

Correlation of Homocysteine Levels with Birth asphyxia

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Abstract

Introduction: Despite major advances in monitoring technology and knowledge of fetal and neonatal pathologies, perinatal asphyxia, remains a serious condition that causes significant mortality and long-term morbidity.

Aims and Objectives: To measure the plasma homocysteine levels in neonates with asphyxia and their mothers. To determine the correlation between plasma homocysteine and occurrence of asphyxia.

Materials and Methods: Before the beginning of the study Ethics Committee permission was taken. Informed consent was taken from mothers enrolled in the study. For all the babies in the study and control groups and their mother's thorough history were taken and detailed clinical examination was done.

Observations and Results: Twenty-four asphyxiated neonates and their mothers, 26 normal neonates and their mothers were included in the study. Of the asphyxiated babies 17 (70.8%) were males and 7 (29.2%) were females. Out of 26 control group babies, 13 (50%) were males, and 13 (50%) were females.

Key words: Homocysteine, Neonate, Asphyxia, New born

INTRODUCTION

Despite major advances in monitoring technology and knowledge of fetal and neonatal pathologies, perinatal asphyxia, remains a serious condition that causes significant mortality and long-term morbidity.

The incidence of perinatal asphyxia is approximately 1-1.5% of live births (varies between 1% and 10% in different studies) and is usually related to gestational age and birth weight. It occurs in 0.5% of live born infants >36 weeks gestation and accounts for 20% of perinatal deaths (50% of stillborns are included).

A higher incidence is noted in term infants of diabetic mother (or) toxemic mothers, infants with intrauterine growth restriction, breech presentation, and postdates.

Aims and Objectives of the Study

1. To measure the plasma homocysteine levels in neonates with asphyxia and their mothers.
2. To determine the correlation between plasma homocysteine and occurrence of asphyxia.
 - a. Design: Cross-sectional study.
 - b. Setting: CKM maternity hospital.
 - c. Period of study: February 2011-September 2011.
 - d. Number of subjects:
 - 24 neonates with birth asphyxia and 26 normal neonates and their mothers, all of them being hospital deliveries were selected at random. Cases of asphyxia were diagnosed according to the following clinical criteria.
 - Need for assisted ventilation for the establishment of adequate spontaneous respiratory effort.
 - Neonates were selected irrespective of their sex.

Inclusion Criteria

Term babies with asphyxia were included in this study.

Exclusion Criteria

- Babies with dysmorphic features.

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- Babies with family h/o of inborn errors of metabolism.
- Preterm babies.
- Babies born to mothers with chronic systemic illness.
- Babies born to mothers with malnutrition.
- Babies born to mothers on antenatal steroid or drugs.

MATERIALS AND METHODS

- Before the beginning of the study, Ethics Committee permission was taken.
- Informed consent was taken from mothers enrolled in the study.
- For all the babies in the study and control groups and their mother's thorough history were taken and detailed clinical examination was done.
- Samples were collected from mothers and neonates with birth asphyxia and gestation matched controls and their mothers within 8-48 h after delivery.
- Blood samples were drawn from each one of them into vacutainers and within 30 min of collection, vacutainers were centrifuged at 1000 rpm for 10-15 min to separate serum for homocysteine levels which was stored in a deep freezer at -20°C till analysis.

Plasma Homocysteine Measurement

- Plasma samples were treated with tri-N-butyl phosphine and incubated at 40°C for 60 min before precipitation with trichloroacetic acid and centrifuged for 10 min to separate proteins. The supernatant fluid was incubated with borate and EDTA-sodium hydroxide buffer, and fluorescent 7-benzo-2-oxa-1,3-diazole-4 sulfonic acid solution for 60 min at 60°C . The solution was cooled to room temperature, filtered and injected into solution was cooled to room temperature, filtered and injected into high-performance liquid chromatography (HPLC), agilent 1100 series, and fluorescence intensities were measured with excitation at 385 nm and emissions at 515 nm. Agilent chemstation software was used for quantitation of total homocysteine (tHcy). Supelcosil TMLC-18-DB HPLC column (150 mm \times 4 mm \times 6 mm) was used for the separation, and an isocratic mobile phase of potassium dihydrogen phosphate buffer (10 mm, pH 2.1) containing 4% acetonitrile was used with a flow rate of 2.0 ml/min. After spiking the sample with the standard the recovery of tHcy was 104.5%. Plasma homocysteine levels were done by HPLC fluorescence detection at the Biochemistry Department of National Institute of Nutrition, Hyderabad.

