

Diagnostic value of cardiac troponin I (cTnI), creatine kinase (CK), and aspartate amino transferase (AST) in selenium deficiency in lambs

F. ATAOLLAHI¹, M. MOHRI^{2*}, H. A. SEIFI²

¹Department of Clinical Sciences, School of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, IRAN

²Department of Clinical Sciences and Center of Excellence in Ruminant Abortion and Neonatal Mortality, School of Veterinary Medicine, Ferdowsi University of Mashhad, P.O. Box: 91775-1793, Mashhad, IRAN

* Corresponding author: mohri@um.ac.ir

SUMMARY

The aims of the present study were to: 1) elucidate the probable relationships between selenium (Se) concentration with cardiac troponin I (cTnI) concentrations and also with the activities of CK, and AST. 2) Determination of diagnostic efficiency and likelihood ratios of cTnI in diagnosis of Se deficiency. Sampling was conducted from 201 lambs with the age of less than one year old. Eleven lambs were rule out from study due to various diseases. Lambs were divided into two groups based on serum Se concentration: deficient lambs with Se concentration of $\leq 50 \mu\text{g/L}$ (n= 36) and control lambs (n=154) with the Se concentration of higher values. The concentration of cTnI was significantly higher in deficient sheep than control was ($p < 0.05$). There was significant negative correlation between cTnI and Se concentration. The results of Receiver Operative Curve (ROC) analysis to evaluate the diagnostic efficiency of cTnI, AST, and CK were suggested that, the area under the curve (AUC) for cTnI (0.641) was significantly higher than reference line. At selected cut-off value for cTnI (0.06 $\mu\text{g/L}$) the sensitivity and specificity of test were 50% and 73%, respectively. The likelihood ratios (LRs) at selected cut-off point were +LR = 1.85, and -LR = 0.69, for cTnI.

Keywords : Selenium, AST, CK, Sheep, Troponin I, ROC

RESUME

Intérêt diagnostique de la troponine I cardiaque, créatine kinase et aspartate aminotransférase lors de carence en sélénium chez l'agneau

Les objectifs de la présente étude étaient les suivants: 1) élucider les probables relations entre les concentrations en sélénium (Se) et les concentrations en troponine cardiaque I (cTnI), les activités de la CK et AST. 2) Déterminer la pertinence et l'intérêt de la mesure des concentrations en cTnI lors de carence en sélénium. L'échantillonnage a été réalisé à partir de 201 agneaux de moins d'un an. Onze agneaux ont été exclus de l'étude en raison de diverses maladies. Les agneaux ont été divisés en deux groupes en fonction des concentrations sériques en Se: « agneaux déficiente » ayant une concentration en Se $\leq 50 \text{ mg / L}$ (n = 36) et « agneaux témoins » ayant une concentration en Se $> 50 \text{ mg / L}$ (n = 154). La concentration de troponine Ic était significativement plus élevée chez les moutons déficients que les contrôles ($p < 0,05$). On a observé une corrélation négative significative entre la concentration en cTnI et la concentration en Se. Les résultats d'analyse du modèle Receiver Operative Curve (ROC) suggèrent que l'aire sous la courbe (AUC) des concentrations en cTnI était significativement plus élevé que ligne de référence. A la valeur seuil de cTnI de 0,06 mg / L a sensibilité et la spécificité du test était respectivement de 50% et 73%.

Mots-clés : Mots clefs: Sélénium, AST, CK, Mouton, Troponine I, ROC

Introduction

In veterinary medicine, troponins (Tn) measurements have been shown to have the potential to be sensitive indicators of myocardial injury due to both cardiac and non-cardiac disease processes [29]. Changes in serum concentrations of these have been found to be specific and sensitive indicators of myocardium injury in human (with sensitivity of 94 % and specificity of 81%) and dog (with sensitivity of 70-100 % and specificity of 65-81%)[12,24]. Troponins are phylogenetically highly conserved proteins with $> 95\%$ homology between mammals [1]. Thus, assays developed for human serum can be validated for using in other species as performed in previous study for measuring cTnI in blood serum of sheep [15,18].

