

# Calculated Ionized Calcium & Actual Ionized Calcium in Preeclampsia

Faisal Gh. Al-Rubaye MBChB, MSc; PhD.

## **Abstract**

**Background:** Preeclampsia is a form of high blood pressure manifested during pregnancy; however, its etiology is unknown. Also, the status of ionized calcium (Cai) during pregnancy and its complication preeclampsia have not been described adequately. In addition to calculation method described for Cai, the calcium-binding dye murexide has become a widely-used tool for measuring changes in the ionized calcium (Cai) concentration in biological systems.

**Objective:** to demonstrate the level of Cai during preeclampsia with respect to normal pregnancy; and to demonstrate the correlation between calculated Cai and actual Cai.

**Subject and methods:** the present study is a case-control study conducted during the period from February 2007 until the end of June 2007, which includes measurement of total, corrected and ionized calcium (Cai) in 60 patients with preeclampsia that are classified according to gestational age into preeclamptics in the second trimester G1 (n=30) and preeclamptics in the third trimester G2 (n=30).

The results are compared with 60 apparently healthy pregnant controls that are classified according to gestational age into two groups G3 (n=30) and G4 (n=30).

**Results:** the serum corrected total calcium; serum calculated ionized Cai and actual Cai are significantly reduced in preeclamptics in the third trimester (G1) compared with normal pregnant (G4) ( $P < 0.001$ ) and even when compared with preeclamptics in the second trimester (G2). The same significant reduction in serum corrected total calcium ( $P < 0.001$ ); but not in serum ionized Ca (calculated and actual Cai) is found in preeclamptics in the second trimester (G2) compared with normal pregnant (G3). Both calculated Cai and actual Cai are significantly correlated ( $r = 0.7$ ,  $P < 0.001$  in all study groups apart from preeclamptic in the second trimester where  $r = 0.5$ ,  $P < 0.001$ ).

**Conclusion:** all preeclamptics have certain factors that reduce vasodilation, enhance vasospasm. This is supported by the finding of low ionized calcium which is essential for the synthesis of endothelial-derived NO. A mathematical equation can be used in clinical practice for expressing ionized calcium.

**Key words:** preeclampsia, calculated ionized calcium, actual ionized calcium.

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## **Introduction**

Preeclampsia is defined as the onset of hypertension and the presence of proteinuria during pregnancy, usually occurring after the 20th week of gestation in a previously normotensive woman and resolving completely by the sixth week after delivery<sup>(1,2)</sup>.

The dynamics of calcium homeostasis are in fact substantially

altered in pregnancy<sup>(3)</sup>. It is generally accepted that the ionized calcium ( $\text{Cai}^{+2}$ ) is the physiologically active form of calcium in the blood<sup>(3)</sup>. Investigation of  $\text{Cai}^{+2}$  changes in various disease states have been reported, in which it was measured by ion selective electrode<sup>(4)</sup>, but a few reports of  $\text{Cai}^{+2}$  measurement in disease are available by EDTA titration method<sup>(5)</sup>. This paper present data on calcium homeostasis, correlation of ionized calcium measured directly with that calculated from total calcium after correction to total protein and albumin for 60 preeclamptic patients as compared to those of 60 healthy pregnant.

Dept. Chemistry & Biochemistry, College of Medicine, Al-Nahrain University.

Address Correspondence to: Dr. Faisal Gh. Al-Rubaye .

E- mail: [faisal3ghazi@yahoo.com](mailto:faisal3ghazi@yahoo.com)

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### **Subjects & Methods**

A case-control study was conducted during the period from February 2007 until the end of June 2007 on sixty patients with preeclampsia (PE) who attending the Obstetric Consultant-Clinic, Antenatal Clinic, and Labor Ward at Al-Kadhimiya Teaching Hospital, for re-evaluation of newly diagnosed PE, or for delivery. Inclusion criteria were hypertension (absolute BP of 140/90 mmHg twice over 4 hr without prior comparison) <sup>(6)</sup> and proteinuria (21.5 mg of urinary protein per mmol creatinine) <sup>(7)</sup>.

