RESEARCH PAPER

# Postnatal Maturation of Amplitude Integrated Electroencephalography (aEEG) in Preterm Small for Gestational Age Neonates

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**Objective:** The primary objective was to evaluate the postnatal maturation pattern on aEEG during first two weeks of life in clinically stable and neurologically normal preterm small for gestational age (PSGA) and gestation matched (1 week) preterm appropriate for gestational age (PAGA) neonates born between $30^{0/7}$ and $34^{6/7}$ weeks of gestation. **Methods:** Serial aEEG tracings were recorded on 3rd, 7th and 14th day of life. The primary outcome was total aEEG maturation score. Three blinded assessors assigned the scores. **Results:** We analyzed a total of 117 aEEG recordings in 40 (19 PSGA and 21 PAGA) neonates. The baseline characteristics were comparable except for birthweight [1186 (263) *vs* 1666 (230) g]. There was no difference in the mean (SD) total scores on day 3 (9.0 (1.8) *vs*. 9.5 (1.1), *P*=0.32) and day 14 of life, but was lower in PSGA infants on day 7 (8.6 (2.4) *vs*. 10.1 (1.1), *P*=0.02). On multivariate analysis, maturation of PSGA neonates was found to be significantly delayed at any point of life from day 3 to day 14 (mean difference, -0.8, 95 % CI: -1.6 to -0.02, *P*=0.04). **Conclusion:** Lower aEEG maturation score on day 7 possibly indicates delayed maturation in PSGA neonates in the first week of life.

**Keywords:** *Electrophysiology, Neuronal maturation, Outcome.*

Preterm neonates are at a risk of developmental delay, visual and hearing problems, behavioral problems and learning disabilities [1]. Nearly 20% of the preterm neonates are growth retarded thus further increasing their jeopardy [2]. Preterm small for gestational age (PSGA) neonates have decreased cortical volume, myelin reduction, neuronal degeneration in the hippocampus and axonal degeneration in periventricular region [2,3]. The brain electrographic pattern and activity assessed by electroencephalogram (EEG) is an indicator of neuronal activity and differentiation [4].

Amplitude integrated electroencephalogram (aEEG) is used as a bedside device for continuously assessing the cerebral electrical activity and its practical and prognostic use has been demonstrated in many studies in term and preterm infants [5-11]. We hypothesized that postnatal maturation of aEEG would be delayed in PSGA as compared to their AGA counterparts. To date, only three studies from developed countries have assessed the aEEG maturation specifically in PSGA neonates [12-14]. The evidence is thus limited, with aspect to the aEEG maturation pattern in stable preterm neonates which may be important to decipher, given the alteration of aEEG patterns by different morbidities and intracranial insults [15]. Hence, we designed this study to evaluate the aEEG maturation pattern in preterm small for gestational age neonates and compare their maturation with appropriate for gestational age neonates, by using a validated aEEG maturation scoring.

## METHODS

This prospective observational study was conducted in the neonatal intensive unit of our institution from June 2010 to December 2011. Written informed consent was obtained from one of the parents, and the study was approved by the Institutional ethics committee of All India Institute of Medical sciences, New Delhi, India. Clinically stable preterm neonates ($30^{+0/7}$ to $34^{+6/7}$ weeks) were included. Neonates with perinatal insult (Apgar score 2 or less at 5 minutes), major congenital anomalies, any grade of intraventricular hemorrhage (IVH), periventricular leucomalacia (PVL), necrotizing enterocolitis, evidence of central nervous system infection, clinical seizures or suspected metabolic disorders were excluded.

A log book was maintained to keep track of all the neonates. Best gestational age (GA) was assigned based

on the last menstrual period, or the modified Expanded New Ballard score, if the mother was not sure about the dates or if the discrepancy in assessment and dates was more than two weeks [22]. SGA was defined as less than $10^{th}$ centile for gestation according to the AIIMS intrauterine charts [23].

Cranial ultrasound (CUS) was performed on all subjects by the radiologist, as per unit protocol at postnatal age 3, 7 and 14 day of life to rule out intraventricular hemorrhage (IVH), periventricular leucomlacia (PVL) or any cranial malformation.