There are few reports concerning diagnostic value of cTnI in sheep medicine. A number of those described the

diagnostic value of cTnI in experimental cardiac disease in comparative studies and others in diagnosis of acute white muscle disease [5,7,11,17,18,26].

Most regions in Iran are generally Se deficient and white muscle disease (WMD) is frequently diagnosed in lambs based on Se concentration and pathological examination. Diagnosis of deficiency by measurement of Se concentration and glutathione peroxidase activity (as specific markers) are not easily available in developing countries but regional hospitals use cardiac troponin I measurement for diagnosis of human patients with acute myocardial infarction (AMI). Thus, cTnI measurement is more easily available than other mentioned test as test of cardiac involvement in Se deficiency. To the authors' knowledge, studies on the quantitative relationship between serum cTnI and the concentration of selenium and activities of AST and CK and its diagnostic value in sheep have not yet been published. The aims of the

present study were to: 1) elucidate the probable relationships between Se concentration with cTnI concentrations and also with the activities of CK, and AST. 2) Determination of diagnostic values and likelihood ratios of cTnI in diagnosis of Se deficiency.

Materials and Methods

Sampling was conducted from 201 lambs with the age of less than one year old. These lambs were selected from different geographical regions and herds of Khorasan Razavi province (north east of Iran) and examined prior to sampling for any clinical signs. These regions and herds had history of selenium deficiency of soil, hay, and cases of white muscle disease that previously referred to veterinary teaching hospital of school of veterinary medicine, Ferdowsi university of Mashhad. Nine herds with 186 blood samples (minimum number 5 and maximum number 62 depending to the size of herd) were visited. Remaining samples were selected from referred lambs to our teaching hospital. Eleven lambs were rule out from study due to various diseases. These were from different geographical regions and herds. The breeds of sampled lambs were generally Baloochi, Kordi, and Moghani. From sampled herds only two herds had history of irregular injection of selenium/vitamin E or supplementation in the diet of the herd. Except these two herds which feed from pasture and additive all other herds using pasture during year and some alfalfa hay during dry seasons.

Blood samples were collected from jugular vein. Ten milliliter of blood was taken from each lamb by disposable syringes into the plain tube for serum harvesting. All samples were transferred on ice to laboratory. Plain tubes were centrifuged at 1800×g for 10 min followed by removal of serum. Serum was stored at -20°C until analysis. The activities of creatine kinase (CK), and aspartate aminotransferase (AST) were measured by commercial kits (Pars Azmoon, Tehran, Iran); using an autoanalyzer (Biotecnica, Targa 3000, Rome, Italy). Se concentration was determined using atomic absorption spectrophotometry (Perkin Elmer 3030, USA). Cardiac troponin I (cTnI) concentration was measured using ELISA kit (Monobind Inc, Lake Forest, California, USA) and the results were determined according to manufacturer's instructions. The intra and inter assay coefficient of variation (CV) for measured variables were: AST 3.06 % and 1.38 %, CK 0.7 % and 1.04 %, cTnI 3.3% and 7.9%, and selenium 5% and 3%, respectively. Samples were measured every 2 weeks and control serum (Randox control sera, Antrim, UK) was used for controlling measurement accuracy (mean concentrations for AST and CK were 38 IU/L and 169 IU/L, respectively).

Lambs were divided into two groups based on serum Se concentration [23]: deficient lambs with Se concentration of ≤ 50 µg/L (n=36) and control lambs (n=154) with the Se concentration of higher values. There was no significant difference of age between two groups (median of 90 days). All sampled lambs undergone clinical exam and six lambs

had WMD based on clinical signs, which confirmed by necropsy, pathology, and serum Se concentration. Other Se deficient lambs (based on Se concentration) did not show any clinical sign (subclinical).

