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ORIGINAL ARTICLE

Assessment of Blood and Urine Activin A Levels in Neonates with Hypoxic Ischemic Encephalopathy: A Case-Control Study

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ABSTRACT

Objective: We aimed to identify whether Activin A levels are elevated in venous blood and/or urine samples of infants with hypoxic ischemic encephalopathy (HIE), and to determine if Activin A levels may be used as a diagnostic marker of hypoxic ischemic encephalopathy.

Study Design: This was a case-control study.

Place and Duration of Study: This study was performed at Cumhuriyet University Medical Faculty Newborn Clinic from July 2009 to December 2009.

Material and Methods: We included 20 infants with hypoxic ischemic encephalopathy and 20 healthy newborns into this study and measured Activin A levels in venous blood and urine samples on the 1st, 2ndand 3rd days after birth.

Results: Hypoxic ischemic encephalopathy and control groups were similar in terms of gender, pregnancy duration, weight, height, and head circumference. Blood and urine Activin A levels in 1st, 2nd, 3rd days were higher in those with hypoxic ischemic encephalopathy compared to healthy infants.

Conclusion: As a result, Activin A level is an aid parameter that can be used in the diagnosis of HIE.

Key Words: *Infant, Encephalopathy, Activins*

INTRODUCTION

Hypoxic Ischemic Encephalopathy (HIE), a form of brain damage caused by prenatal, natal and postnatal systemic hypoxia and ischemia, is a disease in which blood flow to the brain decreases, leading to serious brain injury. As well as being the most severe consequence of the perinatal asphyxia, it is also among the most serious causes of neonatal mortality and morbidity.[1] Despite all possible treatments (such as hypothermia and intensive care) nearly half of cases (45%) have unfavorable outcome.[2] In the long term, HIE leads to cerebral palsy, mental retardation, epilepsy and learning difficulties. Studies show that perinatal asphyxia (PA) is seen in 0.2–0.4% of full-term births, 20% of these babies suffer from HIE and 25% of the survivors exhibit persistent neuropsychological damages.[3] Although close follow-up is utilized in the post ischemic period, brain damage could be subclinical or symptoms and radiological findings may initially be unrecognizable due to various causes including the effect of sedation.[4] However,

early and accurate diagnosis is vital for targeted treatment after birth, which in turn may decrease morbidity and mortality in this population. Treatment with therapeutic hypothermia within 6 hours of delivery is the gold standard treatment of this disease; therefore, it is apparent that this time limitation for treatment is also a factor that underlines the importance of rapid and accurate diagnose of brain damage.[5]

Activin A, a member of the transforming growth factor-β superfamily, is a glycoprotein comprised of two A subunits and is produced by central nervous system (CNS) for the purpose of maintaining neuron durability in case of brain injury.[6] Apart from stimulating neuron growth, differentiation and survival, it also supports early embryonic development and erythropoiesis.[7] Activin A has three receptor subtypes which possess serine threonine protein kinase activity. Although the first and second receptors are suggested to be the major components of its signaling, the third receptor is also crucial for normal function.[8] In the past decade many studies have reported that Activin A increases during brain damage in various conditions including epileptic seizures and stroke[9,10], and that it has neuroprotective properties.[11] Activin A's main neuroprotective activity stem from its anti-inflammatory and antiapopitotic effects.[6] Taking into account the importance of early diagnosis in HIE, researchers have attempted to find an early diagnostic biomarker in neonates with HIE, one of which is Activin A. Although there are numerous researches concerning the levels of Activin A during a brain damage, there are limited studies about Activin A levels in HIE infants. With this study, we aimed to identify whether Activin A levels are elevated in venous blood and/or urine samples of infants with HIE, and to determine if Activin A levels may be used as a diagnostic marker of HIE.

MATERIAL AND METHODS

This was a case-control study performed at Cumhuriyet University Medical Faculty Newborn Clinic from July 2009 to December 2009. The local ethical committee granted approval for the study (Ethic committee of Cumhuriyet University Medical Faculty 2008-10/4). The study was planned and carried out in accordance with the Helsinki Declaration.

