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# Role of Matrix Metalloproteinase-9 in Neonatal Hypoxic-Ischemic Encephalopathy

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**Keywords:** Neonatal encephalopathy; Matrix metalloproteinase-9; Neonate

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**Abstract**

**BACKGROUND:** Neonatal encephalopathy is a heterogeneous syndrome characterised by signs of central nervous system dysfunction in the newborn. Matrix metalloproteinase-9(MMP-9) increases the blood-brain barrier permeability, and their inhibitors can reduce its damage. MMP-9 has been implicated specifically in cerebral ischemia.

**AIM:** To measure serum MMP-9 in neonatal hypoxic-ischemic encephalopathy and evaluate its correlation to the severity of early prediction and treatment.

**METHODS:** its case-control study. The serum concentration of MMP-9 was determined by ELISA in 100 hypoxic neonates and 50 healthy neonates of matched age and sex who served as controls.

**RESULTS:** In our present study the serum MMP-9 level was significantly higher at p = 0.0001 in hypoxic-ischemic full-term newborns (176.7 ± 68.7 ng/ml)as compared to control newborn (69.4 ± 34.85 ng/ml)and it was significantly higher at p = 0.0075 in hypoxic-ischemic preterm newborn (171.2 ± 132.9 ng/ml) when compared to control newborn (72.54 ± 36.74 ng/ml),also MMP-9 was significantly higher at Sarnat stage III at p = 0.0001.

**CONCLUSION:** Serum MMP-9 level was significantly higher in hypoxic-ischemic newborns, and significantly increased with severity, so we suggest that serum MMP-9 level is important for predicting neurological sequel and severity in neonatal encephalopathy.

## Introduction

Neonatal encephalopathy is a syndrome that has signs of central nervous system dysfunction in newborns and infants. Clinical suspicion of neonatal encephalopathy should be considered in any infant showing convulsions, an abnormal level of consciousness, feeding problems, apnea, aspiration, tone and reflex abnormalities, and hearing abnormalities [1], [2].

Perinatal asphyxia happens in 2 to 10 per 1000 newborn that are born at term, and neurological dysfunctions are present in 25% to 28% of the affected infants. Asphyxia is inversely related to gestational age and birth weight. It occurs in 9% of infants below thirty-six weeks of gestation and 0.5% of infants more than 36 weeks of gestation [3].

The incidence of hypoxic-ischemic encephalopathy in developed countries is 1.5 per 1000 live births, while it varies between 2.3-26.5 per 1000 live births in developing countries [4]. However, in most developing countries, including Egypt, the incidence and outcomes of hypoxic-ischemic encephalopathy are not well documented. One of the studies estimated the rate of neonatal mortality in Egypt as 25 per 1000 live births, almost all of the mortality occurring in the first week of life. Prematurity and its complications were responsible for the largest percentages of deaths (39%), followed by birth asphyxia and birth trauma as major causes [5].

Neonatal encephalopathy was once automatically referred to hypoxia-ischemia, but now it is well known that hypoxia-ischemia is one of many other possible mechanisms of neonatal encephalopathy. Whether a particular newborn's encephalopathy can be attributed to hypoxic-ischemic brain injury is often not clear [6].

Stringent criteria were needed by some investigator for using the term neonatal encephalopathy, as ≥ 2 symptoms of encephalopathy lasing over twenty-four hours [7]. However, others need only a low 5-minute Apgar score [8]. Using of Apgar scores alone is problematic, as it may be low due to analgesia used by the mothers or premature labour or falsely normal in some cases of acute hypoxia-ischemic insult, or can be inflated in actual clinical practice [9].

Neonatal encephalopathy is mostly because of dysfunction of the central nervous system in full and preterm infants when neonatal encephalopathy is due to hypoxic-ischemic brain injury (anoxic brain insult) it is preferred to use the term hypoxic-ischemic encephalopathy (HIE) [10].

Early Estimation of the severity degree of HIE is important for tailoring the medical intervention to decide whether or not to perform potentially neuroprotective therapies [11].