Depending on the gestational age, the patients were divided into two groups:

#### **1. Preeclamptics in the second trimester (G1):**

Includes thirty preeclamptics in their second trimester of pregnancy with an age range was from 18 to 37 years and a gestational age of 20 to 28 weeks as seen in Table 1.

#### **2. Preeclamptics in the third trimester (G2):**

Includes thirty preeclamptics with an age range was from 18 to 40 years and a gestational age range from 29 to 40 weeks as seen in Table 1.

Sixty apparently healthy pregnant attending the Antenatal clinic, and Labor Ward at Al-Kadhimiya Teaching Hospital, for re-evaluation of their pregnancy, or for delivery, matches preeclamptic groups regarding the age, gestational age and depending on the gestational age, the apparently healthy pregnant were subdivided into two groups:

#### **3. Control pregnant in the second trimester (G3):**

They were thirty apparently healthy pregnant in the second trimester of

pregnancy with an age of 15 to 38 years and a gestational age range of 20 to 28 weeks as seen in Table 1.

#### **4. Control pregnant during the third trimester (G4):**

They were thirty pregnant in the third trimester of pregnancy with an age range of 18 to 35 year and a gestational age range of 29 to 40 weeks as seen in Table 1.

Any patients with other medical illnesses that may have an effect on the measured parameters were excluded from the study, such medical illnesses are cardiac, hepatic, endocrine, metabolic diseases, smoking and alcoholism.

Ten milliliters of venous blood were withdrawn into plane test tube from each patient and control, in supine position, without application of tourniquet. The samples were left to clot at room temperature, centrifuged, and the separated sera were transferred into Eppendorf tube and stored at  $-20^{\circ}$  C until analysis of calcium, which was done within one month after collection <sup>(5)</sup>.

The total serum calcium levels were measured using atomic *absorption spectrophotometer* <sup>5</sup>. The *Corrected serum calcium* was calculated according to the formula described by Gowenlock <sup>(8)</sup>:

Adjusted calcium (mmol/L) = Measured calcium concentration (mmol/L) + 0.02 [40 – albumin concentration (g/L)].

Instead of obtaining a crude correction for measured calcium, the same data can be used to calculate the *ionized calcium* according to the formula described by Gowenlock <sup>(8)</sup>:

$$\text{Ionized calcium (mmol/L)} = \frac{\text{measured calcium (mmol/L)} - K'/12 \times 60}{K'+60}$$

where

$$\text{total protein (g/L)} + \times K' = 0.19 \text{ albumin (g/L).}$$

The *actual ionized calcium* was determined by EDTA compleximetric titration method using murexide as an indicator that was employed by Gowenlock<sup>(8)</sup>.

**Results**

The *serum total corrected and ionized calcium concentrations (actual and calculated)* were significantly lowered in the preeclamptic women in third trimester G2 group as compared to healthy controls in the third trimester G4 [P<0.001] and even when compared to the preeclamptic in the second trimester

G1 [P<0.001] as seen in Table 2. The same significant reduction in *corrected but not ionized calcium* was noticed in the second trimester group G1 when compared to the healthy pregnant in the second trimester group G3 [P<0.001 for corrected calcium but greater than 0.05 for ionized calcium] as seen in Table 2. There was no significant difference in *corrected and ionized serum calcium* values between healthy pregnant in each group [P > 0.05].

**Correlation between calculated Cai and actual Cai:**

A significant positive correlation between actual and calculated serum ionized calcium was noticed in different studied groups, in preeclamptics G1 (r=0.7, P < 0.001); and G2 (r=0.5, P < 0.001) also in pregnant control groups G3 and G4 (r=0.7, P < 0.001for both) respectively as in Figures 1, 2, 3, and 4.

