CLINICAL STUDY

Cord Blood Cardiac Troponin I and Creatine Kinase MB Levels in Poor Neonatal Outcomes

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Abstract

Objective: To compare cord blood cardiac-troponin I and creatine kinase-MB levels in fetuses with poor neonatal outcome to completely healthy newborns.
Materials and Methods: Cord blood cardiac-troponin I and creatine-kinase-MB (CK-MB) levels of 398 completely healthy newborns were measured via microparticle enzyme immunoassay. These were compared to the levels of fetuses with acidosis defined as pH <7.1 and/or base excess <-12 mmol/L (n=21), hypoxic ischemic encephalopathy (n=12), fetal anomaly (n=13) and early neonatal mortality (n=8). The median levels were compared using Mann-Whitney U test. Receiver operator characteristics were analyzed to find a cut-off value with best sensitivity and lowest false positive rate.
Results: The median level of troponin I in healthy newborns was 0.2 ng/ml (Range=0-4.4) and CK-MB was 6.0 U/L (Range=0.3-32). These values were 0.8 ng/ml (Range=0-8, p=0.001) and 6.4 U/L (Range=1.4-14.5, p=0.5) for newborns with fetal acidosis. Fetuses that died in the early neonatal period had significantly higher cord blood troponin I levels (Median=0.95 ng/ml, Range=0-6.6; p=0.01) while CK-MB was not significantly different (Median=8 ng/ml; Range=0.1-10.3, p=0.4) than the healthy newborns. The neonates with a diagnosis of hypoxic-ischemic encephalopathy had significantly higher median cardiac troponin I level than the healthy neonates while CK-MB levels were similar ($Median_{CTnl}$=1.0; Range=0-8; p<0.001 and $Median_{CK-MB}$=4.7; Range=1.1-8.4; p=0.1 respectively). The mean level of cardiac troponin I and cardiac-specific creatine kinase among the fetuses with a congenital abnormality were similar when compared with the healthy neonates ($Median_{CTnl}$=0.1; Range=0.2-6; p=0.8 and $Median_{CK-MB}$=5.9; Range=1.1-14.9; p=0.8 respectively). The most sensitive predictive cut-off value with acceptable false positive rate was 0.85 ng/ml for early neonatal mortality and 0.65 ng/ml for hypoxic-ischemic encephalopathy.
Discussion: Cord blood troponin-I but not creatine kinase MB can be used to identify those fetuses with intrapartum hypoxia and forthcoming hypoxic-ischemic encephalopathy and neonatal death.

Keywords: obstetric outcome, troponin I, creatine kinase MB, cord blood, predictive value

Özet

Kötü Neonatal Sonuçları Olan Yenidoğanlarda Kordon Kanı Kardiak Troponin I ve Kreatin Kinaz MB Seviyeleri

Amaç: Kötü neonatal sonuçları olan yenidoğanlarla tamamen sağlıklı yenidoğanların kordon kanı kardiak-troponin I ve kreatin kinaz-MB seviyelerinin karşılaştırılması.
Materyal ve Metot: Tamamen sağlıklı 398 yenidoğana ait kordon kanı kardiak-troponin I ve kreatin-kinaz-MB (CK-MB) seviyeleri mikropartikül enzim immünoesey yöntemi ile ölçüldü. Bu değerler pH <7.1 ve/veya baz açığı <-12 mmol/L olarak tanımlanan fetal hipoksi (n=21), hipoksik-iskemik ensefalopati (n=12), fetal anomali (n=13) ve erken neonatal mortalite (n=8) tespit edilen fetuslara ait değerler ile karşılaştırıldı. Ortanca değerler Mann-Whitney U testi kullanılarak karşılaştırıldı. En düşük yalancı pozitiflik oranıyla birlikte en iyi sensitiviteye sahip bir eşik değer belirlemek üzere ROC analizi yapıldı.

