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Original Article

Serum Cardiac Troponin I Levels in Neonates with Perinatal Asphyxia; A Cross-Sectional Study

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ABSTRACT

Background: Cardiac dysfunction is part of the clinical spectrum of multiple organ dysfunction in asphyxiated newborns. Cardiac troponin I (cTnI) is a biomarker linked to neonatal hypoxic-ischemic encephalopathy (HIE) that can help diagnose perinatal asphyxia and predict the severity of myocardial dysfunction. Therefore, the aim of the present study was to examine the serum levels of cTnI in resuscitated infants suffering from perinatal asphyxia.

Methods: This cross-sectional study was performed on 84 resuscitated infants at Ayatollah Mousavi Hospital in Zanjan, Iran (December 2020-August 2021). A checklist was created that included the demographic data of the infants, Apgar scores at 1, 5, and 10 min, arterial blood gas (ABG) values, and cTnI levels at 72 h postpartum. Quantitative and qualitative variables were compared between the two groups using the independent t-test/Mann-Whitney U test and chi-square/Fisher's exact tests.

Results: Fifty-eight infants (69%) were male and 26 (31%) were female. The mean cTnI levels in infants who underwent advanced resuscitation (38.65 ± 65.63 pg/mL) were significantly higher than in infants who received early resuscitation and positive pressure ventilation (PPV) (18.60 ± 24.47 pg/mL) ($p = 0.013$). It was found that high cTnI levels were more prevalent among infants with base excesses (BEs) greater than -12 mEq/L and infants whose 5-min and 10-min Apgar scores were between 0 and 4 ($p < 0.05$). The results of quantile regression indicated that one week increase in gestational age and one unit rise in the Apgar score at 10 min, Apgar score at 5 min, pH, and BE were associated with a drop of 0.71 ($p = 0.002$), 1.70 ($p = 0.005$), 0.74 ($p = 0.005$), 2.85 ($p = 0.025$), and 0.33 ($p = 0.005$) pg/mL in cTnI levels, respectively.

Conclusion: The results of our study revealed that blood cTnI levels were significantly higher in infants who underwent advanced resuscitation, suggesting that cardiac troponin may serve as a helpful marker in assessing myocardial injury in these individuals.

Keywords: Asphyxia, Newborn resuscitation, Steps of newborn resuscitation, Troponin I

Introduction

The leading causes of infant mortality in developed countries are non-preventable factors, such as congenital anomalies and congenital heart disorders, whereas, in developing countries, preventable factors, namely prematurity, asphyxia, and infections are the most frequent causes of infant death. (1). Of the 136 million births per year around the world, about 1 million newborns die from asphyxia. The majority of these deaths occur in low- and middle-income countries and an estimated 4 to 9 million infants experience asphyxia each year. Asphyxia at birth accounts for 20–40% of all neonatal fatalities. It is predicted that out of 136 million annual births, approximately 10 million newborns (5–10%) respond to simple stimulation to initiate respiratory efforts, 3–6% (6 million) require basic resuscitation, and only less than 1% (< 1 million)

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need advanced resuscitation (2,3).

Prolonged asphyxia raises the risk of oxygen deprivation, CO_2 accumulation, and acidosis, all of which can lead to neurological symptoms in the infant and cause mental retardation and cerebral palsy in the future. Hypoxic-ischemic encephalopathy (HIE) due to asphyxia occurs in 4 million infants annually and results in the death of one million infants. It also causes permanent damage to the brain and nervous system of the fetus, cerebral palsy, and developmental delay. Additionally, if the fetus does not receive enough oxygen, the cardiac cells undergo ischemia and necrosis, and cardiac troponin I (cTnI) is released (2–5).

Studies have demonstrated that cTnI is a beneficial marker for detecting myocardial damage in neonates with respiratory distress syndrome (7). The symptoms of respiratory distress syndrome in patients are very similar to those of cardiovascular disease (6). The first worrisome finding in the initial examination of a newborn is respiratory distress, which is one of the pulmonary disorders that can be life-threatening (8). This condition, which is mainly related to the prematurity of the newborn and affects about 3-7% of live births, is considered one of the most common respiratory diseases of newborns globally (2).

