Original Research Article

Lactate dehydrogenase and CK-MB as predictors of hypoxic ischaemic encephalopathy in newborns with perinatal asphyxia

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Abstract

Background: In developing countries, perinatal asphyxia is a major cause of morbidity and mortality requiring urgent attention. In such poor resource settings bed side diagnostic test having high specificity and sensitivity and also of low cost and feasibility will help in appropriate management. **Aim:** To investigate whether LDH and CK-MB enzyme levels after birth predict the development of HIE in term newborn infants with signs of perinatal asphyxia. **Material And Methods:** The study enrolled 82 newborns in Case and 82 newborns in Controls comprising of asphyxiated and non-asphyxiated neonates, respectively. A serum CK-MB value >92.6 U/L at 8 hours and LDH value >580 U/L at 72 hours was taken as the cut-off level. **Results:** The cut-off LDH value of >580 U/L had 91.67% sensitivity with a specificity of 93.48%. LDH had a positive predictive value of 91.67 % with a negative predictive value of 93.48% in the newborns studied. The cut-off CK-MB value of >92.6U/L had sensitivity of 18.06% with a specificity of 100%. CK-MB had a positive predictive value of 100% with a negative predictive value of 60.93% in newborns studied. **Conclusion:** LDH is having more diagnostic value than CK-MB in neonates with perinatal asphyxia which helps to differentiate asphyxiated from non-asphyxiated neonates. In resource poor settings these markers can be very useful to differentiate HIE newborns from non-HIE newborns.

Key Words: Newborns, perinatal asphyxia, hypoxic ischaemic encephalopathy, Lactate dehydrogenase, CK-MB

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Received Date: 11/06/2019 Revised Date: 03/07/2019 Accepted Date: 09/08/2019
DOI: https://doi.org/10.26611/10141122

Access this article online	
Quick Response Code:	Website: www.medpulse.in
	Accessed Date: 12 August 2019

INTRODUCTION

Perinatal asphyxia is a common problem with the incidence varying from 0.5-2% of live births[1-3] and 98% of these neonatal deaths take place in the developing countries. In developing countries many asphyxiated babies are brought late to hospitals. The signs of asphyxial injury overlap with other illnesses. Thus, there is a need to identify infants who will be at high risk for Hypoxic Ischemic Encephalopathy (HIE) and early neonatal death as a consequence of perinatal hypoxia. A variety of markers have been examined to identify perinatal hypoxia and its complications. Several studies have been conducted to evaluate better markers that help distinguish an asphyxiated from non-asphyxiated neonates. Myocardial dysfunction may occur in any neonate with a history of perinatal asphyxia and injured cells leak intracellular enzymes like lactate dehydrogenase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase muscle-brain fraction (CK-MB) which signals multi-organ dysfunction, so evaluation of these enzymes (LDH and CK-MB) may be used as potential predictors to grade hypoxic ischemic injury in newborns with perinatal asphyxia.[4,5]In developing countries, perinatal asphyxia is

How to cite this article: Sachin Chawla, Rupa Rajbhandari Singh, Nisha Keshary Bhatta. Lactate dehydrogenase and CK-MB as predictors of hypoxic ischaemic encephalopathy in newborns with perinatal asphyxia. *MedPulse International Journal of Pediatrics.* August 2019; 11(2): 58-64. http://medpulse.in/Pediatrics/index.php

a major cause of morbidity and mortality requiring urgent attention. In such poor resource settings bed side diagnostic test having high specificity and sensitivity and also of low cost and feasibility will help in appropriate management. This would lead to better outcome of these children. The LDH and CKMB carry high sensitivity and specificity[6,7] and results of these tests are available immediately to bedside. The substantial rise in LDH and CK-MB after resulting from organ damage following asphyxia[8,9] make these enzymes potential predictors of the severity of the hypoxic-ischaemic insult in the perinatal period. Therefore, we aimed to investigate whether these enzyme levels after birth predict the development of HIE in term newborn infants with signs of perinatal asphyxia.

MATERIAL AND METHODS

This case control study was conducted in the Department of Paediatrics and Adolescent Medicine and Department of Obstetrics and Gynaecology of a tertiary care hospital in Nepal over a period of one year. Ethical approval for the study was obtained from Institutional Ethical Review Board (IERB).

