

## Predictive Value of Serum Amyloid A Protein in Newborn Infants with Hypoxic Ischemic Encephalopathy

ELHAM A. NAWAR, M.D.\*; HOWYDA M.K. SHAABAN, M.D.\*\*; RANIA I. ABD EL-ATY, M.D.\* and HANAN T. EMAM, M.D.\*

The Departments of Pediatric\* and Clinical Pathology\*\*, Faculty of Medicine, Benha University

### Abstract

**Objective:** Hypoxic-Ischemic Encephalopathy (HIE), remains a serious condition, causing significant mortality and long-term morbidity. In spite of major advances in monitoring technology and knowledge of fetal and neonatal pathologies perinatal asphyxia remains a major concern. Serum Amyloid A (SAA) is an acute phase inflammatory marker that is closely associated with ischemic injuries.

This study was aimed to evaluate the serum level of SAA in neonatal HIE and its concentration correlates with the severity of encephalopathy.

**Study Design:** We conducted a prospective case-control study on 44 full-term neonates; 24 cases with evidence of perinatal compromise and 20 healthy controls. Blood samples were collected from cases and controls at postnatal day 1 and day 7, and SAA was measured by ELISA.

**Results:** SAA concentrations ( $\mu\text{g/mL}$ ) were significantly increased in cases when compared with controls at day 1 and at day 7 ( $p < 0.001$ ). SAA concentrations at day 1 were greater in cases who died when compared with those who survived, and correlated significantly with the severity of HIE ( $163.2 \pm 62.4$ ,  $86.8 \pm 30.4$ ,  $54.2 \pm 17.3$ ) in severe, moderate and mild HIE, respectively ( $p = 0.001$ ).

**Conclusions:** The level of SAA is increased in response to hypoxic ischemia of the neonate. The increased concentration correlates with the severity of encephalopathy and is associated with mortality.

**Key Words:** HIE – Birth asphyxia – Neonates – SAA – HIE markers.

### Introduction

**HYPOXIC-ISCHEMIC** Encephalopathy (HIE) in a full-term infant is a clinically defined syndrome of disturbed neurologic function in the earliest days after birth manifested by difficulty in initiating and maintaining respiration, the depression of the muscle tone and reflexes, the subnormal level of consciousness and often seizures. HIE the resultant

condition of a deficit in the oxygen supply to the brain [1].

Despite increasing knowledge about the pathogenesis of asphyxia related disorders, it is often difficult to predict which newborn will develop clinically relevant neurologic problems. The current markers of the development of neurologic problems associated with perinatal asphyxia are less reliable, implicating the need for other predictors of Hypoxic Ischemic Encephalopathy (HIE) and associated disorders [2].

Hypoxia triggers a cascade of adverse events that lead to irreversible neuronal and white matter injury over a period of hours to days. In the primary phase of energy failure, there is cellular loss. After reperfusion and reoxygenation, brain metabolism may recover but deteriorates in a secondary phase 6 to 15 hours later [3]. In human infants, this secondary energy failure is correlated with adverse neurodevelopmental outcome. Lipids and proteins are oxidized, and cytokines and other proteins are overexpressed. In the secondary phase of damage, programmed cell death occurs, and protective and repair mechanisms are activated. The outcome is therefore the result not only of the initial injury but also of the endogenous protective mechanisms [4].

There have been several attempts to find a good marker for the prediction of the severity of HIE. Acute phase Serum Amyloid A (SAA) protein is a multifunctional apolipoprotein that is secreted in the acute phase of inflammation. This protein is found in the high density lipoprotein fraction of the serum and is involved in transporting cholesterol to the liver for the secretion of bile, induction of extracellular matrix degrading enzymes, and chemotactic recruitment of inflammatory cells to

**Correspondence to:** Dr. Elham A. Nawar, The Department of Pediatric, Faculty of Medicine, Benha University

the site of inflammation. Serum Amyloid A protein is involved in the pathogenesis of several chronic inflammatory diseases, such as amyloidosis, atherosclerosis, and rheumatoid arthritis [5].