The primary cause of death related to hypoxia and ischemia in infants is myocardial damage, while fatal cardiac complications can also claim the lives of survivors. Myocardial damage occurs in 28-73% of asphyxiated infants (3). Increased serum levels of cTnI in perinatal asphyxia patients have been reported to indicate increased cardiac involvement, and the level of cTnI is a strong indicator for diagnosing cardiac involvement resulting from perinatal asphyxia and respiratory distress syndrome (3). However, to the best of our knowledge, previous studies have not assessed the association between serum cTnI levels and different stages of resuscitation. Therefore, the present study aimed to investigate the relationship between the serum levels of cTnI and different stages of resuscitation in resuscitated infants.

Methods

This cross-sectional study was conducted on all the infants aged between 26 and 42 weeks who underwent cardiopulmonary resuscitation in the delivery and operating room of Ayatollah Mousavi Hospital in Zanjan, Iran (from December 2020 to August 2021). This study investigated the relationship between serum levels of cTnI in newborns resuscitated in the delivery room and resuscitation stages. The following patients who underwent cardiopulmonary resuscitation steps in the delivery room were included in the study:

1. Infants who received positive pressure ventilation (PPV) using a bag valve mask (Ambu bag) in the delivery room.
2. Infants who underwent PPV and cardiac massage in the delivery room.
3. Infants who were intubated in the delivery room.
4. Infants who underwent PPV, cardiac massage, and intubation and received epinephrine in the delivery room.
5. Infants with parental consent to participate in the study.

Exclusion criteria were evident congenital anomalies, being very premature (< 26 weeks), and a lack of parental consent. This study measured the serum level of cTnI in infants who underwent resuscitation steps. Demographic data (age, gender, and birth weight), Apgar scores (at 1, 5, and 10 min), arterial blood gas (ABG) values, including pH and base excess (BE) of blood samples at the first hour of hospitalization, and resuscitation steps, namely initial steps, PPV intubation, cardiac massage, and drug therapy, were recorded in a checklist. The number of samples was determined using Green's formula as follows. $n>8k+50$, where k denotes the number of independent variables. Based on the aim of the study, four independent variables were considered, and, as a result, a minimum sample of 80 cases was determined.

The test to evaluate serum cTnI levels was performed 72 h postpartum. It should be noted that blood samples are routinely taken from all patients in this center as required on the third to the fifth day of admission; therefore, the patient was not pressured to give additional samples. The obtained samples were centrifuged, and their serum was separated and stored at -20°C until the measurement of cTnI levels. The level of cTnI in the samples was determined using the chemiluminescent microparticle immunoassay (CMIA) and the Architect Abbott device (United States). In this study, the cTnI cut-off point was considered 14 pg/mL based on the manufacturer's guidelines. Finally, the results were analyzed,using demographic data, Apgar scores, ABG values, and resuscitation steps.

Moreover, infants were classified into four groups based on their Apgar scores (9) and the required analyses were performed. Group I

included infants with an Apgar score of 0-2 (the infant had apnea and its heart rate was less than 100 beats per min despite assisted ventilation); group II included infants with an Apgar score of 3-4 (the infant had apnea or a heart rate of less than 100 beats per min); group III included infants with an Apgar score of 5-7 (the infant was breathing on its own, its heart rate was more than 100 beats per min, but its complexion was cyanotic); group IV included infants with an Apgar score of 8-10 (the infant was breathing on its own, the heart rate was more than 100 beats per min, and its complexion was pink).

Statistical analysis

Quantitative and qualitative data are reported as mean ± standard deviation and number (percentage). The independent t-test/Mann-Whitney U test compared quantitative variables between the two groups. Moreover, the chi-square/Fisher's exact test compared qualitative variables between the two groups. In this study, the cTnI level was a dependent variable, and its distribution was non-normal and right-skewed. If the distribution of the dependent variable, as well as the distribution of errors (residuals), in linear regression, is non-normal, or the data of the dependent variable have a large variance, and if there are outlying data (too large or too small) among the dependent variable's data, instead of linear regression, quantile regression is used. Therefore, quantile regression was used to determine the relationship between cTnI levels and independent variables in this study. In addition, to compare the results of linear regression and quantile regression, the data were analyzed with linear regression as well. Data were analyzed using SPSS software version 25.0 for Windows (SPSS Inc., Chicago, IL). The significance level for all tests was considered to be 5%.

