

*Original Article*

# Activin A is Not a Reliable Prognostic Biomarker For Bilirubin Induced Neurotoxicity in Neonates

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## Abstract:

**Background:** Bilirubin induced neurological dysfunction (BIND) remains an important cause of disability in developing countries. Although high total serum bilirubin (TSB) is the main instigator for BIND, different babies may have different neurological outcomes at the same TSB level. This reflects the need for a more specific predictive factor for the neurological outcome, which would allow prompt intervention and prevention of kernicterus.

**Aim of the Work:** To assess the value of serum activin A as a predictor for acute bilirubin neurotoxicity and adverse neurodevelopmental outcomes at one year of life.

**Materials and Methods:** The study enrolled 84 term/near-term infants admitted with indirect hyperbilirubinemia requiring intervention to the Neonatal Intensive Care Unit of Cairo University Children's Hospital. Clinical examination, BIND score and laboratory tests including activin A were performed. Neurodevelopmental outcome was assessed at 3, 6 and 12 months using the Bayley scale of Infant Development II. Correlations between serum activin A, TSB, BIND scores and Bayley scores were studied.

**Results:** Mean TSB level at admission was  $25.92 \pm 7.14$  mg/dL. BIND score at admission ranged from 0-7, and mean serum activin A level was  $109.92 \pm 55$  pg/ml. Activin A did not show significant correlations with TSB or BIND scores. A negative correlation between activin A level and psychomotor developmental index (PDI) at 3 months was detected however all other neurodevelopmental outcomes showed no significant correlation with activin A.

**Conclusion:** In cases of neonatal hyperbilirubinemia, activin A is not a reliable biomarker for predicting acute or chronic bilirubin induced neurotoxicity.

**Level of Evidence of Study: IIA. (1)**

**Keywords:** Hyperbilirubinemia; bilirubin; BIND score; acute bilirubin encephalopathy; Bayley scales of infant development; activin A

**Abbreviations:** ABE: acute bilirubin encephalopathy; BIND: bilirubin induced neurological dysfunction; MDI: mental developmental index; PDI: psychomotor developmental index; TSB: total serum bilirubin.

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## Introduction

Bilirubin induced neurological dysfunction (BIND) still represents a cause of significant morbidity and mortality in developing countries (2, 3). Clinically a BIND score >3 is an indicator of moderate or advanced acute bilirubin encephalopathy with a risk of permanent neurological impairment (4). This occurs if neonatal hyperbilirubinemia is neglected and left to reach extreme levels (5). However, at the same total bilirubin level (TSB), two babies may have completely different neurological outcomes, one may develop frank kernicterus and the other may be spared (6). The availability of sensitive and specific biomarkers for neurological injury, independent of TSB values, can help identify specific babies at risk, thus providing means for clinicians to accelerate therapy and minimize residual brain damage.

Activin A is a glycoprotein member of the transforming growth factor beta protein family, which was first isolated from gonads but was later detected in numerous body cells, and was found to perform various functions in cell repair (7). Activin receptors have been identified in the cerebrum and spinal cord and was recently found to have a major neurotrophic factor in the developing injured brain (8). Activin A level was found to increase soon after neonatal hypoxic ischemic brain injury and was detected in numerous body fluids such as cord blood (9, 10), blood (11), urine (12) and cerebrospinal fluid (13) of hypoxic infants. It was also detected in the urine (14) and blood of infants with intraventricular hemorrhage (15). The surge in activin A levels following brain insults makes it a potentially suitable biochemical index of brain injury [11] and places it among the group of recently identified “brain biomarkers” (16). Since it plays an important biological role in cell repair and angiogenesis; it might also have a possible therapeutic effect on injured neurons (17). The aim of this study was to assess the unknown potential validity of serum activin A as a predictor for acute and chronic bilirubin neurotoxicity in babies with hyperbilirubinemia. This was achieved through correlating serum activin A level with BIND scores as well as neurodevelopmental outcome scores throughout the first year of life.