Data Analysis

- Mean and standard deviation values are calculated for continuous variables.

- Prevalences were calculated for categorical variables.
- Mean values were compared across groups using Students *t*-test.
- Relationships among variables of homocysteine of mother and offspring were calculated using Pearson correlation coefficient level of significance was considered as 0.05.

OBSERVATIONS AND RESULTS

- 24 asphyxiated neonates and their mothers, 26 normal neonates and their mothers were included in the study.
- Of the asphyxiated babies 17 (70.8%) were males and 7 (29.2%) were females. Out of 26 control group babies, 13 (50%) were males, and 13 (50%) were females.

Categorization According to Socioeconomic Status

- Out of 24 mothers of cases, 20 (83.3%) belong to lower class and 4 (16.7%) to middle class.
- Of the 26 mothers of control group 18 (69.2%) were from the lower class and 8 (30.8%) were from the middle class (Table 2 and Figure 2).

Categorization According to Parity

- Out of 24 mothers of cases, 15 (62.5%) were Para 1, and 9 (37.5%) were Para 2 or more.
- Of the 26 control group mothers 6 (23.1) were Para 1 and 20 (76.9%) were Para 2 or more (Table 3 and Figure 3).

Mode of Delivery

- Of the mothers of case group 16 (66.6%) delivered by spontaneous vaginal delivery (SVD), 5 (20.8%) by outlet forceps, and 3 (12.5%) by lower segment Cesarean section (LSCS).
- In control group mothers 23 (88%) were delivered by SVD, 1 (4%) by OF and 2 (8%) by LSCS (Table 4 and Figure 4).

Mode of Delivery in Hypoxic Ischemic Encephalopathy (HIE) Stages

- In HIE Stage 1, SVD was more (85.6%) when compared to of (7.2%) and LSCS (7.2%).
- Of (50%) deliveries were more when compared to SVD (37.3%) and LSCS (12.7%) in HIE Stage 2.
 - In HIE Stage 3 50% deliveries were due to SVD and 50% due to LSCS (Table 5 and Figure 5).

Categorization Depending on Presence of Meconium Aspiration Syndrome

In our study, out of 24 asphyxiated cases, meconium aspiration syndrome (MAS) was found in 8 cases (33.3%) with 4 cases (50%) in Stage 1, 3 cases (37.5%) in Stage 2, and case (12.5%) in Stage 3 (Table 6 and Figure 6).

Plasma Homocysteine Concentration in Mothers

- Plasma homocysteine cutoff higher limit taken was 15 $\mu\text{mol/l}$.
- Out of 24 samples of mothers of the case group analyzed 24 (100%) were within normal range (mean - 6.4 ± 2.80).

Table 1: Categorization of babies according to sex

Cases	Sex	Controls
17	Males	13
7	Females	13
24	Total	26

Table 2: Categorization according to socio economic status

Social status	Cases	Controls
Lower	20	18
Middle	04	08
Total	24	26

Table 3: Categorization according to parity

Parity	Cases	Controls
Para 1	15	6
Para 2/>	9	20
Total	24	26

Table 4: Mode of delivery

Type	Cases	Controls
SVD	16	23
Of	5	1
LSCS	3	2
Total	24	26

SVD: Spontaneous vaginal delivery, LSCS: Lower segment cesarean section

Table 5: Mode of delivery in HIE stages

Type of delivery	HIE Stage 1	HIE Stage 2	HIE Stage 3
SVD	12	3	1
of	1	4	0
LSCS	1	1	1
Total	14	8	2

HIE: Hypoxic ischemic encephalopathy, SVD: Spontaneous vaginal delivery, LSCS: Lower segment cesarean section