Sample size

Sample size was calculated based on expected frequency of HIE in newborns with 5min Apgar score < 6. Since Newborns with 5 minute Apgar score < 6 was found to be a risk factor for HIE in several literatures, where it was found that 30% of newborns with 5 minute Apgar score < 6 had HIE.To detect an ODD's Ratio of 3.68 with 80% power and 95% confidence interval and 1:1 case to control ratio the final sample size using EPI-INFO STATCAL software (version 6) was estimated at 75 cases and 75 controls. Adding 10 % non- response rate, the final sample size was equal to 82 cases and 82 controls.

Inclusion Criteria for Cases

- Written informed consent given by parents.
- Term newborns (37-41 weeks) and Appropriate for gestational age (AGA) with Birth asphyxia as per WHO definition- "failure to initiate and sustain breathing at birth" and based on Apgar score as an Apgar score of <7 at one minute of life. With any one of the following:
  - i. Apgar score of 6 or less at 5 minute (if known).
  - ii. Neonatal arterial blood gas pH < 7.2 at the time of admission (if done)
  - iii. Foetal distress (heart rate of less than 100 beats per minute, late decelerations, or an absence of heart rate variability)
  - iv. Thick, meconium stained amniotic fluid and respiratory depression or bradycardia.
  - v. Resuscitation for more than 1 minute with positive pressure ventilation and oxygen immediately after birth.

Exclusion Criteria for Cases

- Written informed consent not given by parents.
- Babies with major congenital malformation.
- Those born to mothers who had taken Pethidine or Magnesium sulphate within 4 hours prior to delivery.
- Preterm neonates.

Inclusion Criteria for Controls

- Written informed consent given by parents.
- As apparently healthy term (37-41 weeks) neonates, appropriate for gestational age, without any signs of birth asphyxia as evidenced by normal fetal heart rate pattern with
- Clear aminiotic liquor.
- 1 minute or 5 minute Apgar score >7
- Neonatal arterial blood pH > 7.35 at the time of admission (if done).
- Neonate without major congenital malformations.

Exclusion Criteria for Controls

- Written informed consent not given by parents.
- Sick neonates less than 37 weeks of gestation not fulfilling the inclusion criteria

METHODOLOGY

1. Written informed consent was obtained from the parents and /or guardians of all patients before the commencement of study. Detailed antenatal, natal and postnatal history was obtained to elicit evidence of perinatal asphyxia if any.
2. Subjects with symptoms of birth asphyxia were also considered if they presented with symptoms as mentioned in the inclusion criteria.
3. Detailed clinical examination of the newborn was performed to assess the gestational age (as per New Ballard score) and neonates were examined to assess activity, tone, neonatal reflexes along with other systemic evaluation. Following which detailed systemic examinations was conducted. APGAR score at 1, 5 and 10 min. was taken after birth (if inborn).
5. Relevant investigations were carried out: Cranial ultrasound, was taken into account (whenever done) and correlated with the levels of LDH and CK-MB. Other investigations were done as and when required.

Cord blood samples (2ml) from both cases and control groups was drawn for CK-MB and LDH estimation within 72 hrs. if the newborns involved in the study were in NICU/Nursery/Ward.

Method of estimation of Lactate dehydrogenase (LDH): By using Agappe diagnostics limited using semi auto-analyzer.

Method of estimation of Creatine kinase muscle brain fraction (CK-MB): CK-MB level analysis was done by immuno-inhibition method using auto- analyser (Agappe diagnostics limited).

Statistical analysis

Descriptive statistical analysis has been carried out in the present study. Student t-test has been used to find the significance of study parameters on continuous scale between two groups (Inter group analysis) on metric parameters. Chi-square/ Fisher Exact test has been used to find the significance of study parameters on categorical scale between two or more groups of cases and controls. Receiver Operator Characteristics (ROC) curves analysis has been performed to find the diagnostic performance of LDH and CK-MB in neonates with perinatal asphyxia. The Statistical software namely SPSS 21.0, EPI-INFO STATCAL software (version 6).