Under acute inflammatory conditions, SAA is induced to similar extent, causing up to a 1000-fold increase in the SAA concentration. The induction of SAA1 and SAA2 is largely triggered by elevated levels of proinflammatory cytokines, interleukin-1, 6 and tumor necrosis factor A in the circulation [6].

Induction of SAA protein by proinflammatory cytokines has been reported in many other cell types, including macrophages, endothelial cells, intestinal epithelial cells, and the brain of patients with Alzheimer disease [7].

This study was aimed to evaluate, the serum levels of Serum Amyloid A protein in the healthy and asphyxiated newborns during the first week of life, and secondly to assess if the level of this protein correlates with degree of asphyxia.

## Subjects and Methods

### Selection of cases:

This prospective case-control study was conducted in the Department of Pediatrics, Faculty of Medicine, Benha University, from May to November 2015.

#### • Inclusion criteria:

Infants were diagnosed with asphyxia if they had demonstrated at least two of the following:

- Apgar score <3 at 1 minute or <6 at 5 minutes.
- Umbilical cord arterial pH <7.2 with base deficit > 10mmol/l.
- The presence of post natal clinical complications attributed to birth asphyxia, such as seizures, abnormality in state, hypotension requiring inotropic support, severe apnea and oliguria.

#### • Exclusion criteria:

Infants were excluded from the study if they met any of the following conditions:

- If increased SAA was attributed to inflammatory causes other than asphyxia such as sepsis or localized infection.
- If they were diagnosed with life threatening congenital anomalies, inborn errors of metabolism or preterm births.

#### • Ethical consideration:

Consent from all cases and their parents were taken for examination and procedure involved. They were informed of the procedure involved. Also all cases detected with condition that requires medical attention were referred for appropriate management.

### Methods:

#### A- History taking:

Full maternal and perinatal history was obtained for all cases and controls.

#### B- Examination:

- *General examination:* Complete general examination was done with special emphasis on:
  - Respiratory system: Respiratory distress-apnea-meconium aspiration.
  - Cardiovascular system: Bradycardia-heart failure-shock.
  - Renal system: Oliguria.
- *Neurological examination:*
  - Level of consciousness: Alert-lethargy-coma.
  - Motor system:
    - Power.
    - Tone: Normal, hypertonia or hypotonia.
    - Reflexes: Normal, exaggerated or decreased.

#### C- Staging system:

HIE was defined as mild, moderate or severe using the sarnat and sarnat staging system [8]. The assessed elements include, level of consciousness, muscle tone tendon and complex reflexes, seizures, autonomic function and electroencephalogram description.

#### D- Laboratory tests: Laboratory testing was for all subjects and included:

- C reactive protein.
- Complete blood count.
- Liver and kidney function tests.
- Blood gases: PO<sub>2</sub>, PCO<sub>2</sub>, PH, base excess.
- Serum amyloid A by ELIZA technique at a post natal age of 1 and 7 days.

#### SAA assay:

Three milliliters of blood were drawn using standard venipuncture techniques and serum separated from the blood cells as soon as possible. Samples were allowed to clot for one hour at room

temperature, centrifuged for 10 minutes and serum extracted. Separated serum was collected and stored at  $-20^{\circ}\text{C}$ . A quantitative measurement of SAA was performed using an Enzyme-Linked Immunosorbent Assay (ELISA). This SAA Enzyme-linked Immunosorbent Assay (ELISA) applies a technique called quantitative sandwich immunoassay. The microtiter plate provided has been pre-coated with a monoclonal antibody specific for SAA. Standards or samples are then added to the appropriate microtiter plate wells and incubated. SAA if present, will bind and become immobilized by the antibody pre-coated on the wells. The microtiter plate wells are thoroughly washed to remove any unbound SAA and other components of sample. In order to quantitate the amount of SAA present in the sample, a standardized preparation of Horseradish Peroxidase (HRP)-conjugated polyclonal antibody specific for SAA is added to each well to "sandwich" the SAA immobilized during the first incubation. The microtiter plate then undergoes a second incubation. The wells are thoroughly washed to remove all unbound HRP-conjugated antibodies and a TMB (3,3',5,5' tetramethyl-benzidine) substrate solution is added to each well. The enzyme (HRP) and substrate solution are allowed to react over a short incubation period. Only those wells that contain SAA and enzyme-substrate reaction will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the intensity of the color was measured.