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Sonuç: Sağlıklı yenidoğanlarda ortanca troponin I seviyesi 0.2 ng/mL (Aralık=0-4.4) ve CK-MB seviyesi 6.0 U/L (Aralık=0.3-32) olarak tespit edildi. Bu değerler fetal hipoksisi olan yenidoğanlarda ise sırasıyla 0.8 ng/ml (Aralık=0-8, p=0.001) ve 6.4 U/L (Aralık=1.4-14.5, p=0.5) idi. Erken neonatal dönemde ölen fetusların kordon kanı troponin I seviyeleri sağlıklı yenidoğanlardan belirgin olarak yüksek tespit edilirken (Ortanca=0.95 ng/ml, Aralık=0-6.6; p=0.01), CK-MB seviyeleri arasında fark izlenmedi (Ortanca=8 ng/ml; Aralık=0.1-10.3, p=0.4). Hipoksik-iskemik ensefalopati tanısı alan yenidoğanların ortanca kardiak troponin I seviyeleri sağlıklı yenidoğanlardan belirgin olarak yüksek tespit edilirken CK-MB düzeyleri benzer olarak saptandı (sırasıyla $Ortanca_{cTnI}$=1.0; Aralık=0-8; p<0.001 ve $Ortanca_{CK-MB}$=4.7; Aralık=1.1-8.4; p=0.1). Konjenital anomaliye sahip fetusların ortanca kardiak troponin I ve kardiak-spesifik kreatin kinaz seviyelerinin sağlıklı yenidoğanlarla benzer olduğu görüldü (sırasıyla $Ortanca_{cTnI}$=0.1; Aralık=0.2-6; p=0.8 ve $Ortanca_{CK-MB}$=5.9; Aralık=1.1-14.9; p=0.8) . Kabul edilebilir yalancı pozitiflik oranı ile birlikte en duyarlı kestirim değeri erken neonatal mortalite için 0.85 ng/ml ve hipoksik-iskemik ensefalopati için 0.65 ng/ml idi.
Tartışma: Kordon kanı troponin I seviyeleri intrapartum hipoksi ve gelişebilecek neonatal ölüm ve hipoksik-iskemik ensefalopatili fetusların tespit edilmesinde kullanılabilirken CK-MB kullanılamaz.

Anahtar sözcükler: obstetrik sonuç, troponin I, kreatin kinaz MB, kordon kanı, prediktif değer

Introduction

Perinatal hypoxia is a common cause of neonatal morbidity and mortality and the incidence varies between 1 and 5 percent (1). Besides, although asphyxia is associated with multiple organ injuries, especially with adverse neurological outcomes, management still focuses on supportive care. So, if the adverse effects of hypoxia on the newborn is considered, there is a need to identify infants who will be at high risk for hypoxic-ischemic encephalopathy and early neonatal death as a consequence of perinatal hypoxia. A variety of markers have been examined to identify perinatal hypoxia including electronic fetal heart monitorings, low APGAR scores, cord pH, electroencephalograms, computed tomography (CT) and magnetic resonance imaging (MR) scans and Doppler flow studies (2-6). Among these markers, a best predictor has not been determined and this situation has directed the researchers to investigate other tests.

Recently, cardiac troponin I (cTnI) has been an area of interest. Troponin is an inhibitory protein complex located on the actin flament in all striated muscles and consists of three subunits T, C, and I. cTnI is the subunit that inhibits actomyosin ATPase activity, preventing muscle contraction in the absence of Ca^{+2} (7). cTnI is released into the bloodstream after myocardial damage and for this reason cTnI has been used as a marker of myocardial injury (8). In neonates cardiac troponins T (cTnT) and I (cTnI) have been proposed to be biochemical indices of myocardial injury and respiratory distress syndrome in infants (5).

The aim of this study was to compare the levels of cord blood cardiac troponin I and creatine kinase MB (CK-MB) levels in healthy infants to infants with poor neonatal outcomes.

Materials and Methods

The study was performed between February 2004 and February 2005 at Kocaeli University, Department of Obstetrics and Gynecology. A total of 446 newborns were enrolled in the study.