RESULTS

Cases and Controls comprised of newborns with perinatal asphyxia and without perinatal asphyxia, respectively, as defined in the inclusion criteria. The blood samples from 82 neonates comprising the cases and 82 neonates comprising the controls constituted the materials for the study.Among the 82 neonates in case group, there were 56 (68.3%) males and 26 (31.7%) females. Among the control group of 82 neonates, there were 50 (60.9%) males and 32 (39.1%) females. Gender distribution of neonates was statistically similar between the two groups with P value of 0.327.Among the 82 neonates in case group, 49(59.7%) neonates weighed between 2500-3000 g, 26(31.7%) neonates weighed between 3001-3499 g and 7(8.6%) neonates weighed > 3500 g. Among the control group of 82 neonates, 45(54.8%) neonates weighed between 2500-3000 g, 33(40.2%) neonates weighed between 3001-3499 g and 4(5%) neonates weighed >3500 g. The mean weight in case group was 2860 ± 329 g and in control group was 2940 ± 294 g. Birth weight distribution of neonates was statistically similar with P value of 0.101. Among the 82 neonates in case group, 56 (68.3%) neonates were delivered by spontaneous vaginal delivery and 26 (37.1%) neonates were delivered by caesarean section. Among the control group of 82 neonates, 50 (60.9%) were born through spontaneous vaginal delivery, 32(39.1%) neonates were born through caesarean section. Mode of delivery was statistically similar in both cases and controls groups with P value of 0.327. Among the 82 neonates in case group, 41(50%) neonates were having meconium stained liquor and in 41(50%) neonates the amniotic fluid was clear. All the 82(100%) neonates in control group had clear amniotic fluid as per the inclusion criteria. Out of the 82 neonates in case group, 3 (3.7%) neonates had an Apgar score of ≥7 at 5 min and 79 (96.3%) neonates had an Apgar score <7 at 5 min with resuscitation. All the 82 (100%) neonates in control group had an Apgar score >7 at both 1 and 5 min. Incidence of Apgar score <7 at 5 min was more in neonates in case group. Among the 164 neonates studied, 24(14.6%) neonates had mild HIE, 30 (18.3%) neonates had moderate HIE and 18(11%) neonates had severe HIE while 92(56.1%) neonates had No HIE. Among 82 neonates in control group none had features of HIE. Among the 164 neonates studied, 151 neonates had CK-MB levels <92.6 U/L out of which 69 (45.7%) neonates were in case group and 82 (54.3%) neonates were in control group. Remaining 13 neonates were found to have CK-MB levels >92.6 U/L. All the 13 (100%) neonates were from case group. The number of neonates having CK-MB levels of >92.6 U/L was statistically very significant in neonates from case group when compared to neonates of control group with P value <0.001.

Table 1: Comparison of cut-off levels of CK-MB cases and controls

CK-MB cut-off	Cases	Controls	Total	P value
<92.6 U/L	69 (45.7%)	82 (54.3%)	151 (100%)	
>92.6 U/L	13 (100%)	0 (0%)	13(100%)	<0.001
Total	82 (50%)	82 (50%)	164 (100%)	

Among the 164 neonates studied, 92(56.1%) neonates had LDH levels of <580 U/L out of which 11(12%) neonates were from case group and 81(88%) neonates were from control group. Remaining 72(43.9%) neonates were found to have LDH levels >580 U/L out of which 71(98.6%) neonates were in case group and 1(1.4%) neonates were in control group. The number of neonates having LDH levels of >580 U/L was statistically very significant in neonates from case group when compared to neonates of controls group with P value < 0.001.

Table 2: Comparison of cut-off levels of LDH cases and controls

LDH Cut-off	Cases	Controls	Total	P value
<580 U/L	11 (12%)	81 (88%)	92 (100 %)	
>580 U/L	71 (98.6%)	1 (1.4%)	72 (100%)	<0.001
Total	82 (50%)	82 (50%)	164	

The mean LDH was 992.32±437.25 U/L in neonates of case group and 350.17±104.89 U/L in the neonates control group. The mean value was statistically significant in neonates from case group as compared to neonates of controls group with P value <0.001. The mean CK-MB level was 71.082±50.16 U/L in neonates of case group and 44.049±13.79 U/L in the neonates of control group respectively. The mean value was statistically significant in neonates from case group as compared to neonates of control group with P value <0.001.