#### E- Imaging:

Cranial ultrasound and CT scan were done in justified cases.

#### Statistical analysis:

All data were expressed as Mean  $\pm$  SD. Difference between the groups were compared by student *t*-test with *p*-value  $<0.05$  selected as the level of statistical significance [9]. SPSS version 16 was used for statistical analysis.

### Results

A total of 44 infants were studied, of them 24 cases and 20 controls. The demographical and clinical characteristics of the study population are presented in (Tables 1,2). A total of 4 cases died before 7 days and hence SAA at day 7 was not assessed in these infants. Nine infants has normal neurological examinations and 11 infants were abnormal. Neurological abnormalities included decreased muscle tone ( $n=9$ ), increased tone ( $n=2$ ) and abnormal reflexes ( $n=10$ ) mortality was associated with the more severe stages of HIE ( $p=0.05$ ).

SAA at day 1 was significantly higher in cases when compared with controls ( $p<0.001$ ) Fig. (1); within cases, SAA at day 1 was higher in those who died compared with those who survived ( $p=0.002$ ) Fig. (2).

SAA at day 7 was higher in cases as compared with controls ( $p<0.001$ ) Fig. (1), but the difference between survived and died cases can't be determined. SAA at day 1 and day 7 did not associated with mode of delivery, but it significantly correlated with grades of encephalopathy (Table 3).

Using the ROC curve, SAA at day 1 could significantly predict mortality of the studied cases with a sensitivity of 91% and specificity of 85% (cut off point= $93.7 \mu\text{g/ml}$  and area under the curve =0.81%). Fig. (3).

Table (1): Demographical data and Apgar score at 1 minute & 5 minutes among studied groups.

	Cases No.=24	Controls No.=20
Male	13 (54.2%)	10 (50%)#
Female	11 (45.8%)	10 (50%)#
Gestational age (weeks)	$38.6 \pm 1.7$	$38.1 \pm 1.9$ #
Birth weight (kgs)	$3.15 \pm 0.2$	$3.0 \pm 0.17$ #
Medical disease among mothers of studied groups		
Pregnancy induced hypertension	4 (16.6%)	3 (15%)#
Diabetes mellitus	4 (16.6%)	3 (15%)#
Premature rupture	3 (12.5%)	2 (10%)#
Apgar score at 1 minute & 5 minutes among studied groups		
1 minute	$2.1 \pm 0.7$	$5 \pm 1.6^*$
5 minutes	$4.5 \pm 1.7$	$8.1 \pm 3.5^{**}$

\*\*\* : Significant difference between cases and controls ( $p<0.01$ ).

\* : Significant difference between cases and controls ( $p<0.001$ ).

# : Non significant difference between cases and controls ( $p>0.05$ ).

Table (2): Clinical manifestations among studied cases.

Clinical manifestations	Number	%
Respiratory Distress (RD)	14	58.3
Oliguria	8	33.3
Bradycardia	5	20.8
<i>Conscious level:</i>		
Alert	15	62.5
Lethargy	5	20.8
Coma	4	16.6
Hypotonia	9	37.5
Hypertonia	2	8.3
Abnormal reflexes	10	41.7
Convulsions	10	41.7

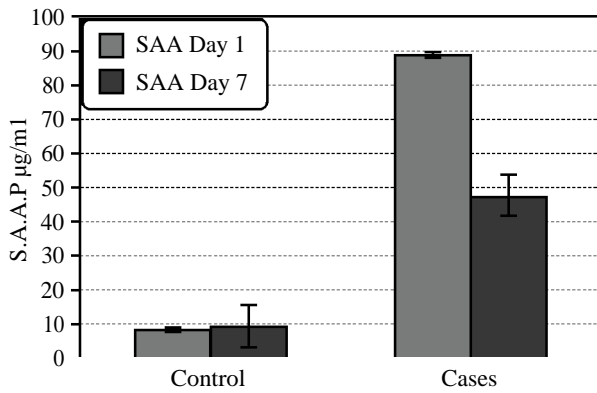


Fig. (1): Level of SAA in day 1 and day 7 in studied groups.