Ethical approval

The study was carried out after approval by the ethics committee of Zanjan University of Medical Sciences (IR.ZUMS.REC.1400.220).

Results

The demographic and clinical data of the studied subjects are displayed in **Table 1**. This cross-sectional study was conducted on 84 infants. In this study, 58 infants (69%) were male and 26 (31%) were female. The mean and standard deviation of the infants' weight and gestational age were 2.5 ± 0.63 kg and 35.7 ± 2.9 weeks, respectively. Regarding resuscitation, 11.9% underwent advanced resuscitation, whereas the rest (88.1%) underwent only initial resuscitation and PPV. The average 1-min, 5-min, and 10-min Apgar scores were 4.7 ± 2.2, 7.3 ± 1.9, and 8.7 ± 1.5, respectively. Moreover, the average pH, PCO_2, and BE were 7.3 ± 0.13, 38 ± 9.2, and -9.4 ± 4.0, respectively. The mean cTnI level of

Table 1. Frequency distribution of the demographic and clinical characteristics of mothers and neonates

		Number (percentage)/ mean ± SD
Gestational age (week)		35.7 ± 2.9
Weight (kg)		2.5 ± 0.63
Gender	Girl	26 (31)
	Boy	58 (69)
Delivery typ	Natural	20 (23.8)
	Cesarean section	64 (76.2)
Therapeutic measure	Oxygen hood	31 (37)
	CPAP	23 (27)
	NIV	17 (21)
	Intubation	13 (16)
Resuscitation	Advanced (PPV, cardiac massage, intubation ± epinephrine)	10 (11.9)
	Primary + PPV	74 (88.1)
Apgar score	1 min	4.7 ±2.2
	5 min	7.3 ± 1.9
	10 min	8.7 ± 1.5
pH		7.3 ± 0.13
PCO_2 (mmHg)		38.0 ± 9.2
BE (mEq/L)		- 9.4 ± 4.0
Cardiac troponin I (pg/mL)		

CPAP; Continuous positive airway pressure, NIV; Non-invasive ventilation, PPV; Positive pressure ventilation, BE; base excess.

Table 2. Indicators of cardiac troponin I measurement in patients according to the type of delivery, gender, and treatment measures.

Variables		Number	Mean (pg/mL)	Median	Standard deviation	10th percentile	90th percentile
Delivery type	Natural	20	11.3	4.6	16.0	35.5	64
	Cesarean section	64	23.3	14.9	35.5	26	43.5
Gender	Girl	26	23.3	8.0	46.5	0.1	65.3
	Boy	58	19.2	14.9	23.7	0.2	39.4
Therapeutic measures	Oxygen hood	31	11	11	10.3	0.1	28.6
	CPAP	13	19.2	15.2	18.9	0.11	54.1
	NIV	23	25.5	12	37.3	0.2	83.7
	Intubation	17	31.8	16	51.8	1.3	92.8

CPAP; Continuous positive airway pressure, NIV; Non-invasive ventilation.

newborns was 20.98 ± 32.19 pg/mL (range of 0.1–223 pg/mL) (Table 1). The indicators of mean, median, standard deviation, 10th percentile, and 90th percentile of cTnI levels according to the type of delivery, sex of the newborn, and treatment measure are presented in Table 2.

In this study, all infants had undergone initial resuscitation and PPV. Evaluating cTnI levels in infants based on advanced resuscitation showed that infants who needed advanced resuscitation had significantly higher cTnI levels than infants without advanced resuscitation (p = 0.013). In addition, the number of infants who had cTnI levels above the normal range in the advanced resuscitation group (70 infants) was significantly more than that in the group with initial resuscitation and PPV (46 infants) (p = 0.045). The mean cTnI level in the group that underwent initial resuscitation and PPV was significantly lower than the advanced resuscitation (p=0.03).