## Subjects and Methods

This prospective cohort study was conducted at the Neonatal Intensive Care Unit of Cairo University Children’s Hospital in the period between October 2014 and February 2016 as part of a bigger study for neurodevelopmental follow up of babies with neonatal hyperbilirubinemia (18). Newborns were enrolled over the course of 5 months, and follow up was performed until all infants reached one year of age. The study was approved by the ethical committee of Cairo University Pediatric Department. An informed consent was obtained from the patients’ parents.

### Participants

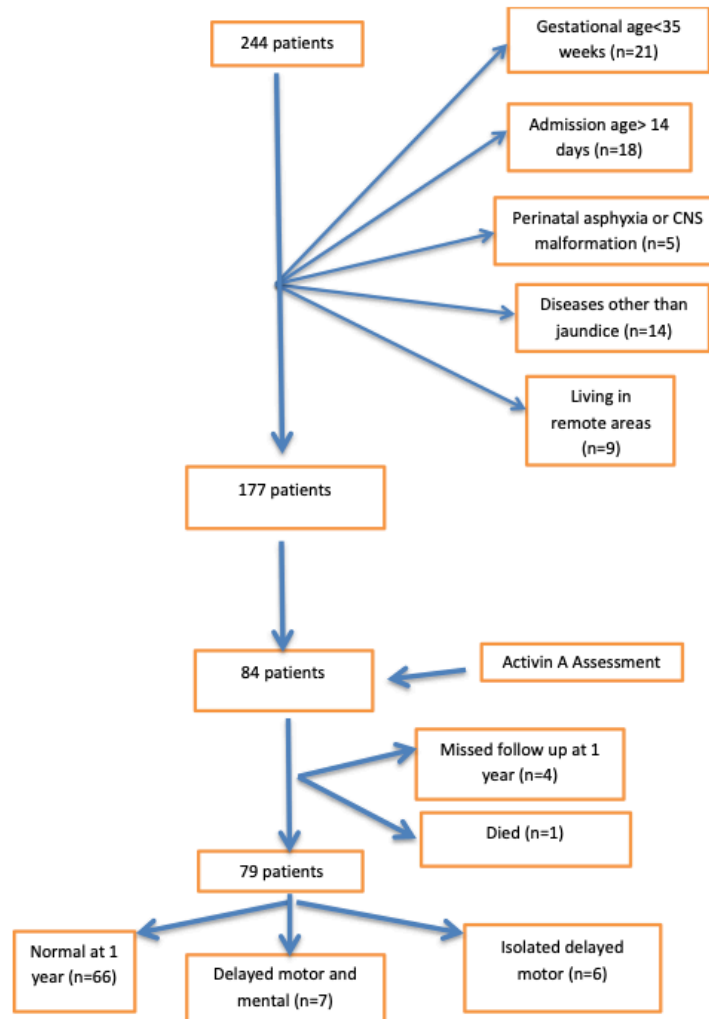
This study included newborns admitted to the Neonatal Intensive Care Unit with indirect hyperbilirubinemia requiring intervention according to the American Academy of Pediatrics guidelines (19). Any baby with gestational age <35 weeks (n=21), admission age >14 days (n=18), history suggestive of perinatal asphyxia or proven central nervous system malformation (n=5), clinically proven disease besides jaundice (n=14) or living in remote areas prohibiting follow up (n=9) were excluded from the study, leaving 177/244 in the inclusion pool. Based on an initial pilot study, median activin values of approximately 100 pg/ml in neonates with BIND ≤ 3 and 115 pg/ml in those > 3 were expected. A sample size of 80 was calculated to have a power of 80% and a p value of 0.05. Eighty-four newborns were finally included in the study. (Figure 1).

All included newborns were subjected to full history taking and thorough clinical examination. Gestational age was verified using the Ballard score (20) since our study population was comprised of out-born neonates with no documentation of accurate gestational age. Anthropometric measurements, and signs and degree of acute bilirubin encephalopathy using the BIND score (4) were recorded for all infants. The BIND score was used to assess for acute bilirubin encephalopathy (ABE). It is a clinical tool used to detect and grade ABE in babies with hyperbilirubinemia by assessing mental state, muscle tone and cry pattern. Scores of (1–3), (4–6), (7–9) indicate mild, moderate and severe acute bilirubin encephalopathy respectively.