The aEEG tracings were recorded using the amplitude integrated EEG machine (Nicolet, Viasys, San Diego CA, United States), as per standard methodology [24]. Each recording was done for at least 4-hour duration to ensure good sleep-wake cycling recording [25]. Continuous impedance check was done throughout the recording. The tracings with impedance >20 kOhm were discarded.

The primary outcome variable was total aEEG maturation score as per Burdjalov, *et al*. [7]. Each tracing of the subject was analyzed by three independent assessors. The methods adopted for scoring the aEEG traces are summarized in ***Box* I**. The secondary outcome variables were the scores of the four individual parameters of maturation.

According to the study done by Burdjalov, *et al.* [7] the maturation score in neonates with 30-34 weeks of gestation on day 3 of life was 10. A study on conventional EEG in term SGA neonates versus the term AGA neonates followed till three months of age revealed 50% less amplitude in SGA cohort as compared to AGA cohort because there was no study on mean total score (summation of all four components of the aEEG tracing) evaluation in preterm infants prior to the initiation of this study we assumed the difference in mean total score to be the same as the score in amplitude alone in conventional EEG study. To observe the difference in mean total score of at least 50% among the two cohorts, with alpha error 5% and power of study 80 %, we needed to enroll 20 neonates in each group.

*Statistical analysis*: The data were analyzed using Stata version 11 (Stata Corp, College Station, TX). Normally distributed, continuous data was analyzed by Student *t* test and data which was non-normally distributed was analyzed by Wilcoxon rank sum test. Chi-square or Fisher exact test were used to analyze categorical data. Generalized estimating equation (GEE) analysis was used to analyze the change in score from first aEEG recording to the final one after adjusting for correlated values. P value of <0.05 was considered as significant.

## RESULTS

Of the total 159 neonates assessed for eligibility, 119 neonates were excluded (***Fig.* 1**). The maternal characteristics and baseline neonatal characteristics in PSGA (*n*=19) and PAGA (*n*=21) cohorts were similar in the two groups (***Table* I**). No neonate in either group developed retinopathy of prematurity (ROP) requiring treatment, or patent ductus arteriosus (PDA) needing any intervention. There was no death in either cohort during the study period.

There was no difference in the total scores of the two cohorts on day 3 of life and day 14 (***Table* II**). However, PSGA neonates had lower total score on day 7. On multivariate analysis, maturation score of PSGA neonates was found to be significantly delayed at any point of life from day 3 till day 14 of life (mean difference, -0.8, 95 % CI:

**Box I** METHODS ADOPTED FOR ASSIGNING THE AEEG SCORE

*Step 1: Training of assessors*

Three independent assessors* were trained for analyzing the recorded aEEG's. Each assessor was provided with study material and video CD of aEEG. After the training sessions, assessors were provided with sample aEEG records, of various patients with varying gestational age and relevant clinical scenarios.

*Step 2: Analyzing the aEEG recordings*

The assessors were blinded to gestation, birth weight, and postnatal age at which aEEG was recorded. They analyzed the aEEG tracings individually and assigned the scores to each component of aEEG trace. They submitted their scores to the principal investigator (KA).

*Step 3: Assigning the final scores*

The principal investigator assigned the final scores based on the concordance between the three outcome assessors – if the scores given by all three assessors were the same, that score was recorded as the final score. However, if it was different, the three assessors reviewed the aEEG tracing together to reach a consensus score. This score was then recorded as the final score. Concordance among assessors was >95%.

**The outcome assessment team (three members) included neonatology fellows/faculty level personnel with experience and competency in neonatology.*

