

**INCIDENCE OF NEONATAL CONVULSION/SEIZURES ASSOCIATED WITH PRIMARY DISTURBANCE OF CALCIUM, PHOSPHORUS, AND MAGNESIUM IN UNIVERSITY OF PORT HARCOURT TEACHING HOSPITAL (UPTH)**

**ABSTRACT**

Neonatal seizures are abnormal electrical discharge in the neonates that usually manifest as stereotyped muscle activity of autonomic change. Seizure occur in up to 1.4% of mature infant and 20% of pre-mature infant. Neonate in university of Port Harcourt Teaching Hospital were used for the study, 86 blood samples were collected from the children department, the children include both male and female within the Age range of 1 day to 7 day. Out of the total of 86 sample analyzed for calcium, magnesium and Phosphorus from the results obtained from both calcium and magnesium failed below the reference value, that indicate that calcium and magnesium where the major causes of the neonatal seizures.

**1.0 INTRODUCTION**

Neonatal seizures are abnormal electrical discharge in the neonates that usually manifest as stereotyped muscle activity of autonomic change. The most common causes of Neonatal seizures may occur before delivery or after delivery. Seizure occur in up to 1.4% of mature infant and 20% of pre-mature infant, Neonatal seizure occur as a result of ischemic stroke in Neonate with polycythemia with thrombophiua, a genetic disorder or with severe hypotension., infections such as meningitis and sepsis may cause seizure. In such cases seizure is usually accompanied by

others symptoms and signs [1]. Group B streptococci and gram-negative bacteria are common causes of such infections in neonates. Encephalitis due to cytomegalovirus, herpes, simplex virus, rubella virus, treponema pallidum, or Toxoplasma gondii can also cause seizures.

Neonatal seizures are usually focal and are difficult to recognize; common manifestations include migratory clonic jerks of extremities, alternating hemi-seizures, and primitive subclonic seizure (which cause respiratory arrest, chewing movements, persistent eye dilation, myoclonic movements, and episodic changes in muscle tone) [2].

Generalized tonic-clonic seizures are uncommon. Neonatal seizures have a vast number of possible aetiologies; common aetiologies include neonatal encephalopathy (60%), intracranial infection (5-10%), intracranial hemorrhage, developmental malformations and correctable metabolic disturbances such as hypoglycemia and derangement of electrolytes (example, sodium, calcium and magnesium) [3].

These metabolic disturbances, since they are correctable, early diagnosis is necessary for timely institution of appropriate treatment and important in determining clinical outcome. This project is therefore aimed at early determination of electrolytes that are associated with neonatal seizures.

## **2.0 MATERIALS AND METHODS**

In this cross-analytical study, 86 neonates diagnosed with seizure disorder were used as the subjects. 3mls of blood were collected into heparinized anti-coagulant bottles from the paediatric phlebotomy unit. Each batch collected was taken to me in the laboratory for analysis.

### **2.1. PHOSPHORUS**

#### **Materials and Equipments**

Centrifuge, Hand glove, Anticoagulant free bottles, pipettes (Pasteur pipettes), Test tube racks, spectrophotometer, micro-pipettes (1000pI), phosphorus Reagent (ROT), phosphorus standard (STD), QC, Distilled water, cuvette.

### Reagent Contents

RGT: 2 x 100ml Reagent

Animoniurnheptamolybdate	-	0.3mmol/L
Sulphuric acid (PHL1.0)	-	160mmol/l
Detergent	—	1%

### Activators and stabilizers

STD: 1 x 5ml standard 32

Phosphorus - 10mg/dl or 3.2mmolk

### (Photometric UV test for the Determination of phosphorus)

#### Principle of the Test

Phosphate reacts with molybdate in strong acid medium to form a complex. The absorbance of this complex in the near UV is directly proportional to the phosphate concentration. Reaction principle (simplified).