Matrix metalloproteinases are a group of proteases that physiologically adjust the extracellular matrix and basement membrane reconstruction [12].

Matrix metalloproteinase-9 increases the permeability of the blood-brain barrier. Matrix Metalloproteinases inhibitors can reduce blood-brain barrier damage [13].

Matrix metalloproteinase-9 has been included specifically in cerebral ischemia [14].

Neuroprotection following of MMP-9 inhibitors activation has been demonstrated previously in the brain of an adult after cerebral ischemia by using MMP-9 inhibitors [15]. To our knowledge, there are no studies evaluating the level or role of MMP-9 in neonatal hypoxic-ischemic encephalopathy.

Our study aimed to measure the serum level of Matrix metalloproteinases-9 in neonatal hypoxic-ischemic encephalopathy and evaluate its correlation to the severity for early prediction and treatment, which in turn will reduce the financial burden of the country.

## Subjects and Methods

### *Subjects*

The present study was conducted 150 newborns admitted to neonatal intensive care units in Nasser general hospital.

They were divided into 2 groups

*Group I:*

Included 100 hypoxic neonates 70 of them are full-term, and 30 are preterm with a mean gestational age of ± 38 wks for full-term and ± 34 wks for preterm, 54 are males, and 46 are females, regarding the mode of delivery 37 were delivered with caesarean section and 63 by spontaneous vaginal delivery. They were diagnosed as having HIE as evidenced by the presence of at least 2 of the following criteria:

- Evidence of fetal distress (abnormal fetal heart patterns and meconium-stained amniotic fluid).

- Apgar score < 3 at one minute or < 6 at 5 minutes.

- Evidence of neonatal respiratory distress.

- Umbilical cord arterial pH < 7.2 with base deficit > 10 mmol/l.

- Abnormal neurological signs on examination are denoting HIE according to Sarnat staging [16].

*Exclusion criteria*

- Neonatal sepsis.

- Blood group incompatibility.

- Babies born to preeclamptic mother.

- Infant of diabetic mothers (IDM).

*Group II*

Included 50 healthy neonates 35 of them were full-term and 15 were preterm, 20 of them were males, and 30 were females with a mean age of ± 39 wks for full-term and 35 wks for preterm. Regarding mode of delivery, 28 were delivered with cesarean section and 22 by normal vaginal delivery, without signs of perinatal asphyxia.

### ***Methods***

All newborns included were subjected to the following:

- *Full maternal history taking*

- *Detailed antenatal and perinatal history.*

- *Clinical examination including* 1. Gestational age assessment: modified Ballard scoring system [17]; 2. Apgar scores estimation at 1 & 5 minutes to assess the presence of perinatal asphyxia [18]; 3. Vital signs*:* Blood pressure; 4. Head circumference; 5. Anthropometric measures; 6. Full cardiac, chest, abdominal and neurological examination and 7. Detailed neurological examination including A) Level of consciousness; B) Activity; C) Neuromuscular examination: Tone, power, position and stretch reflex; D) Primitive reflexes (suckling, Moro, grasp, rooting, glabellar and neck rigidity reflexes); E) Irritability; F) Seizures (type, responses to anticonvulsant drugs) and G) Sarnat and Sarnat staging according to [16].

### *Laboratory investigations*

Venous or capillary blood samples were withdrawn on heparinised tubes for blood gas assessment. Moreover, peripheral blood samples were collected three ml venous blood sample was collected from each patient into a plain tube. After clotting for 30 minutes, serum was separated by centrifugation for 15 minutes at approximately 1000 x g, and samples were stored at -20°C till the assay.

### *Methods of assay*

Blood gases were analysed by the GEMpremier3000 system analyser.

### *Determination of serum MMP-9*

The quantitative determination of serum MMP-9 was done using the commercially available ELISA kit supplied by R&D Systems.