Table 6: Categorization depending on presence of meconium aspiration syndrome

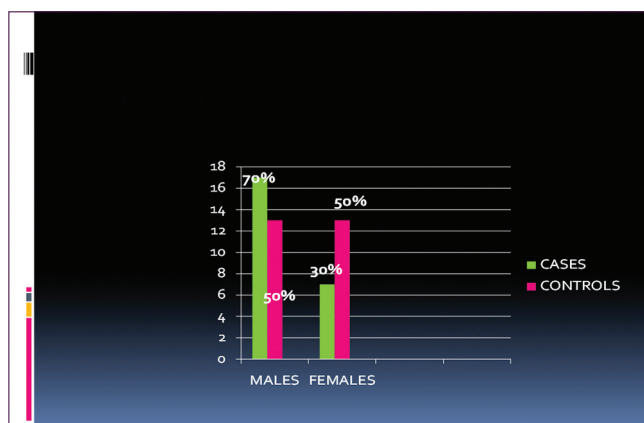
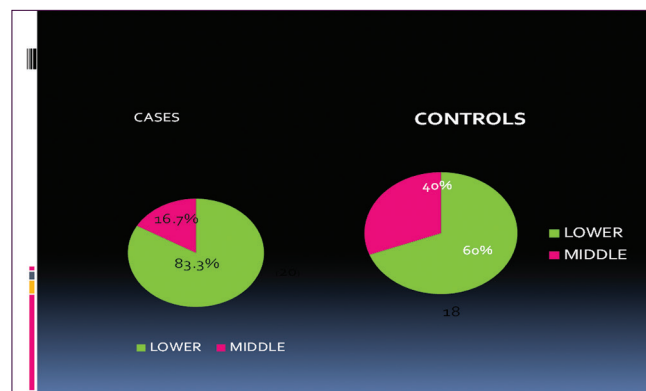
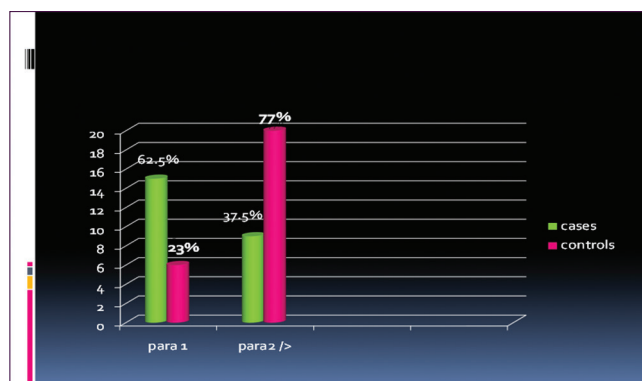
MAS	Cases	Controls
Present	8	0
Absent	16	26

MAS: Meconium aspiration syndrome

- Of the 26 samples of mothers of control group analyzed 26 (100%) were within normal range (mean - 6.1 ± 2.07) (Table 7).

Plasma Homocysteine Concentration in Babies

- Of the 24 samples of asphyxiated babies analyzed 20 (83%) were within normal range, and 4 (17%) had an excess of homocysteine (mean - 11.6 ± 8.72).
- Of the 26 samples of the control group babies analyzed 21 (81%) were with in normal range, and 5 (19%) had excess homocysteine (mean - 11.7 ± 5.37) (Table 8 and Figure 7).

**Figure 1: Categorization of babies according to sex****Figure 2: Categorization according to socioeconomic status****Figure 3: Categorization according to parity**