We included 20 neonates with birth asphyxia (all patients) into this research. We determined asphyxia criteria as: an infant with 1st and 5th minute APGAR (Activity, Pulse, Grimace, Appearance, Respiration) scores of <4 and 7 respectively, cord blood pH<7.20, base excess ≤12 in cord or venous blood within 60 minutes after delivery, requirement for over 3 minutes of resuscitation after delivery, and lastly, requirement for positive pressure ventilation in infants who also fulfilled 3 or more of the above mentioned criteria.[12] We determined 20 babies as control group who were completely healthy in their neurological and physical examination, their APGAR scores were >7 in 1th and 5th minutes after birth, pH> 7.35 of cord or venous blood gases, did not require positive pressure ventilation after birth and did not have CNS infection, chromosomal anomaly, encephalopathy or congenital heart disease. To exclude other metabolic reasons which may cause convulsion, we performed laboratory tests that comprised of complete blood count (CBC), blood glucose, sodium, potassium, chloride, calcium, and phosphorus levels. Infants who had any significant abnormalities in these laboratory tests were excluded from the study. Blood urea nitrogen (BUN) and hepatic function tests; alanine transaminase (ALT), aspartate transaminase (AST) were also measured in both groups.

All participants' type of delivery [cesarean section (C/S) or normal spontaneous vaginal birth (NSVB)], gestational age, gender, APGAR scores, weight, height, head circumference, blood gases, biochemical and hematological parameters were recorded. All babies' spot urine and venous blood samples were collected on the three consecutive days after delivery with appropriate procedures and Activin A levels were measured in both samples. Activin A levels were measured with BOESTER (China) brand kits in a TRITURUS (Italy) device using micro - ELISA by the microbiology laboratory of our hospital.

In the asphyxia group we performed transfontanelle ultrasonography (TFUS), brain Computed Tomography (CT), evoked potentials (EV) such as brainstem auditory evoked potentials (BAEP), magnetic resonance (MR) and

electroencephalography (EEG) according to their clinical findings. These tests were not applied in the control group.

Statistical methods: Data were analyzed using SPSS v14.0 (SPSS Inc., Chicago IL) statistics software. The distribution of continuous variables was assessed using the Kolmogorov–Smirnov test, and values were presented as frequency and percent values (%) for categorical data and as mean ± SD (standard deviation) for continuous data. The comparison of categorical variables was performed using the Pearson Chi-square and Fisher's exact tests. Continuous variables were compared with the Mann-Whitney U test or the Student's t-test, depending on normality of distribution. The level of statistical significance was set at $p<0.05$.

RESULTS

While there were 6 female 14 male infants in the patient group, there were 12 female and 8 male infants in control group. Three of the newborns were delivered under 35 weeks of gestation while 17 were delivered after 36 weeks in both groups. There were no significant differences in terms of gender distribution or pregnancy duration between the groups (p=0.057 and p=1.00 respectively). Additionally, weight (HIE = 2.95 ± 0.82 kg vs. Control = 2.87 ± 0.44 kg), length (HIE = 48.65 ± 4.47 cm vs control = 48.53 ± 2.90 cm), and head circumference measurement (HIE = 33.83 ± 2.88 cm vs control = 33.86 ± 1.91 cm) were similar in both groups as well (p=0.303, p=0.327, p=0.881, respectively). While 40% (n=8) of the HIE group were born via NSVB, 60% (n=12) were born via C/S; similarly, 30% (n=6) of the HIE group was born via NSVB and 70% (n=14) were born via C/S. There was no significant difference between groups (x^2=0.44, p=0.507). In the HIE group, 5 babies had abnormal brainstem auditory evoked potentials (BAEP), 3 babies had abnormal TFUS and 4 babies had abnormal EEG. The results of these tests were normal in the remaining babies. During the follow-up period, 5 of the HIE infants experienced epileptic seizure.