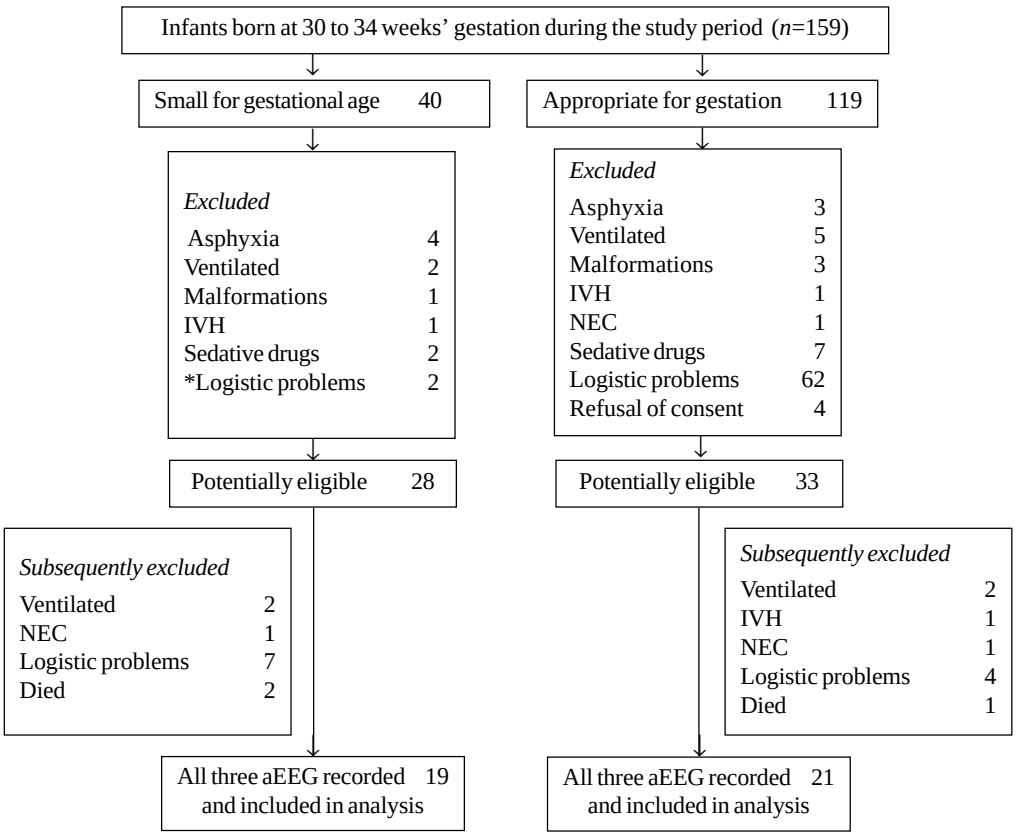


**Fig. 1** *Flow of Patients in the study.*

-1.6 to -0.017, *P*=0.045).

There was no difference in the Continuity scores of the two cohorts at any time point of assessment (***Table* III**). The Cycling score and Lower border score were similar in the two groups on day 3 and day 14. PSGA neonates had lower SWC score and 'Lower border score' on day 7. The bandwidth score in PSGA neonates was lower than the PAGA cohort on day 14 (3.3 (0.6 *vs*.3.7 (0.4, 95% CI 0 to 0.7).

## DISCUSSION

This study evaluates the maturation pattern of aEEG in clinically stable PSGA neonates neonates during the first two weeks of life. This study evaluated both prematurity and the SGA status on maturation pattern using aEEG. PSGA neonates had similar total scores as PAGA neonates on day 3 of life and day 14 and a lower total score on day 7. The maturation score of PSGA neonates in the present study was found to be significantly delayed at any point of life from day 3 till day 14 of life.

The plausible explanation of the delayed maturation score at any time point for PSGA neonates seems to be related to the underlying reasons for growth retardation. The phenomenon for delay at day 7 but not at day 3 or day 14 has not been understood very clearly. The rationale behind this may be the delay in starting the enteral feeds, which may show delay at day 7. Till date no studies have looked at the long term outcome of this transient maturity. Malnutrition during the critical phase of brain development may translate into delayed myelination and subsequently to delayed maturation pattern [3]. A recent study reported similar aEEG total scores in PAGA and PSGA neonates [12]. This study, however, did not specifically comment on the maturation delay at any time point but revealed a higher number of bursts per hour (objective quantitative parameter which indicates slower postnatal adaptation of electrocortical function in PSGA neonates); thus indirectly indicating a delay in electrocortical activity.

