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Original Article

Cerebral blood flow velocity in asymptomatic premature neonates exposed to clinical chorioamnionitis

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ABSTRACT

Aims: To test the hypothesis that cerebral blood flow velocity (CBFV) is altered in asymptomatic premature neonates exposed to clinical chorioamnionitis.

Methods: This prospective observational study included 30 premature (<34 weeks) neonates who were exposed to clinical chorioamnionitis but did not develop any feature of early-onset neonatal sepsis. Thirty gestational-age matched healthy neonates served as controls. Cord blood interleukin (IL)-6 concentrations were measured at birth. Resistance index (RI), pulsatility index (PI), peak systolic flow velocity and vascular diameter of internal carotid, vertebral and middle cerebral arteries were measured by transcranial color doppler ultrasonography within 48 h of delivery. The infants were followed up clinically after discharge and magnetic resonance imaging (MRI) was done at the corrected age of 6 months.

Results: Conventional sepsis screen and blood culture was negative in all. Significantly higher cord blood IL-6 concentrations, lower resistance (RI and PI) and higher blood flow with vasodilation were recorded in all cerebral arteries of the chorioamnionitis group. Significant correlation was observed between the increase in CBFV and the increase of IL-6 concentrations. At the age of 6 months, two cases showed features of delayed developmental milestone and periventricular leucomalacia in MRI.

Conclusions: Asymptomatic premature neonates exposed to clinical chorioamnionitis had significantly increased cord blood IL-6 concentrations and CBFV. The utility of increased

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CBFV as a predictor of adverse neurodevelopmental outcome may be tested in a well designed longitudinal study with larger sample size.

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1. Introduction

Prematurity and sepsis are the two important causes of neonatal mortality all over the world, including India.¹ Preterm birth is a well known risk factor for the development of a spectrum of neurodevelopmental disorders including intracranial hemorrhage, periventricular leukomalacia (PVL), cerebral palsy (CP), autism and schizophrenia.^{2–4} Chorioamnionitis is one of the most common causes of preterm delivery accounting for 30% of preterm births.⁴ Intrauterine inflammation, occurring in approximately 20% of all pregnancies is dramatically increased to approximately 85% in very preterm births.⁵

Recent studies have shown that chorioamnionitis (intrauterine infection and inflammation) is more common than intrapartum hypoxia to cause brain damage in preterm neonates.^{4,6} Infection-induced maternal immune activation leading to fetal inflammatory response (FIRS) has been implicated in fetal neurological damage.⁷ Animal experiments have also shown that despite an absent or limited maternal immune response in low grade intrauterine inflammation, the immune activation of placenta is sufficient to induce a FIRS and subsequent brain injury.⁴

Though it is well known that chorioamnionitis is the forerunner of early-onset neonatal sepsis (EOS), in the resource-limited setting of a developing country, little attention is paid to the neonates who are exposed to chorioamnionitis but do not develop signs and symptoms of EOS. Because of the burden of high patient load, most of them are discharged early and never been followed up. There is no easily available, bedside measure to identify the effects of intrauterine inflammation on developing brain. Measurement of sophisticated cytokines, which are well known parameters for confirming the diagnosis and extent of chorioamnionitis, is beyond the capacity of most of the centers of India. In a previous study, we have seen that neonates with symptomatic EOS develop increased cerebral blood flow velocity (CBFV), which can be utilized as a predictor of brain damage with reasonable accuracy.⁸ The present study was conducted to test the hypothesis that CBFV may also be altered in asymptomatic premature neonates exposed to clinical chorioamnionitis. We have measured the alteration of CBFV in this group and correlated it with cord blood interleukin (IL)-6 concentrations.

2. Methods

This prospective observational study was conducted in Sir Sunderlal Hospital, Institute of Medical Sciences, Banaras Hindu over a period of 15 months. It was approved by the Institute Ethics Committee. The study group comprised of premature (<34 weeks of gestation) inborn neonates delivered

to mothers with clinical chorioamnionitis who did not develop any feature of EOS within 72 h of birth. Gestational-age matched healthy neonates without any exposure to clinical chorioamnionitis formed the control group. Informed consents were taken from all the parents. Clinical chorioamnionitis was defined as prolonged (>18 h) rupture of membrane and intrapartum maternal fever (>37.8 °C in the period from onset of labor to delivery) with two or more of the following features: fetal tachycardia, uterine tenderness, malodorous vaginal discharge or maternal leucocytosis (>15,000 leucocytes/ μ L).⁹ Infants requiring delivery room resuscitation, perinatal asphyxia (1 and 5 min Apgar score ≤ 5), intrauterine infections (TORCH, congenital syphilis, infective hepatitis, Human Immunodeficiency Virus), other systemic or metabolic diseases and congenital malformations were excluded. Those who had any intracranial pathology/malformation since birth, developed signs of sepsis, patent ductus arteriosus or were discharged from hospital before 72 h of life were also excluded.