**Table 1: Demography of different preeclamptics and healthy pregnant groups.**

Group	G1	G2	G3	G4
No	30	30	30	30
Age / year (Mean ± SD)	26.1 ± 6.4	25.1 ± 6.9	24.6 ± 4.5	24.8 ± 4.6
Age range (years)	(18-37)	(18-40)	(15-38)	(18-35)
Gestational age / week (Mean ± SD)	26.3 ± 1.5	35.6 ± 1.9	25.5 ± 1.8	34.6 ± 2.1
Gestational age range (years)	(20-28)	(29-40)	(20-28)	(29-40)

**Table 2: The mean value & standard deviation of calcium (total, corrected Ca<sup>+2</sup>, actual ionized Ca<sup>+2</sup>, calculated ionized Ca<sup>+2</sup>) in the sera of different preeclamptic and pregnant control groups.**

Variable	G1	G2	G3	G4
Total serum calcium (mmol/L)	2.5 ± 0.05	2.4 ± 0.09	2.5 ± 0.1	2.5 ± 0.1
Corrected total serum calcium (mmol/L)	2.3 ± 0.05 <sup>**</sup>	2.2 ± 0.09 <sup>*§</sup>	2.6 ± 0.1	2.6 ± 0.1
Actual serum ionized calcium (mmol/L)	1.9 ± 0.05	1.8 ± 0.12 <sup>*§</sup>	1.9 ± 0.14	2 ± 0.04
Calculated serum ionized calcium (mmol/L)	1.2 ± 0.08	1.1 ± 0.05 <sup>*§</sup>	1.2 ± 0.05	1.2 ± 0.05

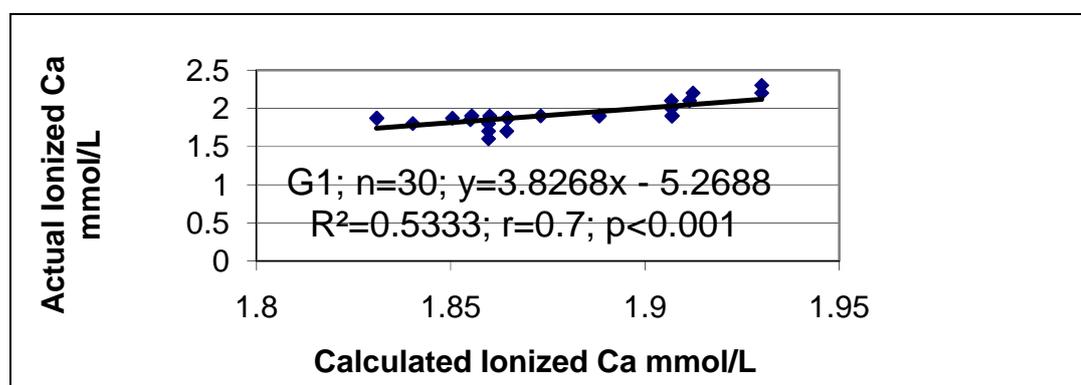
**G1 & G2: Preeclamptics in the second & third semesters of pregnancy.**