### Methods

#### - Clinical Data

Follow up was scheduled at 3, 6 and 12 months of age for neurodevelopmental assessments. Seventy nine of 84 babies completed follow up until 12 months of age (4 dropped out and 1 died at age of 3 months at home of undetermined etiology). Neurodevelopmental assessment was performed by a blinded examiner using the Bayley scales of infant development II. This is composed of the mental developmental index (MDI) and the psychomotor developmental index (PDI). The MDI is used to evaluate cognition by testing sensory perception, knowledge, memory, problem solving and early language, whereas the PDI is used to assess gross and fine motor skills. Normal developmental indices of PDI and MDI scores are ≥85. Mild delay is defined at scores of 70–84, and severe delay is defined at scores of ≤69 (21).



**Figure 1.** Flowchart of Studied Neonates and their outcomes.

### - Laboratory Data

Blood samples were drawn from all infants at admission. Complete blood picture, TSB, reticulocyte count, and Coomb's test were analyzed. Maternal blood grouping was also performed. Admission TSB was measured after the blood samples were allowed to clot, and centrifuged for 20 minutes at 3000 rpm. Serum bilirubin was measured using AU 480-Chemistry auto-analyzer (Beckman Coulter Diagnostics-USA) and levels were plotted on the curve for phototherapy and exchange transfusion to detect the required intervention (19). Activin A samples were drawn at admission and stored in the central laboratory deep freezer at -20 degrees Celsius (as per the kit's instruction manual). After sample collection was complete at the end of the enrollment period, activin A was then measured using Human activin A (ACV-A) ELISA kit with a measuring range 8-350 pg/ml (NOVA, DaXing industry Zone, Beijing, China) according to the manufacturer's instructions. All laboratory analyses were performed in the central laboratory of Cairo University Children Hospital.

### - Follow up Data

Follow up was scheduled at 3, 6 and 12 months of age for neurodevelopmental assessments. 79/84 babies completed follow up until 12 months of age (1 died and 4 dropped out). Neurodevelopmental assessment was performed by a blinded examiner using the Bayley scales of infant development (BSID). This is composed of the mental developmental index (MDI) and the psychomotor developmental index (PDI). The MDI is used to evaluate cognition by testing sensory perception, knowledge, memory, problem solving and early language, whereas the PDI is used to

assess gross and fine motor skills. Normal developmental indices of PDI and MDI scores are  $\geq 85$ . Mild delay is defined at scores of 70–84, and severe delay is defined at scores of  $\leq 69$  (21, 22).

### Statistical Analysis

Data were analyzed using SPSS© Statistics version 24 (IBM© Corp., Armonk, NY, USA). Numerical data were expressed as arithmetic mean  $\pm$  SD or median (25th – 75th percentiles). Correlations between variables were detected using Spearman's correlation test. P values  $< 0.05$  were considered statistically significant.

### Results

Of the 84 babies studied, 40 % were females, and 60% were males, with a mean birth weight of  $2851 \pm 449$  grams, and a mean gestational age of  $37.7 \pm 1.1$  weeks. BIND scores at admission ranged between 0-7, TSB ranged between 10.5-63 mg/dl with a mean of  $25.92 \pm 7.14$  mg/dL, and serum activin A levels ranged between 21.5 – 299.5 with a mean of  $109.92 \pm 55$  pg/ml (Table I).