Nearly all PSGA neonates in the present study achieved continuous pattern on aEEG at enrollment. These results are similar to previous studies [12,14]. Continuity is one of the earliest parameters to be achieved in aEEG recording [7,8]. It represents the general status of the brain [26]. Our neonates were stable and had no

clinical evidence of neurological dysfunction, so the attainment of a continuous pattern on aEEG is not unexpected at 30-34 weeks.

Our study suggested a comparable cycling pattern in PSGA *vs.* PAGA neonates except on day 7 (second recording). The study by Feler, *et al.* [13] suggested a comparable cycling pattern in the two groups while the study by Schwindt, *et al.* [14] suggested less cycling activity in the SGA neonates. It may be difficult to compare our results with the findings of the latter two studies as both these studies evaluated the SWC pattern at a single time point in the first two weeks which may not be representative of the changes which occur with increasing postnatal age. SWC represents the integration of functions of the higher centers of the brain and a premature sleep wake cycling in PSGA neonates may represent a delay in synaptogenesis of the neurons [22,26]. The study by Feler, *et al.* [13] also reported a higher aEEG bandwidth in the PSGA neonates. The bandwidth decreases and the lower border elevates with increasing maturity [7,13].

The strengths of our study include the evaluation of clinically stable preterm neonates without any major morbidities or requiring sedation [13,23,25]. In addition, low impedance during the recording was ensured by adequate electrode contact, continuous check on impedance and uninterrupted study periods [26]. The duration of each tracing was atleast 4 hours. This helped increase the chance of detecting even immature sleep-

**TABLE I** BASELINE VARIABLES OF THE STUDY POPULATION

| *Variable* | *SGA (n=19)* | *AGA (n=21)* |
|---|---|---|
| *Maternal characteristics* | | |
| Age (y)* | 27.8 (3.3) | 26.8 (3.9) |
| Multigravida[#] | 10 (52) | 16 (76) |
| Booked[#] | 16 (84) | 21 (100) |
| Gestational hypertension[#] | 4 (21) | 2 (9.5) |
| Gestational diabetes | 3 (15.7) | 3 (14.2) |
| PPROM[#] | 1 (5.2) | 2 (9.5) |
| *Neonatal characteristics: At birth* | | |
| Gestation (wk)[$] | 32 (30-34) | 33 (30-34) |
| Weight (g)* | 1186 (263) | 1666 (230) |
| Male[#] | 8 (32) | 15 (60) |
| *Mode of delivery[#]* | | |
| Vaginal delivery | 7 (37) | 12 (57) |
| Twins[#] | 7 (36.8) | 9 (43) |
| APGAR at 5 min[$] | 8 (7-9) | 8 (6-9) |
| Need for oxygen[#] | 2 (10.5) | 3 (14.2) |
| BMV[#] | 2 (10.5) | 0 |
| *Postnatal* | | |
| Sepsis[#] | 6 (31) | 4 (19) |
| NEC (I)[#] | 2 (10.5) | 0 |
| Transient tachypnea of newborn[#] | 7 (36.8) | 9 (42.8) |
| Respiratory distress syndrome | 2 (10.5) | 2 (9.5) |
| CPAP[#] | 5 (26.3) | 3 (14.2) |
| Total parenteral nutrition[#] | 6 (31.5) | 1 (4.7) |
| Bronchopulmonary dysplasia[#] | 1 (5.2) | 0 |
| VH (I)[#] | 1 (5.2) | 0 |

*Data presented as [*]mean (SD), [$]median (range), [#]number (%); PPROM: Preterm premature rupture of membranes; BMV: Bag and mask ventilation.*

**TABLE II** AMPLITUDE-INTEGRATED EEG TOTAL SCORE AMONG SMALL FOR GESTATIONAL AGE (SGA) AND APPROPRIATE FOR GESTATIONAL AGE (AGA) NEONATES

| *Day of Life* | *SGA (n=19)* | *AGA (n=21)* | *Mean difference (95 % CI)[#]* |
|---|---|---|---|
| 3 | 9.0 (1.8 ) | 9.5(1.1) | -0.5 (-1.5 to 0.5) |
| 7 | 8.6 (2.4) | 10.1(1.1) | -1.4 (-2.6 to -0.3)[$] |
| 14 | 10.2 (1.3 ) | 10.7 (0.8) | -0.5 (-1.2 to 0) |