Considering the cut-off point of cTnI, a total of 41 infants (48.8%) exhibited serum cTnI levels above the normal range, and 43 (51.2%) had serum cTnI levels within the normal range. The relationship with cTnI levels was assessed among three groups: infants undergoing advanced resuscitation, those receiving initial resuscitation and PPV, and those not receiving advanced resuscitation. No significant differences were observed in elevated cTnI levels between groups. Additionally, the infants were divided into four groups based on the Apgar score. Accordingly, there was no significant difference between the four Apgar groups at 1, 5, and 10 min regardingthe number of increased cTnI cases (data not shown).

The relationship between cTnI and demographic and clinical characteristics of patients using quantile regression for the 10th and 50th percentiles is shown in Table 3. The cTnI levels increased by a minimum of 3.68 pg/mL and a maximum of 7.76 pg/mL per kg of infant weight gain (p = 0.005). The cTnI levels decreased by a minimum of 0.257 pg/mL and a maximum of 1.15 pg/mL per one-week increase in gestational age (p = 0.002). In addition, the cTnI level decreased

Table 3. The association of cardiac troponin I levels with demographic and clinical characteristics of patients using quantile regression for 10th and 50th percentiles.

Variables	10th percentile					50th percentile				
	Correlation coefficient	Standard error	P-value	95% Confidence interval		Correlation coefficient	Standard error	P-value	95% Confidence interval	
				Lower limit	Upper limit				Lower limit	Upper limit
Infant's weight	5.71	1.02	0.005	3.68	7.76	7.4	4.4	0.103	-1.5	16.3
Gestational age	-0.71	0.22	0.002	-1.15	-0.25	-0.37	0.98	0.708	-2.3	1.5
Type of delivery (natural to cesarean section)	-2.42	1.02	0.02	-4.45	-0.39	-6.5	4.4	0.146	-15.4	2.3
Gender (girl to boy)	0.49	0.96	0.61	-0.42	2.4	-5.5	4.2	0.19	-13.9	2.8
Apgar score (1 min)	0.059	0.127	0.439	-0.09	0.211	-02.9	1.51	0.842	-2.71	-3.32
Apgar score (5 min)	-0.74	0.09	0.005	-2.02	-1.39	-2.41	3.16	0.448	-8.72	3.89
Apgar score (10 min)	-1.70	0.158	0.005	-2.02	-1.39	-2.41	3.16	0.448	-8.72	3.89
pH	-2.85	1.244	0.025	-5.3	-0.37	6.86	24.81	0.783	-42.5	56.31
PCO_2	0.028	0.014	0.061	0.058	0.001	-0.22	0.295	0.314	-0.88	0.288
BE	0.339	0.0399	0.005	0.226	0.441	-0.24	0.795	0.759	-1.82	1.34
Advanced resuscitation	1.08	0.733	0.014	0.382	2.45	18.97	10.7	0.081	-2.39	40.33
Therapeutic measures: Oxygen hood	-1.60	0.663	0.018	-2.92	-0.22	-6.13	7.56	0.420	-21.2	8.93
Therapeutic measures: NIV	-1.42	0.569	0.014	-2.26	-2.56	-4.68	7.49	0.534	-19.6	10.24
Therapeutic measures: CPAP	0.998	0.639	0.126	-2.26	0.285	-1.85	9.24	0.841	-20.2	16.56
Therapeutic measures: Intubation										

CPAP; Continuous positive airway pressure, NIV; Non-invasive ventilation, BE; base excess.

Table 4. The association of cardiac troponin I levels with demographic and clinical characteristics of patients using linear regression

Variables		Correlation coefficient	Standard error	P-value	95% Confidence interval	
					Lower limit	Upper limit
Infant's weight		12.5	8.2	0.13	-3.9	28.9
Gestational age		-6.2	77.7	0.44	-21.48	9.43
Type of delivery (natural to cesarean section)		-2.54	1.82	0.17	-6.17	1.1
Gender (girl to boy)		4.9	8.3	0.55	-11.6	21.4
Apgar score (1 min)		2.26	2.22	0.312	-2.16	6.67
Apgar score (5 min)		-1.51	2.79	0.059	-7.01	4.06
Apgar score (10 min)		-5.47	4.06	0.181	-13.55	2.6
pH		0.58	36.4	0.987	-73	71.8
PCO_2		-0.44	0.44	0.317	-1.32	0.43
BE		-0.44	1.19	0.71	-2.81	1.92
Advanced resuscitation		-2.32	15.61	0.950	-29.32	-26.25
Therapeutic measures	Oxygen hood	-3.63	11.39	0.751	-26.32	19.08
	NIV	6.91	11.29	0.542	-15.58	29.4
	CPAP	3.25	13.9	0.816	-24.5	31
	Intubation	-	-	-	-	-

