhythmia was observed ($p \leq 0.05$), it means that the presence of arrhythmias in the group with selenium deficiency is significantly higher than control groups. There was no significant difference between cTnI and arrhythmia in deficient group ($p > 0.05$). There was no significant difference between the types of arrhythmia in deficient group ($p > 0.05$). Numbers and types of arrhythmia are shown in Table 2. Figures 1-3 show three types of arrhythmias diagnosed in this study.

Discussion

Some researchers have reported that early diagnosis is important in WMD as appropriate treatment and prophylactic protocols are applied (Kozat et al., 2011). There are few reports concerning diagnostic value of cTnI in sheep and goats medicines (Ataollahy et al., 2013; Tharwat et al., 2013). To the authors' knowledge, none of the reports investigated Se deficiency in Markhoz breed goat's kids and no correlations were described between Se with cTnI, AST, CK, electrocardiography and its diagnostic value in field conditions. Ataollahy et al. (2013) described these correlations in lambs except electrocardiography. Concerning the reference value of cTnI, Basbugan et al. (2010)

reported reference value of cTnI concentration in ruminants. According to their report, the mean of cTnI concentration in sheep was 0.0- 0.27 $\mu\text{g/l}$ with no age and sex effects on its concentration. The study of Tunca et al. in 2009 indicates the concentration of troponin I ($0.32 \pm 0.06 \mu\text{g/l}$) in healthy lambs. As mentioned, due to the absence of reports indicating the reference value of cTnI in goat kids with the age of less than one month of Markhoz breed, cTnI of 100 apparently healthy goats less than one month was measured and range of 0.0_ 0.47 $\mu\text{g/l}$ was considered as reference value in the goat kids under study. In this study, serum cTnI concentration was significantly higher in goat kids with Se deficiency, and the results reveal significant negative correlation between selenium deficiency and cardiac troponin, which is similar to the study done by Ataollahy et al. (2013), indicating that cardiac troponin I can be regarded as specific and sensitive marker in the field of determining the damage of cardiac muscle cells in white muscle disease in lambs. The concentration of cTnI in present study was close to Ataollahy et al.'s (2013) report, whereas it was lower than Tunca et al. (2009) and Basbugan et al.'s (2010) reports. Two reasons could be considered for this reference; firstly, acute form of disease was considered in these studies. In the present study, regarding the possibility of following up the animals under study, 3 goat kids of the group with selenium deficiency had the clinical symptoms related to selenium deficiency; after death, their blood selenium concentrations were proved by necropsy findings and histopathology. Secondly, difference in method of measurement of cTnI as suggested by Wells and Sleeper, (2008) must be considered (Ataollahy et al., 2013).

In addition to sheep and goats, various studies were performed in cattle concerning cTnI changes in cardiac and non-cardiac disorders. cTnI concentration in cows with idiopathic pericarditis is reported to be higher than in healthy cows (Gunes et al., 2008). Increased concentration of cTnI was reported significantly higher in calves with FMD compared to control (Koya et al., 2013). In another study, serum cTnI concentration as a Myocarditis marker was significantly higher in lambs with FMD (Aslani et al., 2013). In one study the diagnostic value of increases of troponin I and T concentration were confirmed in cattle with traumatic Reticulopritonitis (TRP) (Gunes et al., 2008). Although other researchers have reported significant increase of CK in the lambs with selenium deficiency (Abood et al., 2012; Kosat et al., 2011; Tunca et al., 2010). The results of this study reveal that there is no significant difference between selenium and CK activity for the group with selenium deficiency compared with the group with normal selenium. Our results were consistent with those of researchers (Ataollahy et al., 2013; Varga et al., 2009).

Two reasons for the results obtained in CK enzyme could be described; firstly, in the present study breed differences must be considered, secondly, increase of CK concentration in blood, following the skeletal muscles damages caused by trauma, injections, falls and transportation must be considered. Therefore, considering the age of the goat kids, the possibility of the appearance of such increase in both groups under study can be considered as another reason for statistical analysis in both groups.

In this study, between the group with selenium deficiency and AST activity no significant relationship was observed, which is

consistent with the results of another study (Ataollahi et al., 2013). In yet another study considerable increases in activities of CK and AST in calf were reported which can be used as indicator of cardiac damage, but they are not specific and can also increase following skeletal muscle damage (Aslani et al., 2013). AST is also an indicator of muscle damage, but is not as reliable as the CK because increased AST levels may indicate liver damage (Radostits et al., 2014). AST enzyme exists in the mitochondria of almost all cells and also in plasma, and increase of this enzyme may be caused by injuries in skeletal and cardiac muscles in addition to liver injuries. CK is a more specific index for muscle damage compared to AST; however, AST is often used as a supplement for CK (Kaneko et al., 1997).