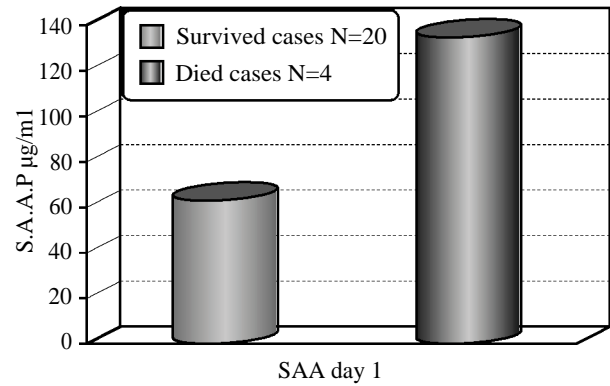


Fig. (2): Level of SAA in survived cases and died cases.

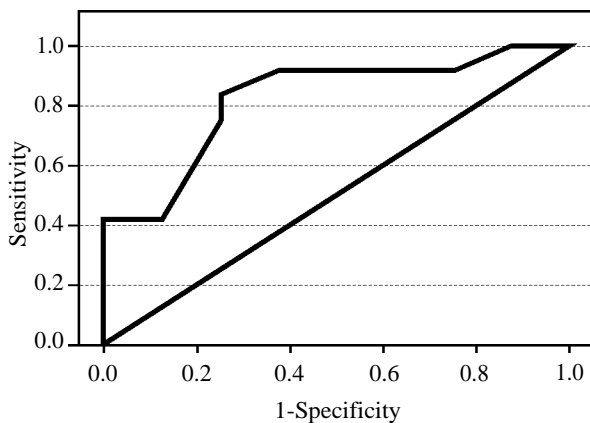


Fig. (3): ROC Curve for sensitivity and specificity.

## Discussion

Hypoxic-Ischemic Encephalopathy (HIE), remains a serious condition, causing significant mortality and long-term morbidity. In spite of major advances in monitoring technology and knowledge of fetal and neonatal pathologies perinatal asphyxia remains a major concern [10].

Prenatal, perinatal, and postnatal factors that have been implicated in the pathophysiology of brain lesions include hypoxic-ischemic insults, maternal infection with overproduction of cytokines and other proinflammatory agents, excessive glutamate release initiating the excitotoxic cascade, oxidative stress, growth factor deficiency, specific drugs, and maternal stress [11]. In addition, recent clinical studies support the existence of genetic susceptibility factors [12].

From the newborns with perinatal hypoxic ischemic encephalopathy 20-25% die within the newborn period and up to 25% of the survivors will exhibit retardation, cerebral palsy and epilepsy [13].

In hypoxic-ischemic cerebral injury the period between the insult and occurrence of cell death may be clinically feasible. However, this 'therapeutic window' is reliability brief and the time of insult "prenatal, natal, postnatal" may not be known exactly. The most effective treatment may be preventing cerebral injury before cell death [2].

A secondary cerebral energy failure occurs from 6-48hrs after the primary event and may involve mitochondrial dysfunction 2ry to extended reactions from primary insult. Some evidence suggests that circulatory and endogenous inflammatory cells/mediators also contribute to ongoing brain injury [14].

The acute phase response (serum amyloid A protein-SAA-is a part of which) is part of the innate defense system of an animal against trauma, inflammation, and infection [15].

The results of the present study revealed that, there was no statistically significant difference between HIE and control groups regarding (gender and mode of delivery).

No gender difference regarding hypoxic insult, this was in accordance with other authors [16] however, others [17,18] found a significant relation between HIE and male gender in their study as risk factors of HIE. They supposed that the male gender is highly vulnerable to any threatening factors such as increasing the risk of sepsis, bronchial hyperresponsiveness, atopy, and mortality of RDS, etc.

There was no statistically significant difference between HIE and control group regarding the mode of delivery this was in accordance with other authors [16] and [19] however, others [20,21] stated that cesarean section was highly associated with HIE and another one [22] found that HIE developed

in 76.5% of vaginal delivered cases, while cesarean section occurred in 23.5% of cases. The variation in different studies concerning mode of delivery may be explained by the fact that neonatal encephalopathy may originate early in the antepartum period in some cases of HIE.