2.1. Sample size calculation

Since there was no similar publication in literature, the present study was conducted as a pilot trial with a convenient sample size of 60 (30 cases and 30 controls).

2.2. Clinical work-up

Detailed antenatal and natal history were recorded and thorough clinical examination was made at birth. Cord blood was collected for the measurement of IL-6 concentrations. Enrolled subjects were followed up for development of clinical signs of sepsis till 72 h of birth. Blood culture and conventional sepsis screen comprising of total leucocyte count (TLC), absolute neutrophil count (ANC), serum C-reactive protein (CRP) and micro-erythrocyte sedimentation rate (μ ESR) were sent after birth. Cut-off values for the parameters of positive sepsis screen were defined as $TLC \leq 5 \times 10^9/L$, $ANC \leq 1.8 \times 10^9/L$, $CRP \geq 10$ mg/L and micro-ESR ≥ 10 mm in 1st hour. Neonates were provided supportive management as per our unit protocol. Progress during hospital stay and outcome was noted. The infants were followed up clinically after discharge and magnetic resonance imaging (MRI) was done at the corrected age of 6 months.

2.3. Collection of samples and laboratory analysis

After complete delivery of the neonate, 10 mL of free flowing cord blood was collected for quantitative estimation of IL-6 in sterilized test tubes from the placental end of the umbilical cord without milking. Plasma was separated immediately by centrifugation and stored at -20 °C until analyzed. IL-6 concentrations were determined by Human IL-6 ELISA kit as per

Table 1 – Comparison of neonatal and maternal demographic parameters.

Parameter	Cases (n = 30)	Controls (n = 30)	p Value
Birth weight (g) (Mean ± SD)	1247 ± 211	1207 ± 164	0.416(NS) ^a
Gestational age (weeks) (Mean ± SD)	30.5 ± 1.9	30.4 ± 1.8	0.947(NS) ^a
Male:Female	16:14	18:12	0.794(NS) ^b
Maternal age (years) (Mean ± SD)	25.5 ± 3.9	24.7 ± 4.2	0.472(NS) ^a
Antenatal care taken (n)	14	17	0.605(NS) ^b
Antenatal betamethasone taken (2 doses) (n)	12	14	0.799(NS) ^b
Gravida, median	2	2	0.411(NS) ^b
Parity, median	1	1	0.875(NS) ^b
Mode of delivery			
Normal (n)	21	18	0.588(NS) ^b
Cesarean section (n)	9	12	
Presentation			
Vertex (n)	27	28	1.000(NS) ^b
Breech (n)	3	2	
Apgar score, median (Range)	8 (7–9)	8 (7–9)	1.000 (NS) ^a

NS indicates not significant.
^a Independent samples T test.
^b Chi square test.

manufacturer's instructions. The kit for research (Dialclone, GEN-PROBE incorporated 10210 Genetic Centre Drive, San Diego, CA92121, USA) was used for the estimation of plasma IL-6 concentrations.

Single-site blood culture was taken within 30 min of birth from a peripheral vein. A 10 mL blood culture bottle containing sheep brain heart infusion broth was inoculated with 1 mL of neonate's blood under full aseptic precautions. Bacterial growth and sensitivity was noted after 48 h and 7 days. Sepsis screen was sent in all neonates between 12 and 24 h after delivery from peripheral venous blood. TLC and ANC were measured in Wipro diagnostic three chamber CBC machine. Quantitative serum CRP was assessed by Nephelometry using the kit of Orion Diagnostica, Finland. Micro-ESR was done in heparinized capillary tube after keeping it vertically for 1 h.

2.4. Assessment of cerebral blood flow velocity (CBFV)

CBFV was assessed by transcranial color doppler (TCD) ultrasound within first 48 h after excluding intracranial pathology. A single observer performed all TCD examinations to avoid any inter-observer variation. Resistance index (RI), pulsatility index (PI), peak systolic velocity (PSV) and vascular diameter were measured in internal carotid (ICA) vertebral (VA) and middle cerebral arteries (MCA) of both sides by using TOSHIBA NEMIO-30 Ultrasound and color doppler machine with high frequency linear (8 MHz for ICA and VA) and curvilinear (3.75 MHz for MCA) array transducer. All measurements were done in thermo-neutral environment without any pressure provocation in quiet neonates, using oral dextrose as pacifier. RI and PI were calculated as per the formulae of ultrasound blood flow imaging technique.¹⁰

2.5. Statistical analysis

The statistical program SPSS version 16.0 (SPSS Inc, Chicago, IL) was used for data entry and analysis. Independent samples T test and Chi Square test were used to compare different variables. Pearson correlation was calculated between IL-6 concentrations CBFV parameters. A p-value of <0.05 was considered statistically significant.