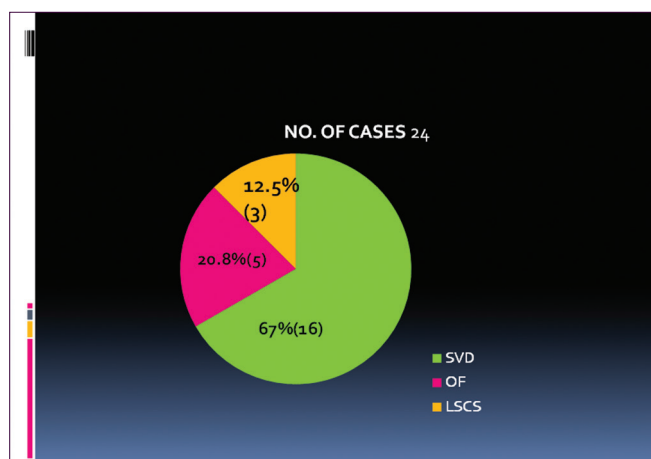


Figure 4: Categorization according to mode of delivery

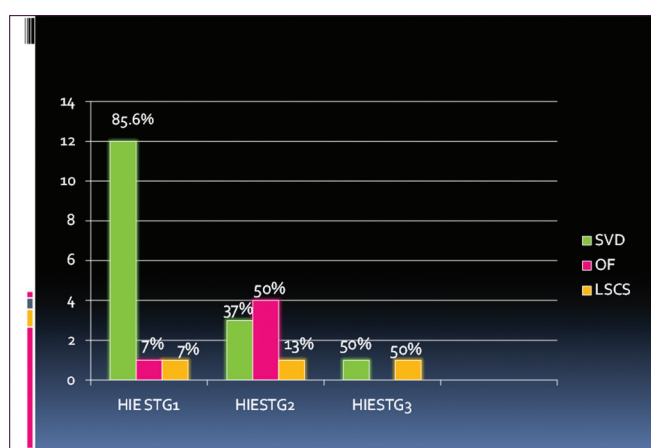


Figure 5: Mode of delivery in hypoxic ischemic encephalopathy stage

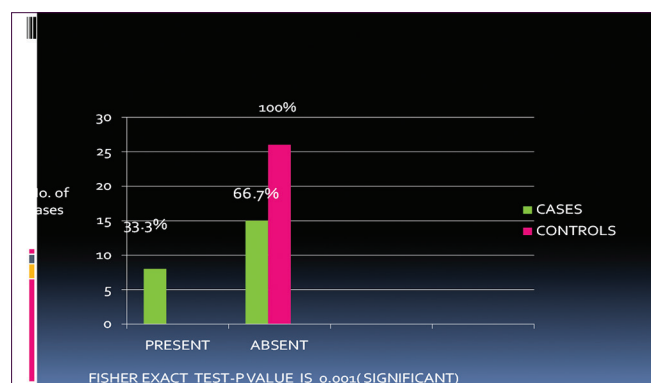


Figure 6: Categorization depending on presence of meconium stained liquor

DISCUSSION

1. Out of 24 samples of mothers of the case group analyzed 24 (100%) were within normal range (mean - 6.4 ± 2.80). Of the 26 samples of mothers of control group analyzed 26 (100%) were within normal

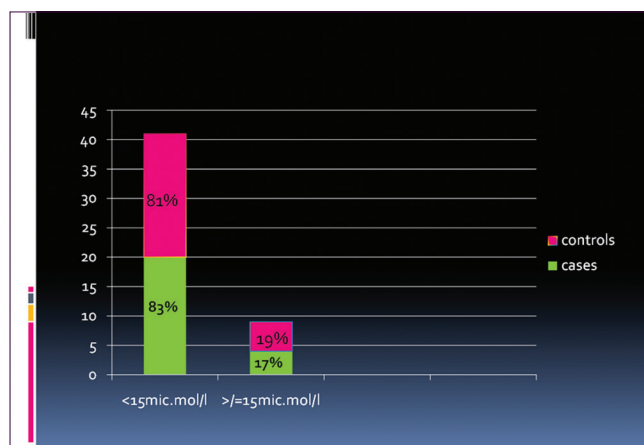


Figure 7: Plasma homocysteine levels in babies

Table 7: Plasma homocysteine concentration in mothers

Homocysteine mothers	Cases	Controls
<15 $\mu\text{mol/l}$	24	26
$\geq 15 \mu\text{mol/l}$	0	0
Total	24	26

Table 8: Plasma homocysteine concentration in babies

Homocysteine babies	Cases	Controls
<15 $\mu\text{mol/l}$	20	21
$\geq 15 \mu\text{mol/l}$	4	5
Total	24	26

Table 9: Mean plasma homocysteine concentrations in $\mu\text{mol/l}$

Homocysteine concentrations	Mean \pm SD
Neonates	
Cases (n=24)	11.61 \pm 8.72
Controls (n=26)	11.73 \pm 5.37
Mother	
Cases (n=24)	6.44 \pm 2.80
Controls	6.18 \pm 2.07

$P > 0.05$ (not significant). SD: Standard deviation

range (mean - 6.1 ± 2.07). There is concentration between mothers of case and control groups.

2. Of the 24 samples of asphyxiated babies analyzed 20 (83%) were within normal range, and 4 (17%) had an excess of homocysteine (mean - 11.6 ± 8.72) of the 26 samples of the range, and 5 (19%) had excess homocysteine (mean - 11.7 ± 5.37). There is no statistically significant difference in plasma homocysteine levels between asphyxiated and control neonates in our study.