Table 3: Comparison of mean values of LDH and CK-MB in cases and controls

LDH	Cases	Controls	P value
Mean	992.32 ± 437.25	350.17 ± 104.89	<0.001
Median (25th–75th centile)	626.50 - 1262	299.25 – 413.75	
CK-MB	Cases	Controls	
Mean	71.082 ± 50.16	44.049 ± 13.79	<0.001
Median (25th–75th centile)	63 (34.75-85)	41 (32-55)	

Among the neonates studied, the cut-off CK-MB value of >92.6U/L had sensitivity of 18.06% with a specificity of 100%. CK-MB has a positive predictive value of 100% with a negative predictive value of 60.93%. The cut-off LDH value of >580 U/L has 91.67% sensitivity with a specificity of 93.48%. LDH has a positive predictive value of 91.67 % with a negative predictive value of 93.48% in the newborns studied. The diagnostic performance of LDH seemed to be better than CK-MB in the newborns with perinatal asphyxia.

Table 4: Sensitivity, specificity and predictive values of CK-MB and LDH

	Cut-off	Sensitivity	Specificity	PPV	NPV
CK-MB	>92.6	18.06%	100%	100%	60.93%
LDH	>580	91.67%	93.48%	91.67%	93.48%

The ROC curve of LDH and CK-MB shows that LDH is having more diagnostic value than CK-MB in neonates with perinatal asphyxia with more Area under ROC (Receiving operating Characteristic) value when compared to CK-MB (0.978vs. 0.731). The cut-off calculated according to ROC curve is 508 U/L for LDH and 75.5 U/L for CK-MB.

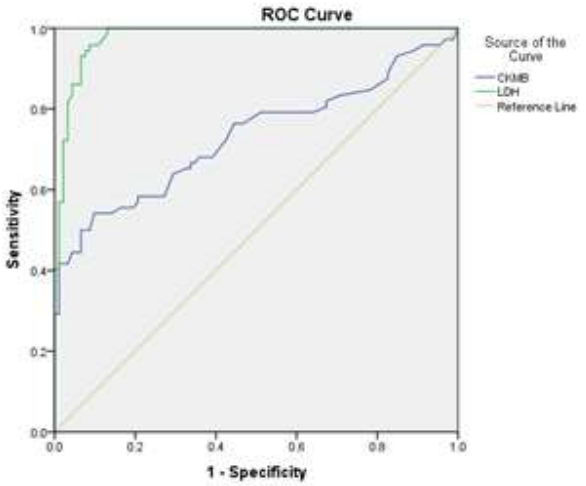

Figure 1: Comparison of ROC curves of LDH and CK-MB

DISCUSSION

Perinatal asphyxia is one of the major causes of neonatal death in developing countries and accounts for an estimated 23% of annual 4 million neonatal deaths.[4] Hypoxic ischaemic insult is seen in various organs of the body with release of several enzymes such as LDH, AST, ALT, CK-MB and Troponins. Among these, LDH is a non-specific enzyme released from various tissues – like heart, red cells, white blood cells, kidney, lung, germ cells, liver, skeletal muscles, lymph nodes, etc. and CK-MB is mainly a cardiac enzyme but it is also released in small amount from skeletal muscle injury. Significant elevation in the levels of CK-MB and LDH can serve as markers of asphyxia. In asphyxiated neonates, hypoxia is often responsible for myocardial ischemia and leads to myocardial damage. When hypoxia is very severe,

peripheral tissues develop oxygen debt which leads to lactic acidosis, due to anaerobic glycolysis. The resulting metabolic acidemia depresses the cardiovascular function resulting in ischemia. Ischemia not only results from hypoxia but also the lack of delivery of substrates resulting in hypoxic ischemic disease, which also increases the lactic acidosis.[10]