Table 2 – Comparison of cord blood Interleukin-6 levels (pg/mL) and cerebral blood flow velocity of different arteries.

Parameter	Cases (n = 30)	Controls (n = 30)	Independent samples T test	
			t-value	p Value
IL-6	93.26 ± 32.26	20.80 ± 12.35	13.42	<0.001
ICA RI	0.63 ± .06	0.85 ± .13	8.326	<0.001
ICAPI	1.12 ± .15	1.56 ± .29	7.287	<0.001
ICA PSV (cm/s)	74.42 ± 8.79	30.84 ± 5.22	23.328	<0.001
ICAD (mm)	2.95 ± 0.15	2.15 ± 0.32	12.249	<0.001
VARI	0.53 ± 0.07	0.86 ± 0.09	15.008	<0.001
VAPI	1.04 ± 0.19	1.52 ± 0.15	10.749	<0.001
VA PSV (cm/s)	51.12 ± 7.15	27.67 ± 5.61	14.129	<0.001
VAD (mm)	2.67 ± 0.40	1.77 ± 0.42	8.325	<0.001
MCARI	0.48 ± 0.11	0.84 ± 0.14	10.931	<0.001
MCAPI	0.94 ± 0.22	1.41 ± 0.19	8.495	<0.001
MCA PSV (cm/s)	42.7 ± 5.35	22.37 ± 4.26	16.244	<0.001
MCAD (mm)	2.65 ± 0.38	1.77 ± 0.29	9.920	<0.001

IL-6 – Interleukin-6, ICA – Internal carotid artery, VA – Vertebral artery, MCA – Middle cerebral artery, RI – Resistance index, PI – Pulsatility index, PSV – Peak systolic velocity, D – Diameter.

3. Results

Thirty premature newborns were included in the asymptomatic chorioamnionitis group as cases and an equal number of matched, healthy neonates, not exposed to clinical chorioamnionitis served as controls. Baseline parameters of cases and controls were comparable. There was no difference between mean 1 and 5 min Apgar score and receipt of two doses of antenatal betamethasone 24 h before delivery (Table 1). No neonate received any postnatal steroid and none had any intracranial hemorrhage at the time of measurement of CBFV. Mean IL-6 concentrations were significantly higher in cases compared to controls (93.26 ± 32.26 vs. 20.80 ± 12.35 pg/mL; $p < 0.001$). In CBFV parameters, compared to controls, cases had significantly decreased RI and PI and increased PSV and vascular diameters in all three cerebral arteries (Table 2). Each CBFV parameter was measured thrice for each reading and the mean value was taken for statistical comparison. The standard error of mean ranged from 0.01 to 0.06 for RI, PI and vascular diameter and 0.5–1.6 for PSV of all arteries. Since there was no statistically significant difference between RI, PI, PSV and diameter of both sides of the same artery in any neonate, a mean value was taken for each patient. There was no difference in CBFV parameters between males and females.

Pearson correlation showed highly significant correlation between IL-6 concentrations and CBFV parameters (Table 3). Only two neonates in the chorioamnionitis group and one in the control group had a CRP >10 mg/L. None of the neonates in either group was positive for any other parameter. Blood culture was negative in all. None of them received any antibiotics.

At the corrected age of 6 months, two infants in the chorioamnionitis group were found to have delayed developmental milestones. On examination, both of them had hypertonia and features of PVL in MRI. CBFV of these two cases was significantly higher compared to the cases with normal outcome (data not shown).

4. Discussion

In the present study, the neonates of the case group had significantly higher mean cord blood IL-6 concentration, suggesting the development of intrauterine inflammation even if they remained asymptomatic after exposure to chorioamnionitis. CBFV was increased and significantly correlated with the rise in IL-6 concentrations. Two of our study neonates developed delayed developmental milestones and PVL in MRI. Both of them had higher CBFV compared to others with normal outcome, but, in a small sample size of 30 neonates it is difficult to assess the statistical validity of CBFV as a predictor of adverse neurological outcome.