CPAP; Continuous positive airway pressure, NIV; Non-invasive ventilation, BE; base excess.

by 0.561 pg/mL and 0.935 pg/mL on average per one unit increase in the Apgar score at 5 min (p = 0.005). The cTnI level decreased by a minimum of 1.392 pg/mL and a maximum of 2.02 pg/mL per one unit increase in the Apgar score at 10 min (p = 0.005). The level of cTnI among infants who underwent advanced resuscitation was, on average, 1.08 pg/mL higher than infants who did not receive advanced resuscitation (p = 0.014). Table 4 indicates the relationship between cTnI levels and demographic and clinical characteristics of patients using linear regression. The level of cTnI in the infants did not show a significant association with these variables (p > 0.05 for all comparisons).

Discussion

The main findings of our study revealed significantly higher levels of cTnI in infants with advanced resuscitation compared to infants with mild/initial resuscitation. Moreover, the neonates under the ventilator had severe and prolonged respiratory distress.

In our study, advanced resuscitation was performed only for about 12% of the infants. Moreover, in the 10th percentile, there was a significant difference in cTnI levels between infants who received advanced resuscitation and those who underwent early resuscitation and PPV, and it was found that cTnI levels were higher in infants with advanced resuscitation, which was in line with previous study conducted by Tanasan et al. In our research, the number of infants with higher than normal cTnI levels was significantly higher in the advanced resuscitation group than in the group that did not receive advanced resuscitation. These results showed that cTnI can be a suitable indicator of the outcome of neonatal resuscitation steps. It could be concluded that when perinatal asphyxia is severe and/or prolonged, the initial protective dive reflex fails to protect vital organs such as the heart and brain. As a result, the myocardium suffers from hypoxic-ischemic injury, causing myocardial cellular death and the release of cTnI into the bloodstream, which leads to an increase in serum cTnI levels.

The neonatal Apgar score was another main variable whose relationship with cTnI levels was evaluated after classification. The Apgar score at 1 min was not significantly associated with the cTnI level in any of the infants receiving early or advanced resuscitation. However, in the case of 5-minute and 10-minute Apgar scores, the number of infants with abnormal cTnI levels was significantly higher in low Apgar groups. All infants whose 5-minute Apgar score was between 0 and 4 and whose 10-minute Apgar score was between 3 and 4 had higher blood cTnI levels than normal. It should be noted that this significant relationship was observed only in infants who had advanced resuscitation and not in infants receiving early resuscitation and PPV. The results of the study by Siddiq *et al*. were in agreement with this finding, which showed that low Apgar scores were associated with abnormal cTnI levels (10). In addition, in the study by Lee *et al*., it was determined that the higher the Apgar score of infants, the lower their cTnI levels (11). These findings were also consistent with those of our study.

According to the results of this study, in the 10th percentile, cTnI had a significant relationship with the infant's weight, gestational age, the type

of delivery, the Apgar score at 5 and 10 min, pH, BE, and the treatment measure. In the present study, the weight of the newborns was directly related to cTnI levels, and with an increase of 1 kg in the weight of newborns, an average of 5.71 pg/mL was added to the level of cTnI. In the study conducted by Gouda, the average weight of asphyxiated infants was significantly higher than that of control infants (12).

In the study by Lee *et al*., no significant relationship was found between the weight of infants and the severity of HIE (13). In the study by Issa *et al*., there was no difference between the HIE group and the control group in terms of birth weight (14). In this study, similar to the study by Lee *et al*., the average weight of infants was 3 kg, which was more than that in our study. Seyyed *et al*. showed that the birth weight of infants had a significant relationship with the severity of HIE, such that with an increase in the severity of HIE, the weight of infants also increased (15). In the study by Michniewicz *et al*., no relation was found between birth weight and HIE. In their study, the average weight of infants was about 1 kg more than that in our study (16). Since the association between cTnI and HIE is nearly established, it can be stated that the findings of these two studies are not in agreement with each other; however, two considerations must be taken into account: One is that the average weight of infants in our study was 2.5, whereas, in Lee's study, it was about 3 kg. In addition, in our study, the average weight of the 10th percentile was used, while the average weight of the 50th percentile had no significant association with cTnI levels.