#### Procedure

1. Four test tubes were arranged into the test tube rack and labeled as test (T), Blank (B) standard (s) and Qc (C)
2. 10 $\mu$ l. of sample was pipetted into the sample tube 33
3. 10  $\mu$ l. of standard was added to standard tube
4. 10  $\mu$ l. of QC was added into the QC tube

5. 1000 $\mu$ L (1.0l) of reagent (RGT) was added to each of the tubes
6. The tubes were mixed, incubated at Room temperature for 1 minute

**Table 1: PROCEDURE TABLE**

Tubes	Reagent Blank	Sample	STD	QC
Sample (s)		10 $\mu$ l	-	-
Standard (STD)			10 $\mu$ l.	
QC	-	-	-	10 $\mu$ l
Reagent	1000 $\mu$ l	1000 $\mu$ l	1000 $\mu$ l	1000 $\mu$ l (1ml)

### Calculation

$$\text{Concentration } C = 10 \times \frac{A_{\text{sample}}}{A_{\text{STD}}} \text{ mg/dl}$$

$$C = 3.2 \times \frac{A_{\text{sample}}}{A_{\text{STD}}} \text{ mmol/L}$$

### Normal Values

Children 4.0 – 70mg/dl – 1.30 – 2.26mm 01/L

## 2.2 CALCIUM (CA)

### Materials and Equipment

Spectrophotometer, test tubes, Distileld water, calcium kit, micro-pipette, test tube rack, 1ml pipettes

## Reagent Composition

R<sub>1</sub> Buffer

2 – amino -2- methyl

Propan -1.01

-3.5m 01/L, PH 10.7

## R2 Chromogen

0 - Cresolphthalein complexone -0.16mmol 35

8 - Hydroxyguinoline - 6.89mmol/L

## Colorimetric Method

**Principle of the Test:** Calcium ions form a violet complex with 0- cresolphthalein complexone in alkaline medium

## Procedure

1. Four test tubes were arranged in the test tube rack as Reagent Blank, standard, sample and Qc.
2. 25 $\mu$ l (0.025ml) of Distilled water was added to the blank tube.
3. 25  $\mu$ l of standard was added to the standard tube.
4. 25  $\mu$ l of sample was added to the sample tube.
5. 25  $\mu$ l of QC was added to Qc tube
6. 0.5ml of reagent (R<sub>1</sub>) was added to each of the tubes
7. 0.5ml of R2 was added to each tube.

8. Each tubes were mixed, the absorbance of the sample (A sample), standard (A Standard), QC was read against the reagent blank of 5 to 50 minutes at room temperature at a wavelength of (550-590nm).

**Table 2: PROCEDURE TABLE**

Tubes	Reagent Blank	Sample	STD	QC
<b>Sample</b>	-	-	25 $\mu$ l.	-
<b>DH<sub>2</sub><sup>0</sup></b>	25 $\mu$ l.	-	-	-
<b>Std</b>	-	25 $\mu$ l.	-	-
<b>QC</b>	-	-	-	25 $\mu$ l.
<b>R<sub>1</sub></b>	0.5ml	0.5ml	0.5ml	0.5ml
<b>R<sub>2</sub></b>	0.5ml	0.5ml	0.5ml	0.5ml

### Calculation

$$\text{Conc. (mmol/L)} = \frac{A_{\text{sample}} \times \text{Standard concentration}}{A_{\text{standard}}}$$

$$\text{Conc. (mg/dl)} = \frac{A_{\text{sample}} \times \text{Standard concentration}}{A_{\text{standard}} \times 37}$$

### Normal Values

Serum = 2.02 – 2.60mmol/l

(8.10 – 10.4mg/d).

## 2.3 MAGNESIUM

### Materials And Equipment Used

Spectrophotometer micro pipette, 10ml pipette, test tubes, test tube rack, magnesium kit

(standadr and Reagent (A) and sample.

## Contents And Composition Of Reagent

A. Reagent 4x50ml. calmagite  $80\mu\text{mol/L}$

EGTA  $60\mu\text{mol/L}$ , diethylamine 0.2mo 1/h

S. Magnesium standard 1 x 5ml.

Magnesium  $2\text{mgld}/(0.82\text{mmO/k})$

## Colorimetric Method

**Principles of the Test:** Magnesium in the sample reacts with calmagite in alkaline medium formign a coloured complex that can be measured by spectrophotometer in the reagent to remove calcium interference.