*Values expressed as mean (SD); [#]Adjusted for correlated values by generalized estimating equation (GEE); [$]P=0.02.*

**TABLE III** aEEG SCORE (CYCLING, CONTINUITY, LOWER BORDER, AND BANDWIDTH) AMONG SMALL FOR GESTATIONAL AGE (SGA) AND APPROPRIATE FOR GESTATIONAL AGE (AGA) NEONATES (*N*=40)

| *aEEG recording* | *SGA[*] (n=19)* | *AGA[*] (n=21)* | *Mean difference (95 % CI)[#]* | *P value* |
|---|---|---|---|---|
| *aEEG continuity score* | | | | |
| Day 3 | 1.7 (0.4) | 1.9 (0.3) | -0.1 (-0.36 to 0) | 0.31 |
| Day 7 | 1.7 (0.5) | 2.0 | -0.2 (-0.4 to 0) | 0.08 |
| Day 14 | 2.0 | 2.0 | -0.5 (-1.2 to 0) | 0.11 |
| *aEEG cycling score* | | | | |
| Day 3 | 2.2 (0.8) | 2.4 (0.7) | -0.1 (-0.6 to -0.3) | 0.61 |
| Day 7 | 2.1 (1.1) | 2.7 (0.7) | -0.6 (-1.2 to 0) | 0.03 |
| Day 14 | 2.8 (0.8) | 3.0 (0.6) | -0.2 (-0.6 to 0) | 0.39 |
| *aEEG lower border score* | | | | |
| Day 3 | 1.8 (0.8) | 2.0 | -0.1 (-0.2 to 0) | 0.13 |
| Day 7 | 1.7 (0.4) | 2.0 | -0.2 (-0.4 to 0) | 0.02 |
| Day 14 | 2.0 | 2.0 | - | - |
| *aEEG bandwidth score* | | | | |
| Day 3 | 3.1 (0.6) | 3.3 (0.4) | -0.1 (-0.5 to 0) | 0.60 |
| Day 7 | 3.0 (0.8) | 3.4 (0.5) | -0.4 (-0.8 to 0) | 0.05 |
| Day 14 | 3.3 (0.6) | 3.7 (0.4) | -0.3 (-0.7 to 0) | 0.03 |

*[*]Values expressed as mean* (SD); *[#]Adjusted for correlated values by generalized estimating equation (GEE).*

**WHAT THIS STUDY ADDS?**

- Preterm SGA neonates show similar total aEEG maturation score as Preterm AGA neonates on day 3 and 14, but a lower score on day 7 possibly indicating a delayed maturation in these neonates in the first week of life.

wake cycles. In addition, the serial recordings on neonates gave an insight in the maturation pattern at different time points, rather than one study time point [22]. We used the controls from the same population rather than other reference standards. The aEEG tracings were assessed by three blinded assessors.

Our study had a few limitations, including a small sample size. It is difficult to enroll stable PSGA neonates because this subgroup of neonates suffers from high risk of perinatal asphyxia, necrotizing enterocolitis and requirement of mechanical ventilation. The second limitation was a short follow up aEEG recordings of these neonates. In addition, ongoing recordings continuing upto term gestation may have helped us to delineate the specific maturation pattern in these neonates.

The maturation score of clinically stable and neurologically normal P-SGA neonates PSGA neonates was found to be significantly delayed at any point of life from day 3 till day 14 of life. Further studies evaluating progressive aEEG maturation till term age and the impact of the maturation pattern on subsequent neuro-developmental outcomes may provide further insight into the developmental pattern in these neonates.

*Contributors*: KA: primary responsibility for protocol development, study implementation, data management and writing the manuscript; AT, JS: development of the protocol and supervised implementation of the study, analysed the tracings, assigned the scores and contributed to writing of the manuscript. JS**:** helped in the statistical analysis. VKP, AKD, SG: protocol development, gave critical inputs in designing and execution of the study, data analysis and in manuscript writing. RA: conceptualized and designed the study, analyzed the aEEG tracings and assigned the scores, finalized the manuscript. He will act as guarantor of paper. All authors have reviewed the final manuscript and approved it.
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