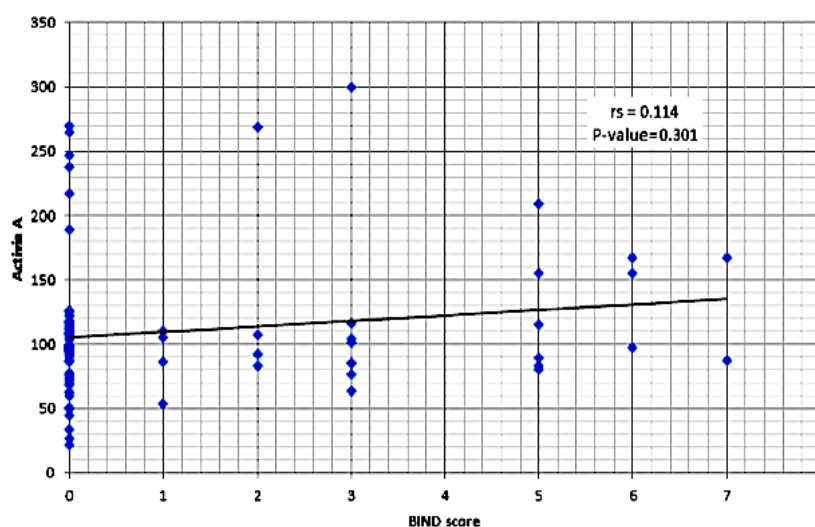
**Table 1.** Demographic, clinical and laboratory data of the studied neonates.

Data	Range	Mean $\pm$ SD
Gestational age (weeks).	35-40	$37.7 \pm 1.1$
Weight (grams).	1820-4065	$2851 \pm 449$
Age of admission (days).	1-14	5(3-7)*
BIND score	0-7	0(0-2)*
TSB (mg/dL)	10.5-63	$25.92 \pm 7.14$
Activin A (pg/ml)	21.5 – 299.5	$109.92 \pm 55$
Hb (gm/dL)	4-22	$13.8 \pm 3.1$

\* Data are represented by median (25th-75th percentile).

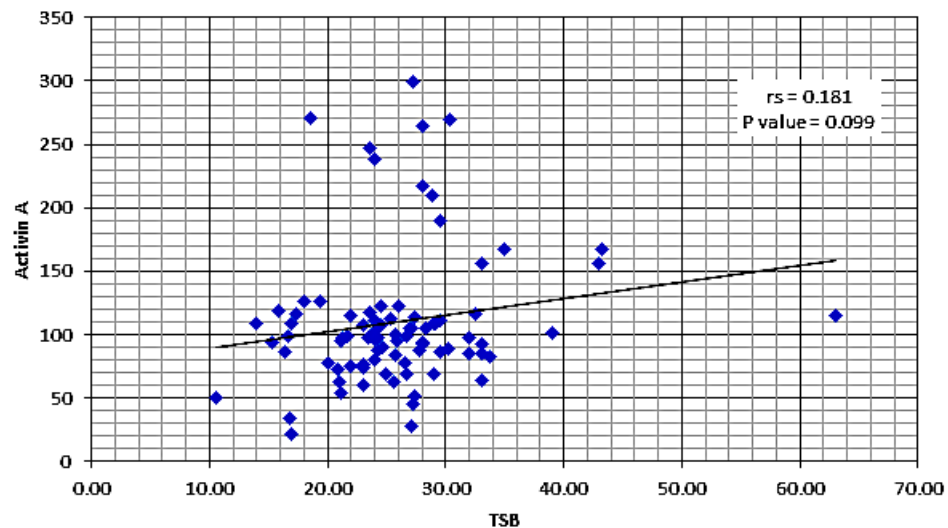
BIND: Bilirubin Induced Neurologic Dysfunction; Hb: Hemoglobin; TSB: Total Serum Bilirubin.

No significant correlation was detected between activin A and BIND score in this study, nor was there any significant correlation between activin A and TSB level (Figures 2,3). In terms of neurodevelopmental outcome, there was a significant correlation between activin A levels and the PDI scores at 3 months of age. Otherwise no significant relationship was shown between activin A levels and PDI at 6 and 12 months, nor MDI scores at any stage of follow up (Figure 4).