3. In Mi *et al.* study serum homocysteine levels were significantly higher in the asphyxia group than in the control group (mean - $14.66 \pm 2.61 \mu\text{mol/l}$ vs. $7.55 \pm 0.50 \mu\text{mol/l}$).¹
4. In our study of the control group, neonates mean homocysteine was high ($11.7 \pm 5.37 \mu\text{mol/l}$) compared to Devi *et al.* study group ($6.99 \pm 0.26 \mu\text{mol/l}$).
5. The mean homocysteine in Japanese² and Italian newborn³ was found to be $2.5 \mu\text{mol/l}$ and $2.92 \mu\text{mol/l}$ (dried blood spot method). The mean homocysteine in Indian newborns is higher than Japanese and Italian newborns.
6. In our study, there were no significant differences in plasma homocysteine between either in the asphyxia group or the control group comparable to Mi *et al.*¹ study and Devi *et al.* study,⁴ gender differences were not observed in the mean plasma homocysteine concentration among newborns. Gender differences becoming prominent after puberty probably indicate the role of reproductive hormones⁵ and body mass index.⁶
7. In our study, mean plasma homocysteine concentrations were higher in neonates ($11.7 \pm 2.44 \mu\text{mol/l}$); this could be due to increased maternal estrogen and increased renal clearance.
8. Maternal plasma homocysteine concentrations were positively correlated ($r = 0.567$, $P < 0.001$) to neonatal plasma homocysteine by Pearson correlation coefficient comparable to study of Bohles *et al.* who reported a positive correlation between homocysteine concentrations in mother and newborn.
9. In our study, male babies with asphyxia were 17 in number (70%), and female babies were 7 (30%) cases with male:female of 2:1.
In Chandra *et al.*,⁷ there were 25 males (49%) and 26 females (51%) with male:female ratio of 0.96:1
In Brown *et al.* study⁸ there were 52 males (55.32%) and 42 female babies (44.68%) with male:female ratio of 1.24:1. Brown *et al.* study were comparable with this study.
10. Primiparas constituted 62.5% (15) of cases in this study. Even in Brown *et al.* study who studied 94 cases of HIE the results were comparable with this study with 62.8% cases being primiparas (59 cases).
11. PROM constituted 3 (12.5%) cases in our study. In Batra *et al.*⁹ there were 22 cases ($n = 109$) (20.2%) which was higher than our study. There were 4 cases of cord around neck (16.6%) in our study.
12. In this study, mode of delivery was SVD in 16 (67%) cases, outlet forceps in 5 (21%) cases, and cesarean in 3 (12%) cases. Chanadra *et al.* showed cesarean sections in 12 cases (25.53%) and outlet forceps in 2 cases (3.9%). Batra *et al.* showed SVD in 74 cases (67.9%)

comparable to our study.

HIE staging - Mode of delivery - comparative table. Table 5 summarize that cesarean deliveries were¹⁰ forceps deliveries were more in Stage 2 (50%) but in Stage 3 no forceps delivery in this study when compared to Deorari *et al.*¹⁰ group (8.33%).

Vaginal deliveries were comparable in Stage 2 in both studies but in stage vaginal deliveries were more (50%) compared to Deorari *et al.* study.

13. MAS was found in 8 cases (33.3%) in our study with 3 cases (37.5%) in Stage 2 and 1 case in Stage 3 (12.5%) Deorari *et al.* study showed 2 cases (6.25%) of MAS in Stage 2 which is less compared to this study and 10 cases (27.78%) in Stage 3 which is more when compared to this study.

CONCLUSION

1. We conclude that mothers of babies with birth asphyxia have not shown hyperhomocysteinemia. There is no statistical correlation between homocysteine and birth asphyxia.
2. There is no correlation between homocysteine and gender of the baby.
3. Maternal plasma homocysteine concentrations were positively correlated to neonatal plasma homocysteine.
4. In our study mean plasma homocysteine concentrations were higher in neonates compared to mothers.
5. In our study asphyxia was found to be more common in primi mothers belonging to the low socioeconomic group.
6. The mode of delivery in the majority of asphyxiated babies was SVD.
7. Asphyxia is more common in males and majority of cases had HIE Stage 1.

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