Cord arterial blood samples were collected immediately at delivery for blood gas analysis, and cord venous blood samples were collected for Troponin I and cardiac-specific creatine kinase (CK-MB) assays after cord clamping but prior to placental separation. None of the specimens that were visibly icteric, lipemic or hemolyzed so, none were excluded from the study. Exclusion criteria for the study included multiple gestations and metabolic disorders (n=11). Approval from the Research and Ethics Committee of the hospital was obtained for the study and those giving written informed consent were enrolled in the study.

cTnI levels were measured using an AxSYM System analyzer using the Abbott cTnI microparticle enzyme immunoassay (Abbott Park, IL, USA). For this assay, the within-run coefficient of variation was 6.6%, and the manufacturer claims minimal cross-reactivity with cardiac troponin C (0.01%), cardiac troponin T (0.34%), and skeletal troponin I (0.04%) at a concentration of 1000 ng/ml. The lower limit of detection for cTnI was 0.1 ng/ml. According to the manufacturer's instructions, a serum cTnI level >2 ng/ml is considered to indicate myocardial injury in adults. Serum CK-MB levels were measured using the Biotrol CK Monoreactive Kit (Biotrol Diagnostic, France) and manufacturer's reagents, employing colorimetric and coupled-enzyme methods. Levels of CK-MB (Diagnostica Merck, Germany) were measured in an Opera autoanalyzer. The coefficient of variation was 3.7%.

Each infant was examined by the same neonatologist (GT). The diagnosis of hypoxic-ischemic encephalopathy was based on Sarnat and Sarnat classification as mild (stage 1), moderate (stage 2), and severe (stage 3) (9). cTnI levels of 398 compeletely healthy newborns were compared with the results of newborns who had fetal acidosis (n=21) defined as cord arterial blood pH ≤7.1 and/or had a base deficit <-12 mmol/L, were diagnosed as hypoxic-ischemic encephalopathy (n=12), had congenital anomalies (n=13), and who had died in the early neonatal period (n=8). Congenital anomalies were, omphalocele, spina bifida, Down syndrome, atrial septal defect, club foot and esophageal atresia. The neonates with more than one poor outcome were analysed in more than one group.

The statistical analysis of the data was performed using statistical software (Statistical Package for the Social Sciences, SPSS Inc, Chicago, IL, USA). As the distribution of cTnI and CK-MB was skewed according to Kolmogorov- Smirnov test of normality, all values were presented as median and range. Cord blood cTnI and CK-MB levels between the two groups were compared by using the Mann-Whitney U test. Probability value (p) <0.05 was considered to be statistically significant. Receiver operator curve (ROC) characteristics of cTnI levels were examined to identify a cut-off value in order to predict early neonatal deaths within 7 days of life. The different cut-off values of cTnI as a predictive variable were identified and sensitivity, specificity, positive predictive value, negative predictive value, false positivity and false negativity were calculated.

Results

Table 1 showes the demographic variables of the study population. The level of cardiac troponin I was Median=0.2 ng/mL (Range=0-4.4) and cardiac-specific creatine kinase was Median =6.0 U/L (R= 0.3-32) for healthy infants (n=398).

The level of cardiac troponin I ($Median_{cTnI}$=0.8 ng/ml, Range=0-8, p=0.001) was significantly higher in infants with fetal acidosis when compared with healthy infants while the level of cardiac-specific creatine kinase ($Median_{CK-MB}$=6.4 U/L, Range=1.4-14.5, p=0.5) was similar.

While the median level of cardiac troponin I in infants whom died in the early neonatal period was higher ($Median_{cTnI}$=0.95; Range=0-6.6; p=0.01) when compared with healthy infants, a significant difference in the mean levels of cardiac-specific creatine kinase was not detected ($Median_{CK-MB}$=8; Range=0.1-10.3; p=0.4) in infants whom died in the early neonatal period when compared with healthy infants.