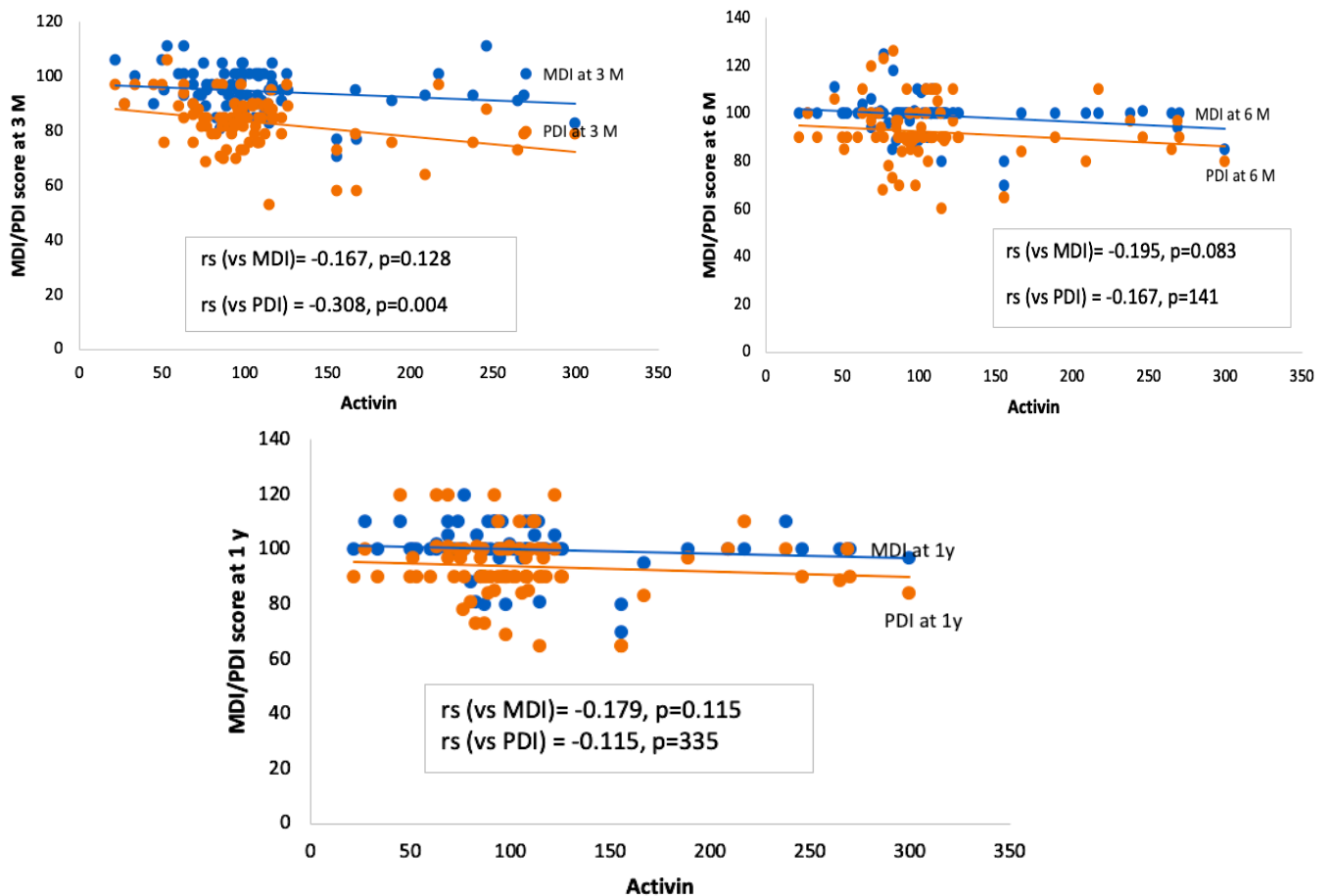


**Figure 2.** Correlation between BIND score and activin A.

BIND: Bilirubin Induced Neurologic Dysfunction; rs: Spearman correlation coefficient.