### *Statistical analysis*

Standard computer program SPSS for Windows, release 23 (SPSS Inc, USA) was used for data entry and analysis. All numeric variables were expressed as mean ± standard deviation (SD). Comparison of different variables in various groups was made using student t-test followed by Duncan's multiple range tests with $P < 0.05$ selected as the level of the statistical significance. Comparisons of multiple subgroups were made, and Data are presented as M ± SEM and analysed by one-way ANOVA followed by Tuckey Kramer post-test using Graph Pad Prism software. For all tests, a probability (p) less than 0.05 was considered significant.

## Results

As mentioned before this study included 100 neonates with HIE and 50 healthy neonates of matched age and sex who served as controls.

**Table 1: Descriptive Data of group I (HIE) (N = 100)**

| | Full Term N = 70 | | Preterm N = 30 | | P Value |
|---|---|---|---|---|---|
| Mean age (weeks) | 38.8 | ± 1.436 | 33.9 | ±0.852 | 0.078 |
| Mean weight (gram) | 3220 | ± 142 | 2205 | ±279.3 | 0.254 |
| Mean apgar 1 min | 2.9 | ± 0.9679 | 2.8 | ±0.8333 | 0.0987 |
| Mean apgar 5 min | 5.8 | ± 0.894 | 5.65 | ±0.8751 | 0.254 |
| Mean PH | 7.173 | ± 0.0233 | 7 | ±0.067 | 0.667 |
| Mean MMP-9 (ng/ml) | 176.7 | ± 168.7 | 171.2 | ±132.9 | 0.98 |

P < 0.05 was significant. Data are presented as mean ± SD; MMP-9: Matrix metalloproteinases-9; HIE: hypoxic-ischemic encephalopathy.

Descriptive, demographic and laboratory data of HIE patients and controls were shown in (Table 1, 2 and 3).

**Table 2: Deceptive data of group II (control) (N = 50)**

| | Full Term N = 35 | Preterm N = 15 | Pvalue |
|---|---|---|---|
| Mean age (weeks) | 39.93±11.335 | 35.25 ± 9.541 | 0.45 |
| Mean weight (gram) | 3981 ± 311 | 2002 ± 258 | 0.142 |
| Mean apgar 1 min | 7.133 ± 0.3519 | 6.65 ± 0.299 | 0.871 |
| Mean apgar 5 min | 9.267 ± 0.4577 | 8.787 ± 0.398 | 0.5412 |
| Mean PH | 7.373 ± 0.0045 | 7.11 ± 0.0023 | 0.0854 |
| Mean MMP- 9 (ng/ml) | 69.41 ± 34.85 | 72.54 ± 36.74 | 0.145 |

P < 0.05 was significant. -Data are presented as mean ±SD ;MMP-9: Matrix metalloproteinases-9.

Table 4 showed that serum MMP-9 was significantly higher in full-term cases than in controls.

**Table 3: Descriptive data of both group regarding gender and mode of delivery**

| | HIE N (%) = 100 | | | Control N (%) = 50 | | |
|---|---|---|---|---|---|---|
| | Full Term N (%) = 70 | Preterm N (%) = 30 | P Value | Full Term N (%) = 35 | Preterm N (%) = 15 | P Value |
| Gender (Male/Female | 38(54.2)/32(45.7) | 16(53.3)/14(46.6) | 0.95 | 11(31.4)/24(68.5) | 9(60)/6(40) | 0.214 |
| Mode of delivery (Normal/C-section) | 56(80)/14(20) | 7(23.3)/23(76.6) | 0.458 | 14(40)/21(60) | 8(53.3)/7(46.6) | 0.121 |

P < 0.05 was significant. -Data are presented frequency (percentage); HIE: hypoxic-ischemic encephalopathy.

Also, there was a significant difference in mean PH and Apgar scores at 1 and 5 minutes between full-term cases and controls as PH was significantly lower in cases than controls, while there was an increase in Apgar score at 1 and 5 minutes in controls.