The median level of cardiac troponin I and cardiac-specific creatine kinase among the fetuses with a congenital abnormality were similar when compared with the healthy neonates ($Median_{cTnI}$=0.1; Range=0.2-6; p=0.8 and $Median_{CK-MB}$=5.9; Range=1.1-14.9; p=0.8 respectively). On the other hand, among the neonates with a diagnosis of hypoxic-ischemic encephalopathy the median cardiac troponin I level was significantly higher than the healthy neonates while CK-MB levels were similar ($Median_{cTnI}$=1.0; Range=0-8; p<0.001 and $Median_{CK-MB}$=4.7; Range=1.1-8.4; p=0.1 respectively).

The ROC characteristics of cTnI to predict early neonatal mortality is presented in Figure 1. Area under the curve (AUC) was 0.74. The ROC characteristics of cTnI to predict hypoxic-isc-

Table 1. Demographic characteristics of the study population (n=398)

Characteristic	Result
Age, years (min-max)	28.5±5.3 (17-46)
Gestational age, weeks (min-max)	37.8±2.6 (24.2-43)
Nulliparity (%)	186 (46.9)
Body mass index, kg/m^2 (min-max)	28.4±4.4 (20.2-43.7)
Tobacco use (%)	56 (14)
Education >8 years (%)	133 (33.6)
Anemia (%)	37 (9.4)
Systemic diseases (%)	33 (8)

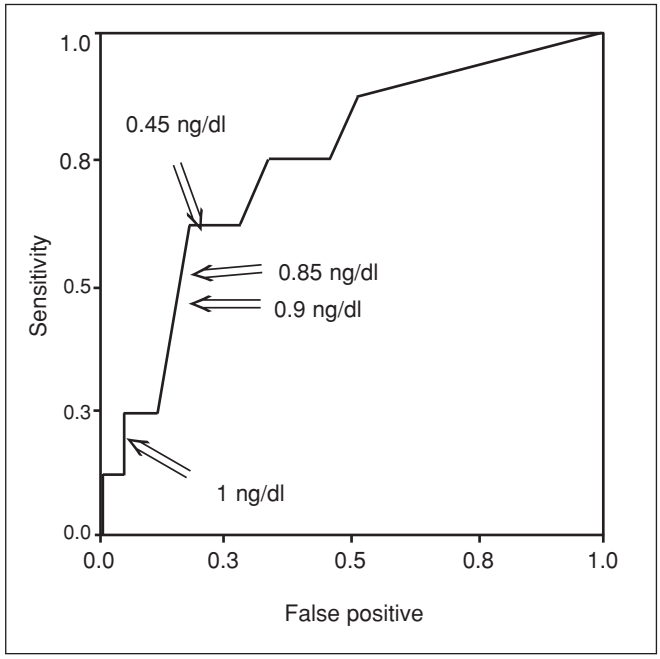

Figure 1. The value of troponin I as a screening test to predict early neonatal mortality. The sensitivity and false positive rate of different Troponin I levels are presented.