Statistical analysis was conducted using SPSS for windows (release 16, SPSS Inc, Chicago, USA). Based on Kolmogorov-Smirnov normality test, non-parametric Mann-Whitney U test was used to investigate significant difference between groups for measured analytes. Linear regression was used to evaluate relationship between selenium as dependent and cTnI, AST, and CK as predictors. Since selenium concentrations in sampled lambs had not normal distribution thus transformation was conducted by square root for normalization. In addition, logistic regression Forward Stepwise (Likelihood Ratio) was used for determining the value of measurements of cTnI, AST, and CK in prediction of Se deficiency in lambs. Receiver Operative Curve (ROC) was used for the detection of area under the curve (AUC), and sensitivity and specificity of measured analytes at selected cut-off point in diagnosis of Se deficiency. Likelihood ratio (LR) positive and negative were calculated by sensitivity/(1-specificity) and (1-sensitivity)/specificity, respectively [8]. For all comparisons $P \leq 0.05$ were considered as significant.

Results

The concentration of cTnI was significantly higher in Se-deficient sheep than control was ($p < 0.001$, Figure 1). The activities of AST and CK (median and range) in Se-deficient lambs and non-deficient ones were [AST (108, 59-1629 IU/L and 113, 23-2217 IU/L), CK (360, 172-14864 IU/L and 368, 84-14280 IU/L)], respectively. There were no significant difference between groups. Regression analysis revealed that weak significant negative relationship existed between selenium and cTnI concentrations [$y = 8.7 - (2x)$, $p = 0.016$, $r^2 = 0.03$]. Scatterplot of selenium and cTnI concentrations is showed in Fig 2. There were no any significant relationship between selenium concentration and AST and CK activities. The statistics of logistic regression in diagnosis of Se deficiency are as follow: Cox and Snell R square: 0.048, Hosmer and Lemeshow test p value: 0.582. Between variables in the equation only cTnI had significant effect in the model (p value: 0.004).

The results of ROC analysis to evaluate the diagnostic efficiency of cTnI is shown in Figure 3. From these results, the area under the curve for cTnI was significantly higher than reference line, although it was low (AUC: 0.641, p value: 0.009, 95% Confidence Interval: 0.533-0.749). The comparison of AUC for measured analytes significantly indicated better diagnostic efficiency for cTnI than AST, and CK for diagnosis of selenium deficiency at herd level. At selected cut-off value for cTnI (0.06 µg/L) the sensitivity and specificity (95% CI) of test were 50% (33-66) and 73% (66-80), respectively. The LR_s (95% CI) at selected cut-off point were +LR = 1.85 (1.2-2.84), and -LR = 0.69 (0.49-0.97). According to AUC, cut-off

values and likelihood ratios were not calculated for CK and AST because there were not any significant differences with reference line.

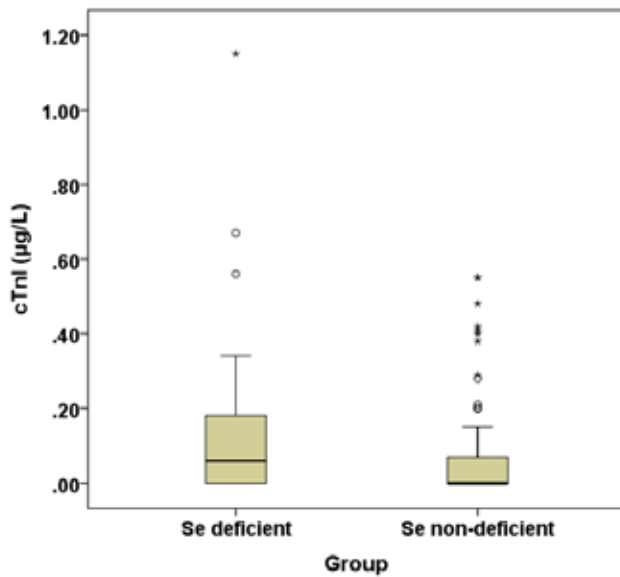


FIGURE 1: Box and whisker plot of serum cTnI concentrations in Se deficient and non-deficient lambs. Significant difference was seen between two groups of lambs ($p < 0.001$)