The mean Apgar score of control group was statistically significant higher than HIE group at 1 and 5 min. [23] noticed that the first and fifth min. Apgar scores of neonates with HIE in their study were significantly lower than those Apgar scores of the control group.

Concerning pH, it was significantly lower in the HIE newborns compared to control newborns. This is in agreement with [24] as they reported low arterial umbilical cord pH had a strong, consistent, and temporal association with neonatal mortality and morbidity (composite of hypoxic ischaemic encephalopathy, seizures, and intraventricular haemorrhage or periventricular leucomalacia) and long term outcome (cerebral palsy).

There are few studies on SAA level as a marker for hypoxic ischemic encephalopathy and evaluation level SAA in different HIE clinical stages caused by perinatal asphyxia.

The results of the present study revealed the following information: SAA is significantly increased in newborn with perinatal compromise and asphyxia when compared with controls, the concentrations of SAA significantly correlate with the severity of encephalopathy, the serum level of SAA continues for the 1-week duration of the study and higher SAA expression is a marker for mortality in these infants.

These results were in agreement with [25] who reported that serum amyloid A as novel prognostic factors in a group of patients with ischemic stroke and shown the relationships between higher levels of inflammation markers, especially serum amyloid A, and the severity of acute ischemic stroke.

Jabor, 2006 [26] revealed that high concentrations of SAA in a non-symptomatic individual are indicative for impending vascular accidents.

SAA concentrations can reflect both the acute phase and the later-onset cell death. Previous studies demonstrated that surgical stress, length of operation and possible risks 72h after operation were best predicted by the SAA; during which time other markers of inflammation, such as IL-6 and leukocytic count, were normal [26].

In the present study SAA was capable of predicting mortality in cases. Previous studies suggested several sensitive proinflammatory markers for predicting outcomes of encephalopathy in neonates; however, they lacked specificity. Oppositely, SAA in our study proved to be very reliable in predicting severity of injury that led to mortality. With a sensitivity of 91% a specificity of 85% at cut off point = 93.7 g/g/ml and area under the curve = 0.81%.

These results were in agreement with [18] who revealed that at day 1 could significantly predict mortality of the studied cases with a sensitivity of 75% and a specificity of 100%.

It can be concluded that determining SAA level could be a useful marker for early HIE diagnosis in the full-term neonate and also in determining the grade of hypoxia and hence predicting the outcome.

#### *Acknowledgment:*

Deep gratitude and appreciation to Faculty of Medicine, Benha University for great help and valuable constructive cooperation in this work.

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## الملخص العربي

يعد نقص الاكسجين الناتج عن نقص التروية الدماغية من الحالات الخطرة التي تتسبب كثير من الوفيات وأمراض طويلة الامد، وعلى الرغم من التقدم الكبير في تكنولوجيا مراقبة ومعرفة امراض الجنين والاطفال حديثي الولادة فان الاختناق الوليدي لا يزال مصدر قلق كبير.

مصل الاميلويد الف هو احد علامات الالتهاب الحاد الذي يرتبط بشكل وثيق مع اصابات نقص التروية.

وقد أجريت هذه الدراسة على ٤٤ من الاطفال حديثي الولادة ٢٤، حالة يعانون من الاختناق الوليدي الناتج عن نقص التروية و٢٠ من الاصحاء وتم جمع عينات الدم من المرضى والاصحاء في اليوم الاول والسابع بعد الولادة وتم قياس مستوى الاميلويد الف باستخدام الاليزا.

وقد لوحظ زيادة تركيزات الاميلويد الف زيادة كبيرة في الحالات المرضية مقارنة بالاصحاء في اليوم الاول والسابع.

وكان تركيز الاميلويد الف في اليوم الاول اكبر في الحالات التي توفيت مقارنة بالناجين ومرتبطة بشكل وثيق مع شدة الاعتلال الدماغى.

الخلاصة: زيادة مستوى البرتين المصلى اميلويد الف استجابة لنقص التروية وترتبط هذه الزيادة ارتباطاً وثيقاً بشدة الاعتلال الدماغى.