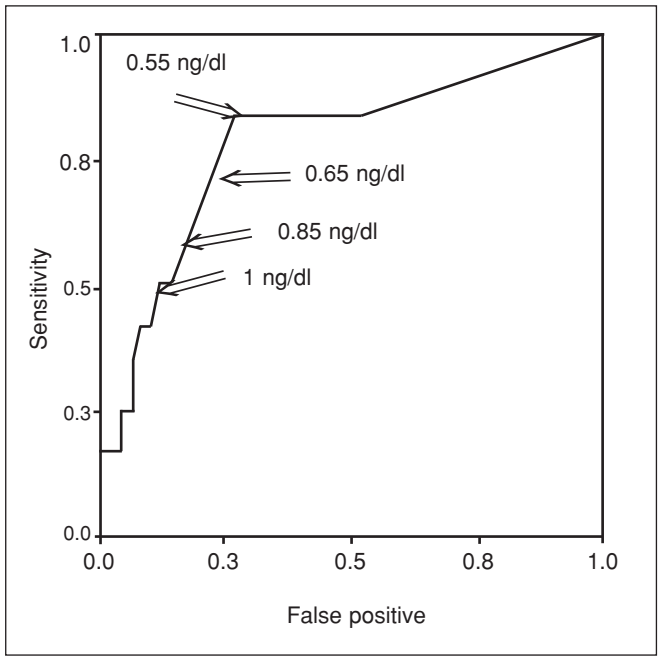

Figure 2. The value of troponin I as a screening test to predict hypoxic-ischemic encephalopathy. The sensitivity and false positive rate of different troponin I levels are presented.

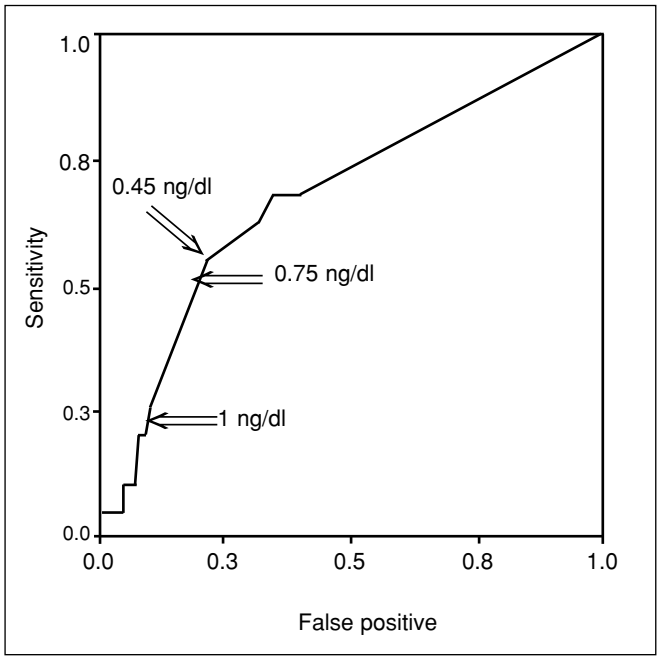

Figure 3. The value of troponin I, as a screening test to predict any adverse neonatal outcome including cord blood pH <7.1 and/or base excess <-12 mmol/L and/or hypoxic ischemic encephalopathy and/or neonatal mortality. The sensitivity and false positive rate of different troponin I levels are presented. Area under the curve is 0.67.

hemic encephalopathy is presented in Figure 2. Area under the curve (AUC) was 0.77. The value of Troponin I as a screening test to predict any adverse neonatal outcome including cord blood pH <7.1 and/or base excess <-12 mmol/L and/or hypoxic ischemic encephalopathy and/or neonatal mortality is presented in Figure 3. Area under the curve was 0.67.

The predictive values of different cTnI cut-off value for early neonatal mortality and hypoxic-ischemic encephalopathy are presented in Table 2. The most sensitive cut-off value with acceptable false positive rate was 0.85 ng/ml for early neonatal mortality and 0.65 ng/ml for hypoxic-ischemic encephalopathy.

Discussion

Cardiac troponin I was measured as an indicator of cardiac injury for a long time, but it has been in interest for the prediction of poor neonatal outcome for a few years. Do we have the chance to predict and early intervene fetuses who are at high risk of poor neonatal outcome, and if "yes", how would that be possible? The relevance of this question has been researched by Turker et al. (10). They analized levels of cord blood cardiac troponin I, cardiac-specific creatine kinase, pH and APGAR scores among the neonates with a diagnosis of hypoxic-ischemic encephalopathy. Their analysis revealed that levels of cardiac troponin I and cardiac-specific creatine kinase were significantly higher in the neonates with the diagnosis of hypoxic-ischemic encephalopathy.