The laboratory and arterial blood gas results of the infants are presented in table 1**.** Blood and urine Activin A levels on the 1st, 2nd and 3rd days were higher in the HIE group compared to the corresponding values of the controls (fig 1). However, there were no significant correlations between arterial blood gas results and Activin A levels or between Activin A levels and APGAR scores (table 2). The Activin A levels in HIE newborns were similar in infants with normal and abnormal BAEP and also in those with normal and abnormal EEG results (p-value >0.05 for both).

TABLE 1: Routine biochemical and arterial blood gases result of the groups

	HIE	Control	P
ALT	61.65 ± 69.57	11.35 ±4.17	0.001
AST	186.00 ±199.60	21.95 ±4.29	0.001
LDH	1173.10 ±662.70	157.30 ±60.66	0.001
BUN	13.00±5.59	11.65±1.95	0.469
Creatinine	1.10 ± 0.29	0.64±0.29	0.001
FBG	79.15 ± 44.75	76.65±13.35	0.787
Ca	8.98 ± 1.11	9.26±0.67	0.250
RBC	5.26 ±0.85	3.95 ± 0.41	0.001
pH	7.14 ± 0.13	7.37 ± 0.04	0.001
HCO3	16.70 ± 2.14	21.24 ± 0.87	0.001
Be	-12.78 ± 2.33	-4.76 ± 0.88	0.001

HIE = Hypoxic ischemic Encephalopathy, **ALT** = Alanine aminotransferase, **AST** = Aspartate Aminotransferase, **LDH** = Lactate dehydrogenase, **BUN** = Blood urea nitrogen **FBG** = Fasting blood glucose, **Ca** = Calcium, **RBC** = Red blood cell, **HCO3** = Bicarbonate, **Be** = Base excess

TABLE 2: Correlation analyses of Activin A levels with blood gases and APGAR scores

	1th day Activin A levels	2th day Activin A levels	3th day Activin A levels
pH	r = 0.12	r = 0.01	r = 0.12
	p= 0.605	p = 0.983	p = 0.591
HCO3	r = 0.11	r = - 0.26	r = 0.15
	p= 0.658	p = 0.263	p = 0.514
Be	r = 0.11	r = - 0.07	r = 0.04
	p = 0.633	p = 0.767	p= 0.863
O2	r = 0.01	r = 0.064	r = - 0.03
	p= 0.983	p = 0.777	p = 0.873
1.min. APGAR score	r = 0.26	r = - 0.02	r = 0.05
	p = 0.252	p = 0.906	p = 0.083
5.min. APGAR score	r = 0.08	r = 0.08	r = 0.09
	p = 0.711	p = 0.715	p= 0.692

r: Correlation coefficient, statistical p value
HCO3 = Bicarbonate, **Be** = Base excess, **APGAR** = Activity, Pulse, Grimace, Appearance, Respiration

