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# ASSESSMENT OF PITUITARY AND THYROID FUNCTION IN NEONATES WITH PERINATAL ASPHYXIA

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## ABSTRACT

**AIM :** The present study was designed to evaluate pituitary and thyroid functions in perinatal asphyxia and their prognostic significance.

**METHOD:** Cord blood samples from 38 cases of perinatal asphyxia (those with APGAR score <6 at 5 minutes ) and 38 healthy controls were assessed for thyroid stimulating hormone (TSH), free thyroxine (FT4), free triiodothyronine (FT3), prolactin and growth hormone (GH). Venous blood samples of perinatal asphyxia cases were reassessed for these parameters after 48-72 hrs .

**RESULTS:** Mean cord blood GH, prolactin , TSH and Free T3 levels were significantly higher in cases and no significant difference was observed in Free T4 levels. Serum Prolactin levels showed significant negative correlation with duration of assisted ventilation. Receiver operating characteristics curve showed that cord blood FT3 level can predict short-term prognosis in perinatal asphyxia at optimum cut off of 2.62pmol/L.

**CONCLUSIONS:** Perinatal asphyxia has significant impact on pituitary and thyroid function.

**KEYWORDS :** Perinatal asphyxia, pituitary function, thyroid function, assisted ventilation

## INTRODUCTION

Perinatal asphyxia is a medical condition resulting from deprivation of oxygen to a newborn during the birth process, which is long enough to cause significant functional and biochemical changes. The effects of perinatal asphyxia mimic 'diving reflex' wherein, the blood perfusion is shunted to heart, adrenal gland and brain, thereby resulting in hypoxic-ischemic insult to other organs like kidney, lungs, GIT etc.

Worldwide perinatal asphyxia is a major clinical problem [1-3] with an incidence of approximately four million per year. It is one of the leading causes of perinatal and neonatal morbidity and mortality especially in developing countries [1,2,4,5]. As per the WHO statistics, 3% of infants (3.6 million babies) suffer from moderate to severe asphyxia in developing countries resulting in nearly 1 million neonatal deaths per year [6].

In India, the incidence reported from community-based studies is 2 to 16.2% [7] and in hospital-based studies it is nearly 12.8% cases of moderate to severe asphyxia have been reported [8]. Many of the perinatal asphyxia cases tend to develop hypoxic ischemic encephalopathy (HIE), which is described as an abnormal neurobehavioral state consisting of decreased level of consciousness, brain stem and/or motor dysfunction. HIE is of foremost concern in an asphyxiated neonate because of its potential to cause serious long-term neuromotor sequelae among survivors [9].

The hypothalamo-hypophyseal axis is believed to be affected in HIE. Few of the recent studies have evaluated thyroid hormones in perinatal asphyxia cases and the results have been conflicting [10-12]. The alterations in pituitary and thyroid hormones may have a prognostic significance because of the primary role of these hormones in growth and development.

The objective of the present study was to evaluate the levels of pituitary (Growth Hormone, Prolactin and TSH) and thyroid hormones (Free T3 and Free T4) in patients with perinatal asphyxia and to correlate these levels with short term prognostic significance in this particular condition.

## METHODOLOGY

This case control study was conducted in a tertiary care hospital in New Delhi after approval by the Institute Ethics Committee. Thirty eight term neonates with evidence of perinatal asphyxia (APGAR score <6 at 5 minutes, need for assisted ventilation at birth for >3 minutes or clinical evidence of HIE) were enrolled as cases. For the control group, thirty-eight healthy term neonates (APGAR score >6 at 5 minutes, no assisted ventilation at birth, clinically well and neurologically normal) born within 24 hours of the index case were selected. The cases and controls were enrolled in the study after obtaining informed consent. Neonates with congenital malformations, intrauterine growth retardation, with history of thyroid dysfunction in mother, and chorio-amnionitis in mother were excluded from the study.

Sample of umbilical cord blood (3-4 ml) was obtained in plain tube from all cases and controls at the time of delivery, immediately after cord clamping. A second venous sample (3-4 ml) was collected from cases only at 48-72 hrs of life . There were ethical concerns for collecting samples at 48-72 hours from healthy controls and hence it was not taken. Serum was separated by centrifugation and stored at – 80°C till further analysis.

The clinical parameters like sex, gestational age, birth weight, mode of delivery, need of resuscitation, fetal heart rate status before delivery, presence or absence of meconium-stained liquor, APGAR score, presence of gestational diabetes, pregnancy-induced hypertension, were noted at the time of birth.