The results of this study show that there is significant difference between the presence of arrhythmia and selenium deficiency. Since the concentration of Troponin was more in the group with selenium deficiency compared to the group with normal selenium, it can be concluded that there was some degree of damage in cardiac muscular cells in group with selenium deficiency that appears as a disorder in electrical or mechanical activity of the heart, and this disorder has been revealed in arrhythmia. But no significant relationship is observed between types of arrhythmia and selenium deficiency. Varga et al. (2009) reported cTnI was significantly associated with left ventricular shortening fraction in cattle with monensin toxicosis, and increased heart rate, lengthening of the duration of QRS complex, prolongation of QT interval, low voltage R wave and IT segment elevation were observed, indicative of myocardial injury. In one study cardiac dysrhythmias and cTnI were evaluated

in low and high yielding dairy cows, cardiac arrhythmias were detected in low yielding dairy cows, probably due to metabolic and electrolyte disorders (Jafary Dehkordi et al., 2014). Fartashvand et al. (2013) reported that serum concentration cTnI was significantly higher in cattle with theileriosis, but there were no pathologic arrhythmias detected in sick animals. This season could be a reason for this result; all cardiac arrhythmias are not caused by heart damage and can have multiple reasons, in addition a number of arrhythmias in ruminants (Markhoz goat breed) are physiologic (Fakour et al., 2013), which could explain the non significance of the relationship of arrhythmias types and troponin concentrations in goats with selenium deficiency.

Considering the fact that one of the types of white muscle disease in young livestock is cardiac muscle dystrophy, the results of this study can indicate some degree of damage to cardiac muscular cells due to the selenium deficiency. Cardiac form of white muscle disease in young animals is acute with the appearance of the least clinical signs. The results of this study show that some degree of damage to cardiac muscular cells can be predicted before developing clinical and acute form, by measuring the cardiac troponin I. cTnI concentration of kids may be increased due to myocardium involvement caused by other cardiac and non-cardiac disorders (Ataollahy et al. 2013). On the other hand, cTnI concentration was not increased in all Se deficiency groups, nevertheless our results reveal significant negative correlation between selenium concentration and cardiac troponin, as serum cTnI concentrations were also significantly higher in goat kids with Se deficiency. In conclusion the evaluation of Cardiac Troponin I would

be useful for diagnosis of Se deficiency in goat kids at herd with better diagnostic efficiency than CK and AST. Also, ECG can be used in the diagnosis of arrhythmia in kids with Se deficiency.

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ارزیابی تروپونین قلبی I، الکتروکاردیوگرافی، کراتین کیناز و آسپاراتات آمینوترانسفراز در تشخیص کمبود سلنیم در بزهای نژاد مرخز

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چکیده

زمینه مطالعه: تروپونین قلبی I بعنوان یک بیومارکر قلبی در تعیین آسیب میوکارد در بیماری‌هایی که عضله قلب را مبتلا می‌کند شناخته شده است. دیستروفی عضلانی تغذیه‌ای یا علت کمبود سلنیم و ویتامین E یکی از این بیماری‌هاست. هدف: هدف از این مطالعه ارزیابی غلظت سرمی تروپونین قلبی I در بزهای نژاد مرخز با کمبود سلنیم و ارتباط آن با پارامترهای الکتروکاردیوگرافی و فعالیت آنزیم‌های CK, AST و همچنین تعیین ارزش تشخیصی تروپونین در آن بیماری است. روش کار: نمونه خون از ۹۴ بز نژاد مرخز زیر یک ماه اخذ گردید. سرم‌های جدا شده از نمونه خون به منظور اندازه‌گیری تروپونین قلبی و CK, AST و ۲ ml خون کامل به منظور اندازه‌گیری غلظت سلنیم استفاده شد. از همه بزها نوار الکتروکاردیوگرام در اشتقاق قاعده ای رأسی اخذ گردید. بزها براساس غلظت سلنیم سرم به دو گروه تقسیم شدند. بزهای با سلنیم کمتر یا مساوی با ۰/۰۵ ppm به عنوان گروه کمبود سلنیم (۳۴ بز) و گروه با سلنیم بالاتر از معیار طبیعی به عنوان گروه کنترل (۶۰ بز). نتایج: غلظت تروپونین در گروه با کمبود سلنیم بطور معنی‌داری از گروه کنترل بالاتر بود ($p < 0/05$). بین غلظت سلنیم و تروپونین ارتباط منفی معنی‌داری بدست آمد. بین غلظت تروپونین و آنزیم‌های CK, AST در دو گروه کنترل و کمبود سلنیم اختلاف معنی‌داری نشان داده نشد ($p > 0/05$). آریتمی در گروه با کمبود سلنیم بطور معنی‌داری از گروه کنترل بالاتر بود ($p < 0/05$). در حالیکه بین غلظت تروپونین و آریتمی در بزهای با کمبود سلنیم ارتباط معنی‌داری وجود نداشت ($p > 0/05$). نتیجه‌گیری نهایی: نتایج مطالعه حاضر نشان می‌دهد تعیین غلظت سرمی تروپونین قلبی I در شناسایی بزهای مبتلا به بیماری عضله سفید به ویژه قبل از بروز نشانه‌های بالینی به هم‌راهم اندازه‌گیری سلنیم خون می‌تواند مفید باشد و الکتروکاردیوگرافی با تشخیص آریتمی می‌تواند مورد استفاده قرار گیرد.

واژه‌های کلیدی: الکتروکاردیوگرافی، آنزیم، بز مرخز، سلنیم، تروپونین

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