**G3 & G4: normal pregnant in the second & third semesters of pregnancy.**

\* T-test; G2 versus G4, P < 0.001

§ T-test; G2 versus G1, P < 0.001

\*\* t-test; G1 versus G3, P < 0.001



**Figure 1: Correlation between actual and calculated serum ionized calcium in second trimester preeclamptics.**

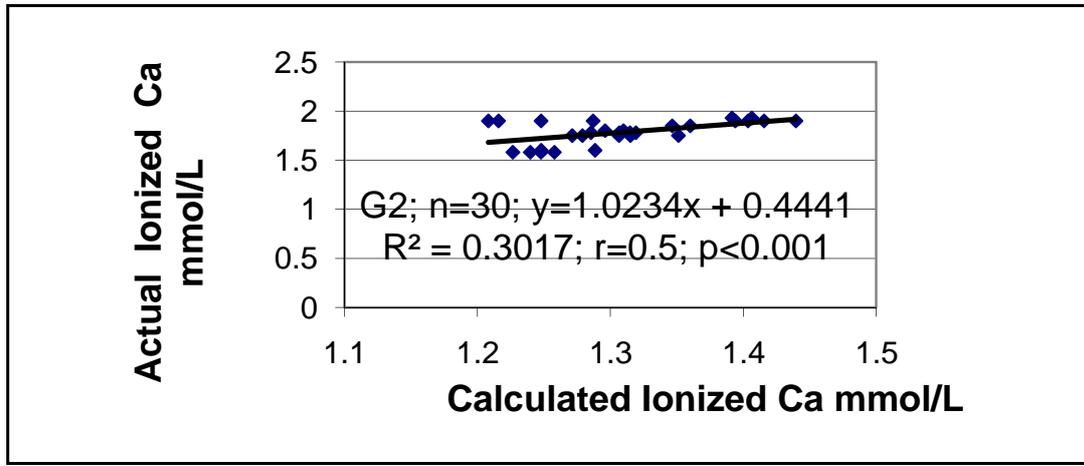


Figure 2: Correlation between actual and calculated serum ionized calcium in third trimester preeclampsics.

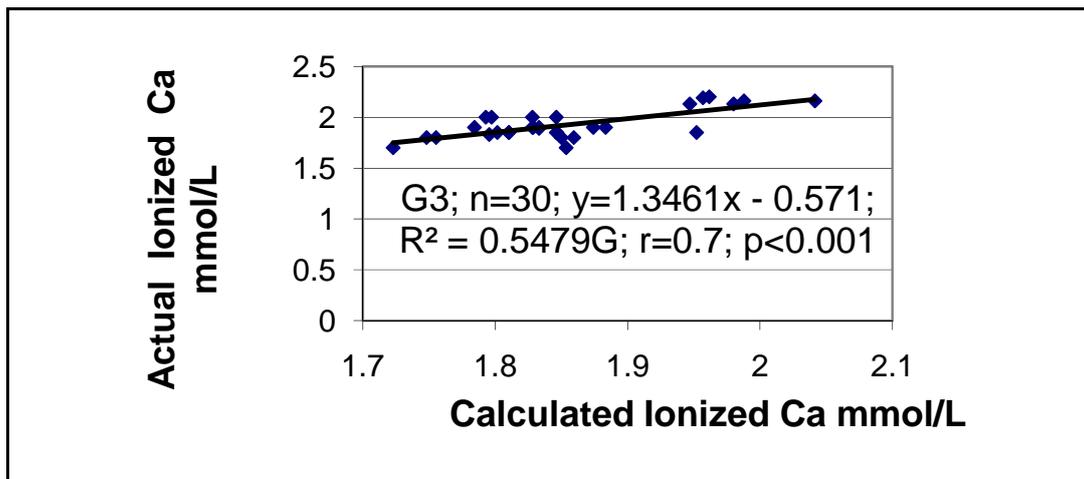


Figure 3: Correlation between actual and calculated serum ionized calcium in second trimester pregnant controls.