Classification of HIE was done using Sarnat and Sarnat scoring and HIE was classified as mild (Grade I), Moderate (Grade II) and severe (Grade III). The scoring was based on the consciousness level, tone of muscles, seizures, pupil size , duration/ respiration in neonates [13].

All the subjects (cases and controls) were followed till admission in the hospital (discharge in healthy state/ discharge with neurological complications /death). Worst HIE during the stay was recorded. Cord blood and venous blood samples were evaluated for following hormones- TSH, Free T3, Free T4 and Prolactin by Electrochemiluminiscence and growth hormone by commercially available ELISA method.

## STATISTICAL ANALYSIS

Statistical analysis was carried out on SPSS 17.0. Kolmogorov-Smirnov test was used to detect parametric nature of data. Results are presented as Mean ± S.D. Comparison of data was done by Student 't' test for parametric data and Mann- Whitney 'U' test for non-parametric data. Spearman's correlation coefficient was used for correlation analysis. ROC (receiver operating characteristics) curve was drawn to determine the optimum cut-off limit of the parameters for predicting their prognostic significance. A p value of less than 0.05 was considered as significant.

## RESULTS

### *MATERNAL CLINICAL AND DEMOGRAPHIC PROFILE*

The neonates enrolled as cases and control were comparable with respect to maternal age (25.66 ± 4.9 vs 25.4 ± 4.5 years ) , gestational age (38.5 ± 1.1 vs 38.7 ± 1.3 weeks) , distribution of parity. However, the difference in mode of delivery (Cesarean versus vaginal), presence of maternal medical illnesses (diabetes, hypertension, severe anemia, heart disease and infection) and obstetric problems (Gestational diabetes mellitus, Pregnancy-induced hypertension and oligohydramnios) between the two groups was statistically significant (p=0.01).

### *CLINICAL AND DEMOGRAPHIC PROFILE OF CASES AND CONTROLS*

Mean birth weight (2.79 ± 5.6 kg vs 2.81 ± 4.5 kg, p=0.867), head circumference (33.5 ±1.78 cm vs 34.73 ± 4.06 cm) and sex distribution (22 (M)/16 (F) vs 20 (M)/18 (F) between the controls and cases were comparable.

Overall 84.2 % (32 out of 38) cases had evidence(s) of fetal distress. The mean APGAR score of the cases at 1 minute was 2.6 ± 0.99 (1-5) which was significantly lower than healthy controls (9.0 ± 0.1). Even at 5 and 10 minutes, the APGAR score continued to be significantly low in cases [median value 4 (1-6) and 6 (3-9)] respectively. In cases with perinatal asphyxia, Twenty eight subjects developed HIE [fourteen patients presented in HIE stage 3 (36.8%) followed by stage 1 (26.3%) and stage 2 (10.5%)].

Among the cases, 26.3% (stage I ) did not show any features of encephalopathy. 63.1 % cases with perinatal asphyxia (Stage I and Stage II) were discharged from the hospital, out of which 52.6% had normal neurological function and 10.5 % had neurological abnormalities at the time of discharge. All patients presenting in HIE Stage III had an adverse outcome and could not survive.

### *PITUITARY FUNCTION TESTS*

The mean growth hormone levels of controls and cases at birth were 17.28 ± 12.93 ng/ml (range: 1.92-52.29 ng/ml) and 26.25 ± 13.74 ng/ml (range: 3.59-48.18 ng/ml) respectively and at 48-72 hours after birth it was 26.0 ± 14.3 ng/ml (range: 0.5-49.1 ng/ml) in perinatal asphyxia cases.. The growth hormone levels at birth were significantly higher in cases as compared to the controls (p=0.014). (Table 1). Elevated levels of growth hormone were seen in majority of cases (37/38) and controls (29/38). However, there was no significant change in growth hormone levels after 48-72 hours of birth. (p=0.947)

The mean prolactin levels of controls and cases at birth were 368.6 ± 137.5 ng/ml (range: 49.7- 570.9 ng/ml) and 517.7 ± 265.9 ng/ml (range: 64.7 -1257.9 ng /ml) respectively ( p=0.012). At 48-72 hours after birth, prolactin levels in cases were 406.1 ± 250.4 ng/ml (range: 18.6 -1015.7 ng /ml). The decrease in prolactin levels at 48-72 hours in perinatal asphyxia cases was not found significant. (Table 2).

### *THYROID FUNCTION TESTS*

The mean serum TSH levels of controls and cases at birth were 10.76 ± 8.65 mIU /L (range: 3.22-32.48 mIU /L) and 20.08 ± 22.94 mIU /L (range: 2.09-98.4 mIU /L) respectively (p=0.047) (Table 1). At 48-72 hours after birth, it was 8.5 ± 9.89 mIU /L (range: 0.382-41.34 mIU /L) in cases. The decrease in TSH levels at 48-72 hours after birth was statistically significant (p=0.002) (Table 2).