**Table 4: Comparison between Full Term cases and control**

| | Cases (Full Term) N = 70 | Control (Full Term) N = 35 | P value |
|---|---|---|---|
| Mean age (weeks) | 38.8 ± 18.436 | 39.93 ± 19.335 | 0.087 |
| Mean weight (gram) | 3220 ± 142 | 3981 ± 311 | 0.74 |
| Mean apgar 1 min | 2.9 ± 0.9679 | 7.133 ± 0.3519 | 0.0001 |
| Mean apgar 5 min | 5.8 ± 0.894 | 9.267 ± 0.4577 | 0.0001 |
| Mean PH | 7.173 ± 0.0233 | 7.373 ± 0.0045 | 0.0001 |
| Mean MMP-9 (ng/ml) | 176.7 ± 68.7 | 69.41 ± 34.85 | 0.0001 |

P < 0.05 was significant; Data are presented as mean ±SD; MMP-9: Matrix metalloproteineases-9.

Table 5 showed that serum MMP-9 was significantly higher in preterm cases than in controls. Also, there was a significant difference regarding mean PH and Apgar scores as PH was significantly lower in cases than controls while there was an increase in Apgar score at 1 and 5 minutes in controls.

**Table 5: Comparison between Preterm cases and control**

| | Cases Preterm N = 30 | Control Preterm N = 15 | P value |
|---|---|---|---|
| Mean age (weeks) | 33.9 ± 0.852 | 35.25 ± 9.541 | 0.98 |
| Mean weight (gram) | 2205 ± 279.3 | 2002 ± 258 | 0.254 |
| Mean apgar 1 min | 2.8 ± 0.8333 | 6.65 ± 0.299 | 0.0001 |
| Mean apgar 5 min | 5.65 ± 0.8751 | 8.787 ± 0.398 | 0.0001 |
| Mean PH | 7 ± 0.067 | 7.11 ± 0.0023 | 0.0001 |
| Mean MMP-9 (ng/ml) | 171.2 ± 132.9 | 72.54 ± 36.74 | 0.0075 |

P < 0.05 was significant; Data are presented as mean ± SD.; MMP-9: Matrix metalloproteinases-9.

According to Sarnat stages of HIE, There was a significant difference in mean MMP-9 in different stages as it was significantly higher in stage 3 when compared with stage 2 and 1 (Table 6).

**Table 6: Comparison between MMP-9 levels in HIE group regarding Sarnat stages**

| | Stage 1 | Stage 2 | Stage 3 | P-value |
|---|---|---|---|---|
| Mean MMP-9 (ng/ml) | 123.5 ± 74.54 | 134.2 ± 89.39 | 189.2 ± 139.8 | 0.0001 |

P < 0.05 was significant; Data are presented as mean ± SD; MMP-9: Matrix metalloproteinases-9; HIE: hypoxic-ischemic encephalopathy.

## Discussion

Neonatal encephalopathy is a syndrome that has signs of central nervous system dysfunction in newborns and infants. Clinical suspicion of neonatal encephalopathy should be considered in any infant showing convulsions, an abnormal level of consciousness, feeding problems, apnea, aspiration, tone and reflex abnormalities, and hearing abnormalities [1], [2].

Prematurity and its complications were responsible for the largest percentages of deaths (39%), followed by birth asphyxia and birth trauma as major causes [5].

Early Estimation of the severity degree of HIE is important for tailoring the medical intervention to decide whether or not to perform potentially neuroprotective therapies [11].

Matrix metalloproteinases are a group of proteases that physiologically adjust the extracellular matrix and basement membrane reconstruction [12].

Matrix metalloproteinase-9 increases the permeability of the blood-brain barrier. Inhibitors of matrix Metalloproteinases can reduce the damage to the blood-brain barrier [13].

Matrix metalloproteinase-9 has been implicated specifically in cerebral ischemia [14].

Neuroprotection following inhibition of MMP-9 activation has previously been demonstrated in the adult brain after cerebral ischemia by using MMP-9 inhibitors [15].

The present study was conducted 150 newborns admitted to neonatal intensive care units in Nasser general hospital.

Included 100 hypoxic neonates 70 of them are full-term, and 30 are preterm with a mean gestational age of ± 38 wks for full-term and ± 34 wks for preterm, 54 are males, and 46 are females, regarding the mode of delivery 37 were delivered with cesarean section and 63 by spontaneous vaginal delivery.