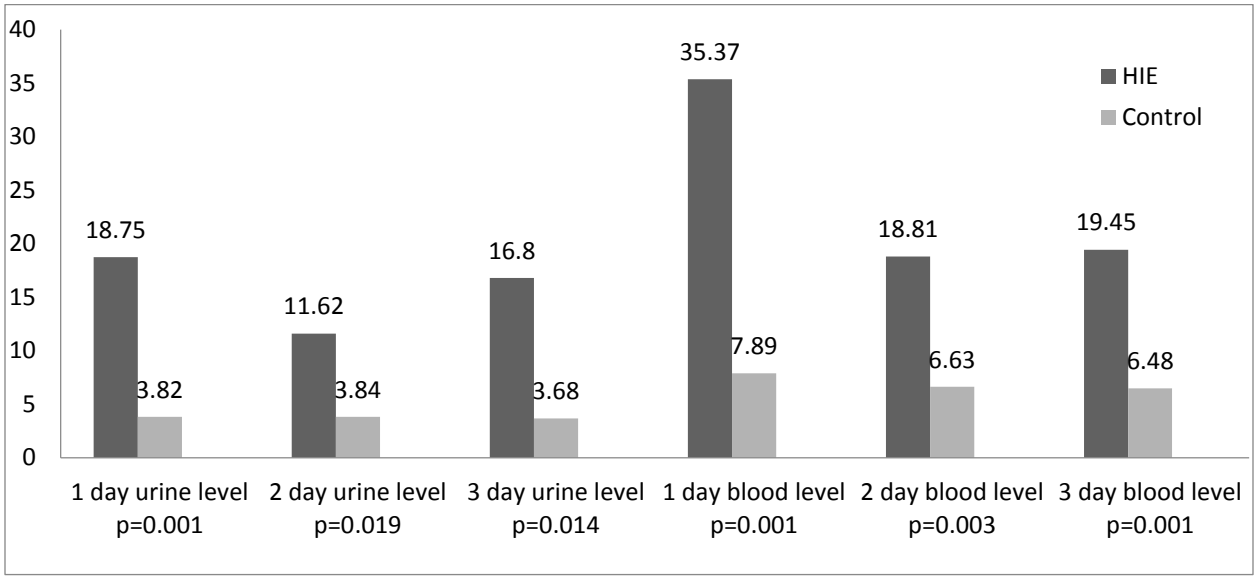

Fig 1: Activin A concentrations in HIE and control groups

DISCUSSION

The main finding of this study was that, Activin A levels are increased significantly in HIE, in both blood and urine samples. However, we did not find any significant correlations between Activin A levels and parameters such as APGAR scores or arterial blood gas results, and there were no relationships between Activin A concentrations and either radiologic or electrophysiologic test results. These negative results may have been caused by the low number of patients included in our study. As mentioned previously in the literature, Activin A is a neuroprotectıve glycoprotein, our results provide data which may support this theory as well. Apart from its neuroprotective activities it can be inferred from our study that Activin A may be used as an early diagnostic biomarker for HIE development.

During the past decade there is a growing interest in the early diagnosis of HIE, mostly due to the fact that early diagnosis would lead to early treatment and lower morbidity/mortality. In recent years, Activin A has particularly drawn attention for the early diagnosis of this condition. In 2004, Florio et al. for the first time showed that cerebrospinal flood (CSF) Activin A levels were significantly higher in both mild and moderate full term HIE infants compared to healthy infant

controls. They also found that an Activin A concentration higher than 1.3 µg/L had a positive predictive value of 100% for HIE diagnosis,[13] they claimed that increased CSF Activin A concentrations are reasonably a direct expression of increased production by the CNS. After this investigation, the same research team reported in 2006 that urine Activin A levels of patients with severe and moderate HIE were significantly higher than that of healthy controls and those with mild HIE. Activin A concentrations were >0.08 µg/L at first urination with moderate or severe HIE.[14] Our results were similar with these findings. In the current study, HIE infants were found to have higher Activin A concentrations in both venous blood and urine samples. However, we could not determine any correlation between urine or blood Activin A levels and parameters such as blood gas levels or APGAR scores. In accordance with our results, Tong et al. also did not find any significant relationship between umbilical artery Activin A concentration and fetal pH.[15]

This study has some important limitations that must be noted. Firstly, our study population was small and due to technical insufficiencies we could not investigate the mechanism by which Activin A exhibits its neuroprotective effects. Another limitation is the fact that, while many factors that may have affected HIE development were assessed, there may be numerous fetal or maternal factors that influence Activin A production and function during pregnancy and birth. However, this limitation is true for all studies on this topic and avoiding these factors may not be feasibly possible. On the other hand, this is one of the few studies that have assessed Activin A levels in both venous blood and urine. We found that Activin A concentrations were higher than controls which were characteristically and demographically similar to the patient group.

CONCLUSION

As a result, Activin A level was measured at 3 different times, on the 1st, 2nd, and 3rd days, and the Activin A level was found to be high in HIE patients in all measurements. Therefore, we think that the Activin A level is an aid parameter that can be used in the diagnosis of HIE. Future studies with a larger number of patients are required to clarify the diagnostic utility of Activin A. Furthermore, research is needed to determine the mechanism of action of Activin A.

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