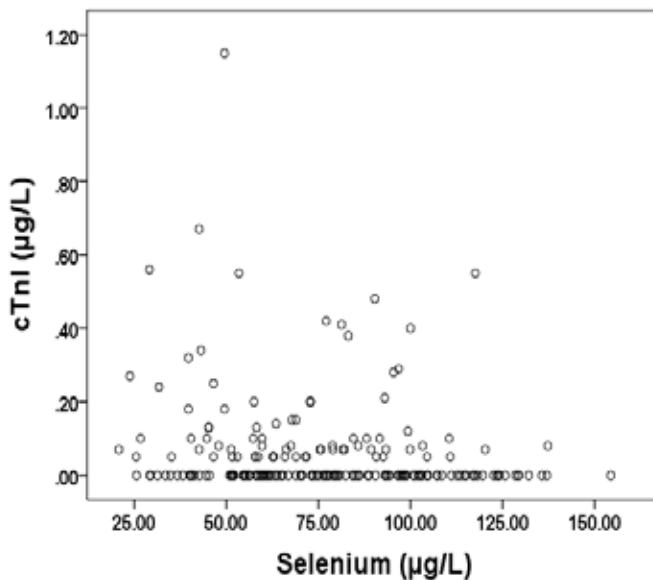


FIGURE 2: Scatterplot of selenium with cTnI concentrations

Discussion

There are few reports concerning diagnostic value of cTnI in sheep medicine. A number of those studied the concentrations of cTnI in experimental cardiac disease in comparative studies and others in diagnosis of acute white muscle disease [5,7,11,17,18,26]. To the knowledge of the authors, none of them described correlations between Se with

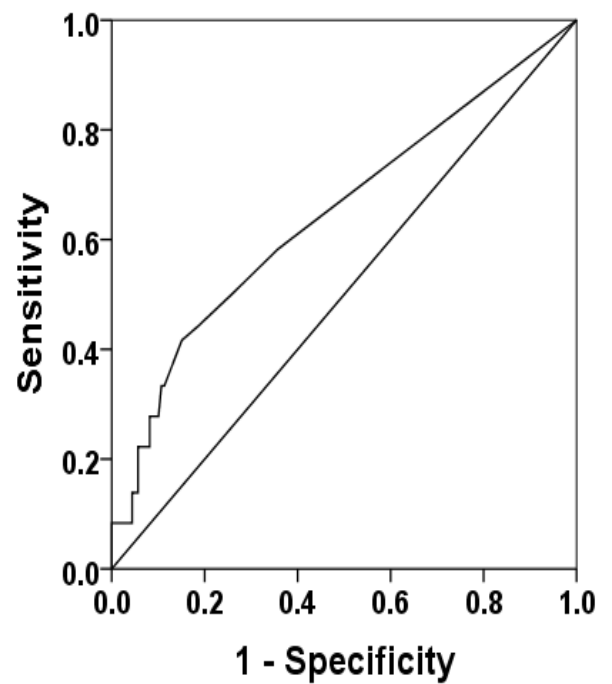


FIGURE 3: Comparative non-parametric receiver operating characteristic plot (ROC) for serum cTnI in diagnosis of selenium deficiency in lambs (AUC: 0.641, p value: 0.009, 95% Confidence Interval: 0.533-0.749)

cTnI, AST, CK and its diagnostic value in field conditions. Concerning to reference value of cTn, Başbuğan et al. [2] reported reference value of cTnI concentration in ruminants. According to their report, the mean of cTnI concentration in sheep was $0.15 \mu\text{g/L}$ with range of $0.0-0.21 \mu\text{g/L}$ with no age and sex effects on its concentrations. Leonardi et al. [18] suggested commercial kits for measurement of human cTnI was appropriately react with ovine cTnI and its concentration started to increase day after coronary ligation. Basement value of cTnI was $0.06 \pm 0.03 \mu\text{g/L}$ increased to $16.93 \pm 9.21 \mu\text{g/L}$ at day 1 after ligation. The difference between basement values in cTnI concentration was apparent between these two studies with our report.