As ischemia progresses, creatine phosphate reserves are used up, adenosine triphosphate levels fall, and cardiac tissue becomes more acidic as lactate and other acidic intermediates of glycolysis accumulate.[10] Up to 15 to 20 minutes after ischaemic incident, the tissue will recover if it receives oxygen supply in time. However, after about 20 minutes of ischaemic insult, over 60% of the cellular adenosine triphosphate gets used up and the amount of lactate in wet myocardial tissue is 12 times its normal aerobic level. In addition, all cellular glycogen also gets used up. Once all the glycogen and creatine phosphate reserves have been used, dramatic structural changes occur, indicating irreversible cell damage. This also causes damage to cell membrane cytosolic enzymes (CK-MB and LDH) which are released into the blood stream.[11,12] The extent of myocardial involvement and lactic acidosis in asphyxiated newborn infants has not been studied in detail. In our study, out of the 164 neonates in both cases and controls groups 92 (56.1%) neonates did not suffer from HIE. Twenty-four (14.6%), 30(18.3%) and 18(11%) neonates suffered from mild, moderate and severe HIE respectively. The incidence is lower compared to the study of Rajakumar PS *et al*[13] in which 100% of the cases included had HIE. The incidence in the present study is slightly higher when compared to the study by Karunatilaka DH *et al*[14] in which 25.71% neonates of the case group had HIE. The differences in the incidence of HIE and involvement of other organ systems in birth asphyxia in different studies could be attributable to various factors such as major differences in the inclusion criteria for the cases, grading system used, meconium-stained amniotic fluid, the extent and severity of hypoxic-ischemic injury to brain and other organs, initiation and effectiveness of resuscitative measures at birth, level of neonatal, intensive care, post asphyxial monitoring and management of the asphyxiated newborns. Persistence of stage II for more than 7 days or stage III at any time is associated with later neurological impairment or death.[15] Other researchers have confirmed that although the overall incidence of death or sequelae is 27%, if the neurological manifestation is mild there are no deficits found later in life. When the neurologic syndrome was severe, 80% of infants died and the remaining 20% had significant sequelae.[16,17] Our study showed that amongst the 164 neonates studied, 92(56.1%) neonates had LDH levels of <580 U/L out of which 11(12%) neonates were from case group and 81(88%) neonates were from control group. Remaining 72(43.9%) neonates were found to have LDH levels >580 U/L out of which 71(98.6%) neonates were from case group and one (1.4%) neonate was from control group. The number of neonates having LDH levels of >580 U/L was statistically very significant in neonates from case group when compared to neonates of controls group with P value < 0.001. This is similar to the study by Reddy S *et al*[6] in which 100% of cases had LDH levels >580U/L. In our study the mean LDH levels in neonates from cases group (992.32 ± 437.25 U/L) were significantly higher as compared to neonates from controls group (350.17 ± 104.89 U/L) with P<0.001 which is comparable to the results of study by Reddy S *et al*.[6] In the present study, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of LDH were 91.67%, 93.48%, 91.67%, and 93.48% respectively. This is comparable to Reddy S *et al*[6] in which sensitivity, specificity, PPV and NPV were 100%, 89%, 92% and 100% respectively. In study by Rajakumar PS *et al*[13] sensitivity and specificity of LDH were 56.5% and 75.7 % respectively which is similar to our study. The area under the ROC curve for LDH is 0.978 (excellent test) in the present study which is comparable to 0.998 (excellent test) in study of Reddy S *et al*.[6] Barberi *et al*[18] reported that CK, CK-MB, CK-MB/CK ratio and LDH were all increased in the asphyxiated group, while in a group of neonates with respiratory distress; only CK-MB and the CK-MB/CK ratio were abnormal. Lackmann *et al*[19] found that newborn infants with asphyxia have significantly higher values of SGOT, LDH and hydroxybutyrate compared to neonates with only respiratory distress syndrome (RDS), and presence of RDS among asphyxiated neonates did not alter the enzyme levels. Karlsson M *et al*[7] in their clinical and experimental study done in 2008 on evaluation of organ damage in perinatal asphyxia concluded that in asphyxiated infants with differing degree of HIE and in infants where there had been signs of fetal distress during birth, a cut off level of 1049 U/L for LDH was the most suitable predictor of mild, moderate, and severe HIE with a sensitivity of 100% and specificity of 97%. This study shows that estimation of CK-MB at 8 hours of life and LDH at 72 hours of life can help distinguish an asphyxiated from a non-asphyxiated term neonate with reasonable degree of accuracy. LDH is having more diagnostic value than CK-MB with more Area under ROC curve value when compared to CK- MB (0.978 vs. 0.731) in our study. Out the 164 neonates in the present study, 151 neonates had CK-MB levels <92.6 U/L out of which 69(45.7%) neonates were from case group and 82(54.3%) neonates were from control group. Remaining 13 neonates were