When they defined fetal acidemia as an umblical artery pH of ≤7.20, some authors suggested that cTnI is the best predictor of perinatal hypoxia and they found a statistically significant negative correlation between the arterial blood gas analyses and cTnI levels (10,11). Turker et al. suggested a negative correlation between base deficit and cTnI and CK-MB in another study and this study was similar in many ways with our study (11). Our results for cTnI was significantly higher in the group with fetal acidosis. We found significantly higher levels of cTnI in the hypoxic-ischemic encephalopathy group but, we did not find a relation between CK-MB levels and the studied poor neonatal outcome parameters.

Turker et al. investigated the correlation between serum cTnI and the severity of HIE (10), and they suggested that the optimal cTnI cut-off value for the prediction of mortality among newborns with severe hypoxic-ischemic encephalopathy was 4.6 ng/ml. Although it was suggested that the main predictor of fetal outcome is cerebral damage instead of myocardial dysfunction which can be reversed with appropriate inotropic support and oxygenation, our results revealed that cTnI can be assessed as a predictor of early neonatal mortality and hypoxic-ischemic encephalopathy. Based on these findings, it is reasonable to suggest that neonatal mor-

Table 2. Different troponin I cut-off values to predict early neonatal mortality and hypoxic-ischemic encephalopathy

Troponin I cut-off value (ng/dl)	Sensitivity	Specificity	Positive predictive value	Negative predictive value	False positive	False negative
Neonatal Mortality						
cTnI ≥1	50	85.6	5.9	99	14.3	50
cTnI ≥0.9	62.5	83.5	6	99	17.3	37.5
cTnI ≥0.85	62.5	82.6	6.1	99	17.3	37.5
Hypoxic-ischemic encephalopathy						
cTnI ≥1	50	85	8.9	98	14	50
cTnI ≥0.85	58	83	8.6	98.6	17	41.6
cTnI ≥0.65	75	73	7.1	99	27	25

tality does not only result from cerebral damage, but also there might be other responsible factors like the extend of myocardial damage.

Engin et al. examined the levels of cardiac troponin I from pregnancies complicated by hypertensive disorders and from neonates who were exposed to magnesium sulfate in utero, and showed significantly elevated levels of cTnI (12). They also showed a significant relation between the levels of cTnI and neonatal outcomes. All of these reports may reflect the probable role of hypoxia on cardiac musculature.

No relation was found between CK-MB and perinatal hypoxia parameters, HIE and early neonatal deaths. Our study differed from Turker et al.'s study in that we did not demonstrate a relation between hypoxia and CK-MB similar to Moller et al. (5). When we consider that CK-MB levels increase within 48 h of life in the perinatal asphyxia group, we suggest to reevaluate the levels of CK-MB to rule out the possibility of undetectable levels in cord blood after ischemic damage.

Our study is the first to adress a cut-off value for cTnI among a non-selected group of neonates to predict poor neonatal outcomes, such as early neonatal mortality and hypoxic-ischemic encephalopathy. We found that cTnI level of 0.85 ng/dl can be used to predict neonatal mortality and 0.65 ng/dl can be used to predict hypoxic-ischemic encephalopathy. The positive predictive value of Troponin I is too low to use it as a screening test which points out important overlapping in Troponin I levels between healthy and pathological conditions. If these cut-off values will be used we should keep in mind that, this will not harm a neonate but will increase the work of neonatology unit. The high negative predictive value of the test might ensure the clinician that the neonate will be almost 99% healthy in selected cases.

Results will be rewarding as upto 75% of fetuses at risk would be diagnosed as early as the first few minutes of life with cord blood cTnI screening. Further studies may limit cord blood cTnI screening to fetuses at risk for poor neonatal outcome to increase the sensitivity and positive predictive value of this marker.

In conclusion, we believe that by determining the levels of cardiac Troponin I in cord blood immediately after birth, we may screen the neonates who are at high risk for intrauterine hypoxia and forthcoming neonatal death and make supportive interventions earlier. Cardiac troponin I appears to be a sensitive and specific predictor of obstetrical complications and for this reason, it may be used in early prediction of survival in newborns.

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