Fifty healthy neonates 35 of them were full-term, and 15 were preterm, 20 of them were males, and 30 were females with a mean age of ± 39 wks for full-term and 35 wks for preterm. Regarding mode of delivery, 28 were delivered with cesarean section and 22 by normal vaginal delivery, without signs of perinatal asphyxia.

Apgar score is one of the essential criteria for the diagnosis of perinatal asphyxia. Moreover, Apgar scores at 1 and 5 minutes were not significantly different between the cases and control in group l and group 2 newborns (Table 1 and 2). Table 4 & 5 showed that Apgar score at 1 minute (2.9 ± 0.9679) in full-term HIE newborn which was significantly lower at p = 0.0001 compared to control newborn (7.1 ± 0.351) ,and (2.8 ± 0.83) in preterm HIE newborn which was significantly lower compared to control newborn (6.6 ± 0.29), also at 5 minutes, it was (5.8 ± 0.89) in full-term HIE newborn which was significantly lower at p =0.0001 compared to control full-term newborn (9.26 ± 0.45) and it was (5.65 ± 0.87) in preterm HIE newborn who was significantly lower compared to control preterm newborn (8.78 ± 0.39). These results are in agreement with Mostert al., [19] who reported that Apgar score could differentiate between the control groups and hypoxic groups.

In our study pH was significantly different in preterm HIE newborns (7.0 ± 0.06) compared to control newborns (7.1 ± 0.0023) at p = 0.0001, also there was significant difference in HIE full-term newborns PH (7.1 ± 0.02) compared to control newborns (7.3 ± 0.0045) at p = 0.0001.These results are in a disagreement with khan et al., [20] who reported the insignificant difference in pH level in hypoxic and control groups. On the other hand this agrees with *Johnston et al.,* [21]*,Ferriero,* [22] *and Fahey and King,* [23] as they reported that 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 in the form of cerebral palsy.

In our present study the mean serum MMP-9 level was significantly higher at p = 0.0001 in HIE full-term newborns (176.7 ± 68.7 ng/ml)as compared to control newborn (69.4 ± 34.85 ng/ml)and level of serum MMP-9 was significantly higher at p = 0.0075 in HIE preterm newborn (171.2 ± 132.9 ng/ml) when compared to control newborn (72.54 ± 36.74 ng/ml). These results are in agreement with *Ashai et al.,* [15] who reported that on studying animal models of cerebral ischemia MMP-9 is regulated up early in injured tissue suggesting its involvement in neuronal death and brain damage. Moreover, MMP-9 inhibition through knockout models or drug treatments reduces infarction volume. On the other hand, this disagrees with *Sunagawa et al.,* [24] who studied serum levels of MMP-9/TIMP on the day of birth in asphyxiated newborns and they demonstrated no statistically significant difference in MMP-9 levels between asphyxiated newborns and controls.

According to Sarnat staging of HIE serum level of MMP-9 was 123.5 ± 74.54 ng/ml, 134.2 ± 89.39 ng/ml and 189.2 ± 139.8 ng/ml in stages I, II and III respectively, which was significantly higher at p = 0.0001 with worsening of symptoms and signs in hypoxic newborns. These results are in agreement with *Sunagawa et al*., [24] who reported that MMP-9 levels in asphyxiated newborns with neurological insult on birthday were significantly higher than those in asphyxiated newborn without sequelae also he suggested that high concentrations of MMP-9 on day of birth could injure the blood-brain barrier and causes

irreversible brain damage. Also, *Tsuji et al.,* [25] who tested MMP-9 upregulation after focal cerebral ischemia, found that patients with high plasma levels of MMP-9 experience more brain injury with poor outcomes after cerebral ischemia.

In conclusion, serum MMP-9 level is significantly higher in hypoxic-ischemic newborns, and significantly increased with severity, so we suggest that serum MMP-9 level is important for predicting neurological sequel and severity in neonatal encephalopathy.

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