**Figure 3.** Correlation between TSB and activin A.  
rs: Spearman correlation coefficient; TSB: Total Serum Bilirubin



**Figure 4.** Correlation between activin A and neurodevelopmental outcome at 3, 6 and 12 months of age.

\* A significant statistical correlation was present at 3 months between activin A and PDI. There was no correlation at 6 or 12 months. MDI: mental developmental index; PDI: psychomotor developmental index; rs: Spearman correlation coefficient.



## Discussion

The aim of this study was to test the reliability of activin A as a serum biomarker for bilirubin induced neurotoxicity and as a predictor of neurodevelopment outcome. Since ABE is a preventable cause of brain damage and remains a global health burden, especially in underdeveloped countries (2, 3), accurate and timely identification of cases with severe hyperbilirubinemia prone to develop neurological sequelae is of utmost importance for early intervention, and prevention of complications.

Previous studies have suggested that activin A could be a marker of neurological injury (14–16); accordingly, a relationship between activin A and bilirubin, as well as between activin A and BIND score was pursued. No significant correlation was detected between TSB and activin A levels in this study. A possible explanation is that free bilirubin and not TSB is the neurotoxic agent. This is demonstrated by the fact that different infants react to the same total serum bilirubin level in different ways and some babies have normal developmental outcome despite extreme hyperbilirubinemia (6). This may be due to variations in the level of free bilirubin reaching the brain cells and/or the diverse genetic susceptibilities that may predispose some but not all babies to severe neurological damage (23).

The BIND score, a proven method for the clinical assessment of ABE has been previously shown to correlate well with neurological outcome in hyperbilirubinemic babies, where high BIND scores are predictors of poor outcome (24). This study tested the sensitivity of activin A as a biomarker for acute brain injury in bilirubin encephalopathy but failed to find any correlation between the BIND score and activin A levels, negating a relationship between activin A and ABE.

Another aim of the study was to detect whether activin A levels could predict long term neurological outcome in neonatal hyperbilirubinemia. Untreated ABE can adversely affect motor development as well as hearing, and might impact mental development to a much lesser extent (25). Although our results showed a significant negative correlation between activin A levels and PDI scores at 3 months, this relation was not noted at the 6 or 12 months follow up. Furthermore, there was no parallel correlation with BIND score; and since BIND has been previously shown to reflect neurological injury (24), the evidence provided by the solitary correlation of PDI at 3 months was too weak. Essentially as no other studies have been published to date regarding activin and neonatal hyperbilirubinemia. Hence, it was not possible to corroborate these findings with others; and certainly, future larger studies will be of value in validating or negating these results.

Despite studies showing activin A as a good marker of ischemic brain injury, few other studies failed to show any correlation between activin A and brain injury; Tong et al., reported non-significant correlation between umbilical artery activin and fetal oxygenation or risk of hypoxic-ischemic encephalopathy (26). Also, Lai et al., reported that only severe hypoxia associated with convulsions increased expression of activin A in rat brains (27). It could be that brain insults that involve ischemic vascular injury rather than neuronal injury are the stimulators for increased activin A (28), therefore the pathophysiology of bilirubin induced brain injury which does not induce these ischemic changes may be the reason that activin A levels do not significantly increase in neonatal hyperbilirubinemia. Additionally, other factors exist that may affect activin A levels, including different maternal factors (29, 30) as well as varying gestational (31) and post-natal ages (28).

This study has several limitations including our inability to assess other factors influencing the improvement in developmental scores over time such as environmental factors, rehabilitation programs or early installation of hearing aids. Also sample size might be considered a limitation in view of its small size. To our knowledge this is the first study investigating the role of activin A as a biomarker for bilirubin induced brain injury.

## Conclusion

The results of this study suggest that activin A is not a reliable serum biomarker for prediction of acute or chronic bilirubin encephalopathy. Further and larger studies may be needed to corroborate these results but it is our recommendation that activin A has no role as part of a “bilirubin panel of investigations” in neonatal hyperbilirubinemia-induced neurotoxicity detection.

**Author Contributions:** All authors searched medical literature, databases, conceptualized, conducted the case review and reviewed the final manuscript. All authors have read and agreed to the published version of the manuscript.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest in connection with the reported study. Authors declare veracity of information.

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