The mean Free $T_3$ levels of controls and cases at birth were 2.8 ± 0.59 pmol/L (range: 1.87-4.2 pmol/L) and 3.49 ± 0.9 pmol/L (range: 2.17-5.89 pmol/L) respectively (p=0.001) . At 48-72 hours after birth it was 4.3 ±1.9 pmol /L (range: 2.08-9.76 pmol /L) in cases. The increase in FT3 levels at 48-72 hours after birth was found to be significant (p=0.046).

The mean Free $T_4$ levels of controls and cases at birth were 16.6 ±2.4 pmol/L and 17.3±4.4pmol /L respectively (p=0.479) and at 48-72 hours after birth for cases, it was 19.96 ±7.2pmol /L. (Table 2).

**Table 1: Comparison Of Cord Blood Pituitary And Thyroid Function Tests Between Cases And Controls At Birth**

| | Controls (n=38) | Perinatal asphyxia Cases (n=38) | p-value |
|---|---|---|---|
| Growth Hormone (ng/ml) | 17.28 ± 12.93 | 26.25 ± 13.74 | 0.014 |
| Prolactin levels (ng/ml) | 368.6 ± 137.5 | 517.7 ± 265.9 | 0.012 |
| TSH levels (mIU/L) | 10.76 ± 8.65 | 20.08 ± 22.94 | 0.047 |
| FT3 levels (pmol/L) | 2.8 ± 0.59 | 3.49 ± 0.9 | 0.001 |
| FT4 levels (pmol/L) | 16.6 ± 2.4 | 17.3 ± 4.4 | 0.479 |

**Table 2: Comparison Of Pituitary And Thyroid Function Tests At Birth And At 48-72 Hours In Perinatal Asphyxia Cases**

| | Cord blood values at birth (n=38) | Venous blood values at 48-72 hours (n=38) | p-value |
|---|---|---|---|
| Growth Hormone (ng/ml) | 26.25 ± 13.74 | 26.0 ± 14.3 | 0.947 |
| Prolactin levels (ng/ml) | 517.7 ± 265.9 | 406.1 ± 250.4 | 0.120 |
| TSH levels (mIU/L) | 20.08 ± 22.94 | 8.5 ± 9.89 | 0.002 |
| FT3 levels (pmol/L) | 3.49 ± 0.9 | 4.3 ± 1.9 | 0.046 |
| FT4 levels (pmol/L) | 17.3 ± 4.4 | 19.96 ± 7.2 | 0.179 |

The APGAR score (at 5 minutes) in perinatal asphyxia cases did not show any significant correlation with any of the pituitary or thyroid hormones. However, the duration of assisted ventilation showed a significant negative correlation with cord blood prolactin levels (r = -0.386,p=0.045).

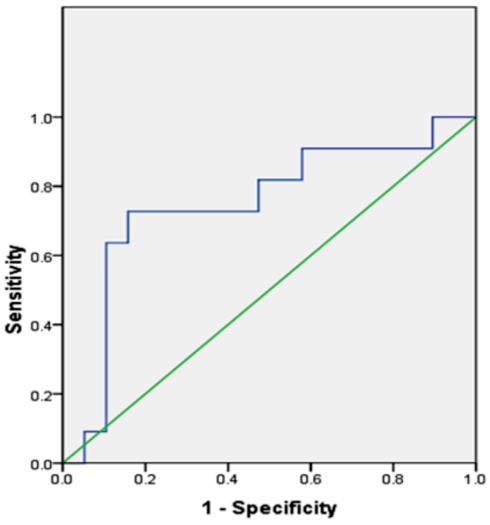
Prognostic significance of pituitary function and thyroid function test parameters in perinatal asphyxia was evaluated by ROC curve (Table 3). Only serum free T3 level from cord blood was found to be predictive of prognosis in perinatal asphyxia. At an optimum cut-off level of 2.62 pmol/l, serum free T3 level was found to have 91% sensitivity with 89% specificity in predicting death/ HIE stage 3 in this condition. It was also found that none of these parameters are suitable for discriminating stage 2 and 3 HIE from stage 0 (no encephalopathy) and stage 1(Figure 1).