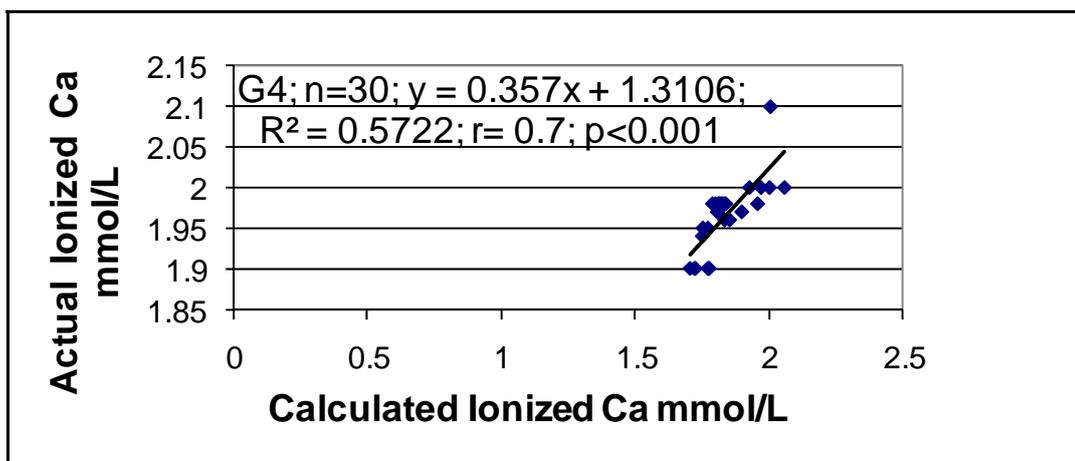


Figure 4: Correlation between actual and calculated serum ionized calcium in third trimester pregnant controls.

### Discussion

Regarding the ionized fraction of calcium which is crucial for the synthesis of vasoactive substances in the endothelium as prostacyclin and nitric oxide<sup>(9)</sup>. The finding of significant reduction in this fraction, as seen in Table 2 is consistent with those reported by Seely et al<sup>(10)</sup>, who revealed that a low level of active vitamin D (1, 25-(OH)<sub>2</sub> D) in preeclampsics, may contribute to suboptimal intestinal absorption of calcium during a time of increased calcium demand resulting in lower ionized calcium, increased parathyroid hormone (PTH), and hypocalciuria in preeclampsia<sup>(11)</sup>. Abnormalities in calcium homeostasis may contribute to the increased vascular sensitivity documented in preeclampsia. In contradiction to the reported difference in ionized calcium between normal and preeclamptic patients, other authors like Sanders et al<sup>(12)</sup>, Siddiqui & Rana<sup>(13)</sup>, Richards et al<sup>(14)</sup> found no difference in serum ionized calcium.

The question of which-actual ionized calcium or calculated ionized calcium-should be reported to the physician is unresolved, and there is no

international recommendation. Bowers et al<sup>(15)</sup> advocate the use of corrected ionized calcium, because of pH values in venous samples that fell outside the reference limits (loss of CO<sub>2</sub> during sample handling lowering the calcium ion concentration in vitro from what it was in vivo), whereas others<sup>(16)</sup> have questioned the use of corrected value for ionized calcium, both analytically and clinically.

We have found a close relationship between calculated ionized calcium and the actual values for ionized calcium in patients with preeclampsia. From theoretical and clinical point of view, we expect actual ionized calcium values to be superior to measurement of the calculated ionized calcium because of parathyrin-induced increases in the renal excretion of bicarbonate, thus giving rise to values for pH and ionized calcium different from those at pH 7.4<sup>(17)</sup>. Our study shows that calculated ionized calcium provides the same clinical information as actual ionized calcium, even in preeclampsia.

In conclusion, biochemical changes in preeclampsia appear to involve

calcium metabolism leading to the appearance of the typical pattern which may cause vasospasm of eclampsia. These changes would include low serum ionized calcium. We also conclude that calculated ionized calcium for a sample is as useful as actual ionized calcium in the evaluation of patients with disorder of calcium metabolism. Clinical superiority of actual ionized calcium is expected only in patients with severe acid-base derangement. Calculated ionized calcium appears to be a good choice for establishing reference values for healthy adults; however, due to the lack of gold standard method for measuring ionized calcium (ion selective electrode) in Iraqi laboratories; the accuracy (sensitivity and specificity) of compleximetric method for measurement of ionized calcium cannot be determined in this study. Further study of intracellular calcium and calcium pumps to explore their potential role in the pathogenesis of preeclampsia is required for future work.