Gestational age had an inverse relationship with cTnI, such that with each week's increase in gestational age, on average, cTnI decreased by 0.71 pg/mL. Moreover, the cTnI level in mothers who had natural delivery was, on average, 2.42 pg/mL lower than in mothers who had a cesarean delivery. In the study by Seyyed *et al*., a significant relation was observed between the type of delivery and the severity of HIE. They reported that the severity of HIE was significantly higher among infants born by cesarean section (15). In the study by Gouda *et al*., no relationship was observed between the type of delivery and infant asphyxia (12). Therefore, it may be concluded that cesarean delivery by raising cTnI levels can be an indicator of the increased risk of HIE in this group of resuscitated infants.

In our study, the Apgar score at 5 min was also directly related to cTnI. For each unit increase in the 5-minute Apgar score, the average cTnI level decreased by 0.74 pg/mL. In the study by Lee *et al*., the 5-min Apgar score showed a significant relationship with HIE, such that this score was lower in infants with higher HIE intensity (13). Additionally, in the study by Gouda *et al*., a significant difference was observed between the asphyxia group and the control group in terms of the 5-min Apgar score (12). Other investigations also reported a significant association between the Apgar score at 5 min and asphyxia of newborns, and it was indicated that the 5-min Apgar scores of non-asphyxiated infants were significantly higher than those of asphyxiated infants (17–19). Moreover, the 10-minute Apgar score had an inverse relationship with cTnI, and for an increase of one unit in the 10-minute Apgar score, cTnI decreased on average by 1.7 pg/mL. This finding, similar to the findings regarding 5-min Apgar, was consistent with the results of other studies. Lee *et al*. showed that the 10-min Apgar score was inversely associated with HIE (13). Therefore, it can be concluded that with a decrease in the Apgar score, or with an increase in the intensity of respiratory and cardiac symptoms of the newborn, as well as an increase in cardiac involvement, the level of cTnI rises.

Furthermore, pH had an inverse relationship with the cTnI level. Each unit increase in pH caused a 2.85 pg/mL decrease in cTnI levels. Gouda's study showed a significant difference between asphyxiated and non-asphyxiated infants in terms of blood pH, such that the blood pH of asphyxiated infants was significantly lower (12). In the study by Michniewicz *et al*., although the blood pH level of infants in the severe HIE group was lower than in the moderate HIE group, this difference was not significant (16). The results reported by other studies were also in line with those of our research (17,18).

A significant association was also observed between BE and cTnI. Accordingly, each unit increase in BE was associated with an average rise of 0.33 pg/mL in cTnI levels. In Michniewicz's study, the mean BE in the severe HIE group was reported to be about -19, and in the moderate group, it was about -15, and the difference between the two groups was significant (16). In Trevisanuto's study, it was found that the BE of infants in the asphyxia group was significantly lower than that of the control

group (17).

The present study had several limitations. One of them was the small sample size, which may have reduced the generalizability of our findings. In addition, this research was conducted in only one center, and for more accurate results, it is suggested to conduct multicenter studies. Since in our study for the 50th percentile, cTnI had no significant relationship with any of the variables, the results were evaluated in the 10th percentile. These statistical analyses were not carried out in any of the other studies, which might explain some discrepancies between the results of our research and those of other investigations. Almost all the previous studies investigated the association between cTnI and the severity of HIE, while one of the prominent features of our study was that we examined the relationship between different variables and cTnI.

Conclusion

The results of this study showed a significant relationship between the serum levels of cTnI in newborns and advanced resuscitation, suggesting that cardiac troponin may serve as a helpful marker in assessing myocardial injury in these individuals. However, multicenter studies with a larger sample size should be conducted for more accurate results.

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Conflicts of interest

The authors declare no competing interests.

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