## Procedures

1. Four test tubes int eh test tue rack labelled blank, samle, standard and Q.C.
2.  $10\mu\text{l}$  of standard was added into standard tube
3.  $10\mu\text{l}$  of sample was added into sampel tube
4.  $10\mu\text{l}$  of QC was added to QC tube.
5. 1ml off reagent (A) was added to each of the tubes.
6. The tubes were mixed and allowed to stand for 2 minutes at room temperature.
7. The absorbance of standard, sample and QC was read at 520nm against the reagent Blank.

**Table 3: Procedure Table**

Tubes	Reagent Blank	Sample	STD	QC
<b>Magnesium standard</b>	-	$10\mu\text{l}$ .	-	-

<b>Sample</b>	-	-	10 $\mu$ l.	-
<b>QC</b>	-	-	-	10 $\mu$ l.
<b>Reagent</b>	1.0ml	1.0ml	1.0ml	1.0ml

### Calculation

$$\frac{A_{\text{sample}} \times \text{standard}}{A_{\text{standard}}} = (\text{Sample concentration})$$

$$\frac{A_{\text{sample}} \times 2}{A_{\text{standard}}} = \text{mg/dl}$$

$$\frac{A_{\text{sample}} \times 0.82}{A_{\text{standard}}} = \text{mmol/dl}$$

### Reference Values

Serum and plasma 1.7 – 2.4mg/dl = 0.70 – 0.98mmol/140

## 3.0 RESULTS AND DISCUSSION

**Table 4: For Calcium**

(x)	Freq. (f)	Mode	Mean (x)	Standard Deviation ( $\delta$ )
<b>1.4 – 1.6</b>	13	`		
<b>1.7 – 1.9</b>	15	51		
<b>2.0 – 2.3</b>	22		1.82	



**Table 5: Magnesium**

	Ranking (x)		(x)		(x)
<b>1</b>	10	34	0.5	67	0.5
<b>2</b>	0.8	35	0.8	68	0.6
<b>3</b>	1.0	36	0.5	69	0.6
<b>4</b>	0.7	37	0.4	70	0.8
<b>5</b>	0.5	38	0.9	71	0.6
<b>6</b>	0.6	39	0.4	72	0.6
<b>7</b>	0.8	40	0.6	73	0.5
<b>8</b>	0.9	41	0.7	74	0.7
<b>9</b>	0.7	42	0.8	75	1.0
<b>10</b>	0.5	43	0.7	76	0.8
<b>11</b>	0.4	44	0.6	77	0.4
<b>12</b>	0.9	45	0.6	78	0.8
<b>13</b>	0.4	46	0.5	79	0.7
<b>14</b>	0.7	47	1.0	80	0.8
<b>15</b>	0.9	48	0.6	81	0.7
<b>16</b>	0.8	49	0.5	82	0.8
<b>17</b>	1.1	50	0.7	83	1.0
<b>18</b>	0.6	51	0.9	85	0.5
<b>19</b>	0.7	52	0.7	85	0.5

<b>20</b>	0.5	53	0.6	86	0.8
<b>21</b>	0.6	54	0.5		
<b>22</b>	0.6	55	0.8		
<b>23</b>	0.8	56	0.7		
<b>24</b>	0.6	57	0.6		
<b>25</b>	0.7	58	0.4		
<b>26</b>	0.8	59	1.0		
<b>27</b>	0.7	60	1.1		
<b>28</b>	1.2	61	0.8		
<b>29</b>	0.8	62	0.6		
<b>30</b>	0.6	63	0.8		
<b>31</b>	0.5	64	0.7		
<b>32</b>	0.4	65	0.6		
<b>33</b>	0.7	66	0.8		

$$\sum x = 59.4$$

$$\text{Mean } \bar{x} = \frac{\sum}{n}$$

$$= \frac{59.4}{86}$$

$$= \mathbf{0.69}$$

Standard deviation

$$\delta = \text{Log } x = \sqrt{\frac{\sum (10x - \log x)^2}{n - 1}}$$

$$\sqrt{\frac{(2.023 - 1)^2}{86 - 1}}$$

$$\sqrt{\frac{(1.023)^3}{85}}$$

$$\sqrt{\frac{1.0465}{85}} = 0.012$$

$$\delta = 0.046$$

**Table 6. Magnesium**

(x)	Freq. (f)	Mode	Mean (x)	Standard Deviation ( $\delta$ )
<b>0.4 – 0.6</b>	39			
<b>0.7 – 0.9</b>	38	39		
<b>1.0 – 1.9</b>	9			
			0.69e	0.012