#### References

1. Baker PN. Editor. Obstetrics by Ten Teacher. 18<sup>th</sup> edition. Hodder Arnold; 2006, P. 159-161.
2. Parry S, and Marchiano D. Hypertension in pregnancy. In: Mark-M and Sam-S. Editors. NMS (National medical series for independent study) / Obstetrics & gynecology. 5<sup>th</sup> ed. Lippincott Williams & Wilkins; 2005. P.169.
3. Dunlop W. Normal pregnancy: physiology and endocrinology. In: Edmonds, DK. Editor. Dewhurst Textbook of Obstetrics and Gynecology for postgraduates. 6<sup>th</sup> ed. Blackwell Science; 1999. P. 81-3.
4. Weeke E and Friis Th. Serum fractions of calcium and phosphorus in uremia. *Acta Med Scand*, 1979; 189: 79.
5. Endres DB and Rude RK. Mineral and Bone Metabolism. In: Carel, AB and Edward, RA. Editors. Tietz Textbook of Clinical Chemistry. 3<sup>rd</sup> ed. Saunders Company, Philadelphia; 1999. P. 1395-1412.
6. Parry S and Marchiano D. Hypertension in pregnancy. In: Mark-M and Sam-S. (Eds.). NMS (National medical series for independent study) / Obstetrics & gynecology, 5<sup>th</sup> ed. 2005, P: 169. Lippincott Williams & Wilkins.
7. Yamasmit Water, Chaithongwogwathana S, Charoenvidhya D, Uerpairojkit B, Tolosa J. Random urinary protein-creatinine ratio for prediction of significant proteinuria in women with preeclampsia. *J-Matern-Fetal-Neonatal-Med*. 2004; 16:257-9.
8. Gowenlock AH, McMurray JR, McLaughlan D. Editors. Varley's Practical Clinical Biochemistry. 6<sup>th</sup> ed. Heinemann Medical Books, London; 1984. P. 868-873.
9. Lopez P. Prevention of preeclampsia with calcium supplementation and its relation with the L-arginine: nitric oxide pathway. *Braz J Med Biol Res (BRAZIL)*. 1996; 29: 731-41.
10. Seely EW, Wood RJ, Brown EM, Graves SW. Lower serum ionized calcium and abnormal calcitropic hormone levels in preeclampsia. *J Clin Endocrinol Metab*. 1992; 74:1436-40.
11. Kisters K, Barenbroka M, Louwenb F, Hausberga M, Rahna KH, Koscha M. Membrane, intracellular, and plasma magnesium, and calcium concentrations in preeclampsia. *AM J Hyperten*. 2000; 13: 765-769.
12. Sanders R, Koijnenberg A, Huijgen HJ, Wolf H, Boer K, Sanders GT. Intracellular and extracellular ionized and total magnesium in preeclampsia and uncomplicated pregnancy. *Clin-Chem-Lab-Med*. 1999; 37: 55-9.
13. Siddiqui JA, Rana IA. Mineral and parathyroid hormone inter-relationships in normal pregnancy and pregnancy-induced hypertension. *J Pak Med Assoc*. 1993; 43: 92-5.
14. Richards SR, Nelson DM, Zuspan FP. Calcium levels in normal and hypertensive pregnant patients. *Am J Obstet Gynecol*. 1984; 149: 168-71.
15. Bowers JG, Brassard C, Sena SF. Measurement of ion calcium in serum with ion selective electrodes: a mature technology that can meet the daily service need. *Clin Chem*. 1986; 32: 1437-47.
16. Wandrup J, Kancir C, Hyltoft P. Ionized calcium and acid-base status in arterial and venous whole blood during general anesthesia. *Scand J Clin Lab Invest*. 1988; 48: 115-22.
17. Thode J, Holmegaard SN, Transbel I, Fogh Andersen N and Siggaard Andersen O. Adjusted ionized calcium (at pH 7.4) and actual ionized calcium (at actual pH) in capillary blood compared for clinical valuation of patients with disorders of calcium metabolism. *Clin Chem*. 1990; 36/3, 41-4.