found to have CK-MB levels >92.6 U/L. All the 13(100%) neonates were from case group. The number of neonates having CK-MB levels of >92.6 U/L was statistically very significant in neonates from case group when compared to neonates of control group with P value <0.001. In our study, the mean CK-MB levels in neonates from case group (71.082 ± 50.16 U/L) were significantly higher as compared to neonates from controls group (44.049 ± 13.79 U/L) with P<0.001 which is similar to the study conducted by Reddy S *et al*[6] and Rajakumar PS *et al*.[13] In our study, the sensitivity, specificity, PPV and NPV of CK-MB were 18.06%, 100%, 100% and 60.93% respectively which is comparable to the result of study 36%, 100%, 100% and 52%. The area under the ROC curve for CK-MB is 0.731 (Fair test) in the present study when compared to 0.82 (good test) found in Reddy S *et al*.[6] This difference can be attributed to peak rise of CK-MB at 8th hour of life as shown in various studies. Primhak *et al*[20] observed that the CK-MB in both normal (n=43) and asphyxiated (n=20) neonates, reached the maximum level at 8 hours and fell by 72 hours. Absolute and percentage CK-MB levels were higher in asphyxiated babies. Omokhodion SI *et al*[11] studied the creatine kinase (CK) and CK-MB activities in 23 perinatally asphyxiated newborns and 12 healthy controls during the first 100hrs of life. The asphyxiated infants had significantly elevated mean CK and absolute CK-MB. The healthy controls showed a steady decline in the activities of these enzymes from birth. Fonseca E *et al*[21] found that antepartum fetal distress is associated with release of CK-MB, and particularly CK-MB; therefore, these biochemical markers may indicate either brain or myocardial damage. Cuestas[22] observed that in healthy neonate's serum CK-BB activity declines rapidly after birth, reaching a stable value after 6hrs to 15 days, whereas neonates with severe asphyxia and neurologic damage showed a rapid increase in CK-MB concentration. In a study done by Walsh and colleagues[23] they monitored CK-MB activity in cord blood over 6hrs to 80hrs post-partum, in normal and severely asphyxiated neonates, CK-MB was 2.5 standard deviations higher than the control-group which correctly predicted subsequent neurologic abnormality in 17 (77%) of the 22 asphyxiated neonates, and in 11 (92%) of the 12 asphyxiated neonates who survived the neonatal period. Serum CK-MB in cord blood between 6 and 12 h post-partum period is an effective predictor of neurological prognosis following severe neonatal asphyxia. In their independent trials, Nagdyman Nicole *et al* in a study examined CK-MB, protein S-100, and neuron-specific enolase in cord blood at 2, 6, 12, and 24 h after birth in 29 asphyxiated and 20 control infants. A combination of serum protein S-100 and CK-MB at 2 hours after birth had the highest predictive value and specificity of predicting moderate and severe HIE in newborns with perinatal asphyxia. Cord blood pH and cord blood base deficit increased the predictive value of protein S- 100 and CK-MB.[24] In developing countries, perinatal asphyxia is a major cause of morbidity and mortality requiring urgent attention. In such poor resource settings bed side diagnostic test having high specificity and sensitivity and also of low cost and feasibility will help in appropriate management of newborns with perinatal asphyxia. This would lead to better outcome of these newborns. The substantial rise in LDH and CK-MB after resulting from organ damage following asphyxia make these enzymes potential predictors of the severity of the hypoxic- ischaemic insult in the perinatal period. Hence, these markers can be very useful to differentiate HIE newborns from non-HIE newborns and as a predictor of severity of HIE. This prediction may also be helpful to decrease morbidity and mortality due to HIE in newborns with perinatal asphyxia.

CONCLUSION

LDH is having more diagnostic value than CK-MB in neonates with perinatal asphyxia which helps to differentiate asphyxiated from non-asphyxiated neonates. In resource poor settings these markers (LDH and CK-MB) can be very useful to differentiate HIE newborns from non-HIE newborns which will help in appropriate management and better outcome of these newborns.

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Source of Support: None Declared
Conflict of Interest: None Declared