**Table 7: Phosphorus**

	Ranking (x)		(x)		(x)
<b>1</b>	1.9	34	2.4	68	2.4
<b>2</b>	1.8	35	2.9	69	1.8
<b>3</b>	3.0	36	3.2	70	2.4
<b>4</b>	2.1	37	2.4	71	3.1
<b>5</b>	1.8	38	2.8	72	2.7

<b>6</b>	2.1	39	3.1	73	2.6
<b>7</b>	2.4	40	2.6	74	2.3
<b>8</b>	1.9	41	2.8	75	2.4
<b>9</b>	2.3	42	2.3	76	3.1
<b>10</b>	3.0	43	2.6	77	2.5
<b>11</b>	1.8	44	3.0	78	2.5
<b>12</b>	2.5	45	2.6	79	2.5
<b>13</b>	2.9	46	3.0	80	1.8
<b>14</b>	1.7	47	2.5	81	2.0
<b>15</b>	2.4	48	2.9	82	1.7
<b>16</b>	2.4	49	2.5	83	1.6
<b>17</b>	2.4	50	2.5	84	2.4
<b>18</b>	2.3	51	1.6	85	2.8
<b>19</b>	2.4	52	1.6	86	2.7
<b>20</b>	2.0	53	2.9		
<b>21</b>	2.1	54	1.8		
<b>22</b>	1.8	55	2.0		
<b>23</b>	2.6	57	2.6		
<b>24</b>	2.4	57	2.6		
<b>25</b>	2.6	58	2.0		
<b>26</b>	2.3	59	2.3		
<b>27</b>	2.7	60	2.3		
<b>28</b>	1.8	61	1.6		

<b>29</b>	2.4	62	1.7
<b>30</b>	2.9	63	2.3
<b>31</b>	2.5	64	2.0
<b>32</b>	1.6	65	2.1
<b>33</b>	1.7	66	2.6

$$\sum x = 200.6$$

$$\text{Mean } \bar{x} = \frac{\sum x}{n}$$

$$= \frac{200.6}{86} = 2.33$$

**Standard deviation  $\delta$**

$$\delta = \text{Log } x = \sum \frac{(10x - \log x)^2}{n - 1}$$

$$\sqrt{\frac{(2.30 - 0.37)^2}{85}}$$

$$\sqrt{\frac{3.75}{85}}$$

$$\sqrt{\frac{12.64}{85}} = 0.03$$

**Table 8: For Phosphorus**

(x)	Freq. (f)	Mode	Mean (x)	Standard Deviation ( $\delta$ )
<b>1.6 – 1.9</b>	19			

<b>2.0 – 2.5</b>	42	42
<b>2.6 – 2.9</b>	18	
<b>3.0 – 3.3</b>	7	2.33
		0.03

## DISCUSSION

The results obtained from the study shows that, the seizure diagnosed by the pediatrician resulted from low level of calcium and magnesium concentration in the neonate's plasma.

Therefore, the results revealed that neonatal seizure is mainly caused by hypocalcemia and hypomagnesemia. This is consistent with the work of Mcinerney et al, 2008 [4] [5].

Also the study show that increase in phosphorus may lead to decrease in calcium and magnesium as the mean value of phosphorus above the reference value.

## 4.0 CONCLUSION

This work revealed a significant low level in calcium and magnesium.

There is an exceptionally important role for patient education pregnant mothers on diet support, electrolytes screening, culture and sensitivity, blood Glucose should be a routing test for all pregnant mothers.

Also the neonates diagnosed of seizure should be treated for hypocalcemia and hypomagnesemia by the paediatrician.

It can be safely concluded that pregnant mothers should develop the habit of attending antenatal clinic, so that the baby state of health can be closely monitored within this period

## RECOMMENDATION

Enlightenment campaign on neonatal seizure associated with electrolyte disturbance should be done using various information media as Television, Newspaper, Radio and seminars. Further work may be necessary on neonatal seizures such as glucose test, neurocranial x-ray, sodium determination etc.

## REFERENCES

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