**Table 3: Roc Curve Analysis Of Pituitary Function And Oxidative Stress Parameters In Predicting Death/severe Brain Damage (hie Stage 3) In Cases Of Perinatal Asphyxia.**

| Parameter | AUC | p value | Optimum cut-off value | Sensitivity | Specificity |
|---|---|---|---|---|---|
| Cord Blood FT3 | 0.743 | 0.02 | 2.62 pmol/l | 91% | 89% |
| FT3 (48-72 hrs) | 0.6 | 0.3 | ----- | ----- | ----- |
| TSH (cord blood) | 0.62 | 0.2 | ----- | ----- | ----- |
| TSH (48-72 hrs) | 0.5 | 0.9 | ----- | ----- | ----- |
| FT4 (cord blood) | 0.6 | 0.1 | ----- | ----- | ----- |
| FT4 (48-72 hrs) | 0.5 | 0.6 | ----- | ----- | ----- |

| | | | | | |
|---|---|---|---|---|---|
| Prolactin (cord blood) | 0.4 | 0.7 | ----- | ----- | ----- |
| Prolactin (48-72 hrs) | 0.3 | 0.1 | ----- | ----- | ----- |
| GH (cord blood) | 0.6 | 0.3 | ----- | ----- | ----- |
| GH (48-72 hrs) | 0.46 | 0.7 | ----- | ----- | ----- |

**Figure 1: Cord Blood Free T3 Levels In Cases Predicting Death Due To Perinatal Asphyxia**




## DISCUSSION

The present study evaluated the effect of perinatal asphyxia on endocrine parameters (pituitary and thyroid hormones) and their short term prognostic significance on. The maternal and neonatal clinico-demographic profile was comparable in both the groups except for the mode of delivery and presence of maternal medical illnesses in the study group.

Growth hormone and prolactin levels were significantly higher at birth in cord blood of perinatal asphyxia cases in comparison to that of controls. Even after 48-72 hours, the levels of growth hormone continued to be high in asphyxia cases whereas prolactin levels decreased.

Similar results for growth hormone and prolactin in perinatal asphyxia were reported by Varvarigou et al [14]. However, one of the recent studies evaluating Serum Growth Hormone and IGF-1 levels in neonates admitted with various grades of HIE did not show any significant differences in growth Hormone levels between cases and controls [15]. The metabolic acidosis resulting from perinatal asphyxia may result in prolonged half-life and diminished metabolic clearance of Growth Hormone [16]. In a recent study by Tani etal, CSF prolactin levels were found to be raised in deaths associated with ischemia/ hypoxia [17]. Usually in a term neonate, serum GH and prolactin levels fall in the immediate neonatal period because of rise in free fatty acids secondary to onset of non-shivering thermogenesis. The rise in cord blood prolactin and GH levels in perinatal asphyxia might be due to stress consequent to dysfunction of the hypothalamic opiate-like peptidergic pathways [18,19]. Interestingly in the current study, only serum GH levels remained high till 48-72 hours. The cause of this differential kinetic pattern in these two hormones is worth investigating.

Cord blood prolactin levels showed a significant negative correlation with the duration of assisted ventilation in asphyxiated subjects . Some of the recent studies provide evidence to the role of prolactin hormone in lung maturation [20,21] and association of low levels of prolactin with respiratory distress, which supports our observation in this study.

Cord blood TSH levels showed a significant difference ($p<0.05$) between the neonates with perinatal asphyxia and control group. Similar results were seen in several recent studies where low APGAR score at 1 minute and requirement for resuscitation was associated with raised cord blood TSH levels [22-24]. After 48-72 hours a significant decline was observed in serum TSH levels as compared to cord blood levels at birth. Borges etal in their study also demonstrated elevated TSH levels in asphyxiated babies 5 minutes after birth which declined progressively to baseline levels after 48 hours [25].

No significant difference was observed in cord blood free T4 levels between the asphyxiated subjects and controls. The levels of free T4 were found to be increased at 48-72 hours after birth but the difference was not found significant. The increase in free T4 may be associated with the decline in TSH levels.

Cord blood Free T3 level was significantly higher in cases as compared to controls at birth. Serum free T3 levels showed a significant increase over next 2-3 days as compared to levels at birth. These findings are in contrast to the findings of Hemasundar etal (2018) where decreased levels of free T3 , free T4 and TSH were observed to be lower than healthy subjects [11].The higher free T3 level in our cases may be explained by increased peripheral conversion of T4 to T3 by deiodinase or increased release of stored thyroid hormones from thyroid gland due to damage induced by perinatal asphyxia or increased release of FT3 from its protein bound form or decreased thyroid hormone binding proteins or a combination of these factors [26,27 ].

The current study has some limitations. Due to time constraints and limited sample size, individual stages of HIE could not be evaluated separately. For ethical issues there were no control values for serum levels of hormones at 48-72 hours of life.

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