Tunca et al. [26] measured cTnI and inducible nitric oxide synthase (iNOS) expression in hearts of the lambs with white muscle disease ($n = 15$) and control healthy lambs ($n=8$). CK, CK-MB, AST, and LDH activities were determined for both groups. Mean cTnI ($10.49 \pm 0.25 \mu\text{g/L}$ concentration was higher in diseased lambs. The concentration of cTnI in our study was much lower than Tunca et al. [26] and Başbuğan et al. [2] reports. Two reasons could be described this difference; firstly, acute form of disease was considered in the study of Tunca et al. [26]. In the present study only 6 of 36 Se deficient lambs had clinical Se deficiency. Secondly, difference in method of measurement of cTnI as suggested by Wells and Sleeper [29] must be considered. In another report, poin-of-care cTn assays were used for diagnosis of acute white muscle disease in lambs. Tests for cardiac troponin I and T were positive for all lambs with disease [11].

In addition to sheep, various studies were performed in cattle concerning to cTnI changes in cardiac and non-cardiac disorders. cTnI is related to myocardial necrosis and severity of myocardial damage in cattle with monensin toxicosis. cTnI could become a useful diagnostic tool in the noninvasive assessment of myocardial injury in cattle with naturally occurring cardiac disease. A serum concentration of cTnI ≥ 1.04 $\mu\text{g/L}$ is an indicator of histopathologically detectable myocardial necrosis in cattle after monensin administration [27]. Increased concentration of cTnI (3.52 $\mu\text{g/L}$) was reported in cow with tricuspid endocarditis [4]. In another study, serum cTnI concentrations were significantly higher in cattle with pericarditis compared with healthy cattle, but were not significantly different from concentrations in cattle with endocarditis, congenital cardiac disease, mediastinal abscess, reticulitis, caudal vena cava thrombosis, or chronic suppurative pneumonia. Based on this report and a previous study, 49 of 53 cattle (92.5%) that were considered clinically normal had serum cTnI concentrations < 0.08 $\mu\text{g/L}$ [19]. From these results it is clear that cTnI could be used for diagnosis of myocardium injuries in cattle but it cannot be able to differentiate cardiac from non-cardiac disorders. While sufficient data would be prepared from studies in cardiac and non-cardiac diseases in sheep this subject must be kept in mind in interpretation of cTnI concentration in sheep.

In one study in human in China, the correlation between glutathione peroxidase activities (as indirect Se estimation) and cTnI concentrations was $r = -0.351$, $p < 0.01$. In addition, cTnI concentration was lower in selenium supplemented subjects than un-supplemented ones [21]. Our results are in consistent with their report and indicated cTnI changes in accordance with Se concentration due to myocardial injuries.

It is important to keep in mind that the sensitivity, and specificity of cTnI as diagnostic tool are not ideal although as mentioned were better than AST, and CK. Many studies were reported cTnI concentrations in cardiac and non-cardiac diseases in cattle [4,9,10,14,19,24,28] and horse [3,6,12,16,20,22]. According to these reports, non-cardiac disorders could be able to affect myocardium and increased the concentration of cTnI in serum; and resulted to decrease sensitivity and specificity of cTnI measurement. In the present study, at selected cut-off value for cTnI (0.06 $\mu\text{g/L}$) the sensitivity and specificity of test were 50% and 73%, respectively. Our results are consistent with this opinion that concentration of cTnI in blood serum of lambs may be increased due to myocardium involvement caused by other cardiac and non-cardiac disorders (specificity) as mentioned previously in cattle and horse. On the other hand, cTnI concentration was not increased in all cases of Se deficiency (sensitivity). It seems in this situation chronicity of the injuries of myocardium and also mass of remaining intact tissue must be considered.

In conclusion cTnI measurement could be considered in laboratory panel for diagnosis of Se deficiency in lambs at herd concentration with better diagnostic efficiency than AST,

and CK. But, it must be considered that its diagnostic value may be affected by other diseases or conditions. Thus, other procedure like necropsy and pathology must be performed.

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