There is a paucity of literature demonstrating the effect of sepsis on CBFV though early cerebrovascular alteration has been reported.¹¹ Hyperdynamic state and cerebral microcirculatory alterations has been documented in experimental animal models of sepsis.¹² The exact nature of cellular and molecular mechanisms of sepsis-induced encephalopathy is still elusive. The impairment of cerebral autoregulation, damage to blood-brain barrier (BBB), and the direct inflammatory effects of free radicals, oxidative stress, and cytokines on glial cells have been speculated to be the contributory factors.^{13,14}

Production of proinflammatory cytokines and activation of complement are the potential candidates of neuroinflammation causing septic encephalopathy. During infection, elevated levels of proinflammatory cytokines have been detected in amniotic fluid,^{5,15} fetal serum,^{16,17} and fetal brains.^{18,19} The exact mechanism by which these cytokines access to the cerebral compartment to induce injury is still debatable. Transfer of cytokines across BBB,^{15,20} and damage of BBB causing increased permeability²¹ could be the responsible factors. It has also been shown that astrocytes and microglia are capable of producing proinflammatory cytokines during inflammation.²² Once in the cerebral compartment, proinflammatory cytokines may induce excitotoxicity by modifying the re-uptake of glutamate,²³ stimulating the

Table 3 – Pearson correlation between interleukin-6 concentrations (pg/mL) and cerebral blood flow velocity parameters.

	ICARI	ICAPI	ICAPSV	ICAD	MCARI	MCAPI	MCAPSV	MCAD	VARI	VAPI	VAPSV	VAD
IL-6	.597 ^a	.519 ^a	.800 ^a	.757 ^a	.706 ^a	-.649 ^a	.757 ^a	.668 ^a	.767 ^a	.692 ^a	.712 ^a	.674 ^a
ICARI	1	.519 ^a	.660 ^a	.550 ^a	.607 ^a	.440 ^a	.661 ^a	.498 ^a	.740 ^a	.561 ^a	.632 ^a	.609 ^a
ICAPI		1	.689 ^a	.617 ^a	.637 ^a	.529 ^a	.575 ^a	.478 ^a	.677 ^a	.594 ^a	.569 ^a	.504 ^a
ICAPSV			1	.869 ^a	.769 ^a	.674 ^a	.848 ^a	.670 ^a	.834 ^a	.740 ^a	.860 ^a	.753 ^a
ICAD				1	.740 ^a	-.561 ^a	.748 ^a	.644 ^a	.767 ^a	.695 ^a	.809 ^a	.715 ^a
MCARI					1	.647 ^a	.739 ^a	.652 ^a	.805 ^a	.700 ^a	.739 ^a	.647 ^a
MCAPI						1	.688 ^a	.568 ^a	.722 ^a	.699 ^a	.588 ^a	.632 ^a
MCAPSV							1	.760 ^a	.784 ^a	.685 ^a	.797 ^a	.717 ^a
MCAD								1	.580 ^a	.580 ^a	.628 ^a	.599 ^a
VARI									1	.730 ^a	.761 ^a	.736 ^a
VAPI										1	.697 ^a	.630 ^a
VAPSV											1	.720 ^a

IL-6 – Interleukin-6, ICA – Internal carotid artery, VA – Vertebral artery, MCA – Middle cerebral artery, RI – Resistance index, PI – Pulsatility index, PSV – Peak systolic velocity, D – Diameter.

^a Correlation is significant at the 0.01 level (2-tailed).

release of free radicals^{24,25} or induce the local production of nitric oxide, a potent vasodilator.²⁶ Moreover, premature neonates have immature germinal matrix with poor glial support. Cerebral vasodilatation and increased CBFV may make them more prone to intracranial hemorrhage with subsequent neurological complications.

We have chosen ICA, VA and MCA for the assessment of CBFV as these arteries are easily visualized in a tiny newborn. There are a couple of limitations in the present study. Firstly, we did not document the presence of chorioamnionitis histologically. Secondly, we have assessed CBFV only once, serial measurement would have been better to document the changes over time.

To conclude, premature neonates born to mothers with chorioamnionitis may be the potential candidates for future developmental delay even if they remain clinically asymptomatic at birth. Raised cord blood IL-6 concentrations indicate that they have been exposed to intra-uterine inflammation with all its potential consequences. Increased CBFV may be the result of this inflammation which made them vulnerable to neuronal damage. In daily practice, this group of neonates is easily missed as routine investigations are rarely positive in them. In absence of the facility for measuring sophisticated cytokines, the utility of increased CBFV as an inexpensive, non-invasive, easily available bedside tool to predict an adverse neurodevelopmental outcome may be tested in a well designed longitudinal study with larger sample size.

Conflicts of interest

All authors have none to declare.

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