Mineral Homeostasis in Preeclampsia

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Abstract

Background: Preeclampsia is a form of high blood pressure manifested during pregnancy, it is a common major complication causing significant morbidity and mortality; however, its etiology is unknown. Moreover, data on mineral homeostasis and on cation pattern during pregnancy are conflicting. Also, the status of ionized calcium and magnesium during pregnancy and its complication preeclampsia have not been described adequately.

Objective: to demonstrate the pattern of minerals during preeclampsia with respect to normal pregnancy.

Subject and methods: the present study is a cross-sectional case-control study includes measurement of minerals (calcium and magnesium) in 60 patients with preeclampsia. They are classified into two groups according to gestational age:

- Preeclamptics in the second trimester G1: (n=30).
- Preeclamptics in the third trimester G2: (n=30,).

The results are compared with 60 apparently healthy pregnants controls. They are classified according to gestational age into two groups:

- Pregnants in the second trimester G3: (n=30).
- Pregnants in the third trimester G4: (n=30).

Results: show that serum corrected calcium and serum magnesium are significantly reduced in preeclamptics when compared with normal pregnants. In addition, there was a reduction in free calcium and free magnesium that was accompanied by a significant high elevation of the ratio between ionized calcium to ionized magnesium.

Conclusion: preeclamptics (in different gestational age groups) have altered mineral status when compared with healthy pregnants matched with their age and gestational age.

Key words: preeclampsia, calcium, magnesium.

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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 of fetus [1, 2].

The pathophysiology of preeclampsia is thought to represent a defective response to the physiologic demands of normal pregnancy [2, 3]. Normal pregnancy is associated with profound changes in maternal homeostasis [4]. The endpoint of these changes is to provide the fetus with the necessary environment for growth and the mother with adequate protection against pregnancy complications [4].

During normal pregnancy, maternal plasma total calcium concentrations fall, primarily because of the decrease in serum albumin to which the mineral is predominantly bound in the circulation and it seems likely that there is a relatively little change in unbound ionized calcium. However, there is a

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substantial fetal need for calcium [5]. It is now clear that the dynamics of calcium homoeostasis are in fact substantially altered in pregnancy [5]. pregnancy-induced hypomagnesemia has been reported previously. however; the status of ionized magnesium during pregnancy and its relation to other important cations such as ionized calcium have not been described adequately it is the "free" or ionized magnesium that exerts biological activity[6].

It was suggested that a deficiency in magnesium contributed to the development of vasoconstriction in preeclampsia [7]. Also, deficiencies in calcium intake have been linked to preeclampsia/eclampsia, and hypocalciuria and deviations in both 1,25-$(OH)_2D_3$ and PTH have been shown in women with preeclampsia[7].

Subjects & Methods

A-Subjects

The study was a cross-sectional, case-control study conducted on 60 patients with preeclampsia (PE) 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.

The diagnosis of PE was based on clinical criteria that were hypertension (absolute BP of 140/90 mmHg twice over 4 hr without prior comparison) [1, 2] and proteinuria (21.5 mg of urinary protein per mmol creatinine) [8].

The exclusion criteria, which were used for cases and controls, were gestational or chronic hypertension, diabetes mellitus, renal disease, multifetal gestation, intrauterine fetal death, and pregnancy less than 20 weeks of gestation.

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. Age range was from 18 to 37 years (mean age $\pm$ SD = 26.1 $\pm$ 6.4 year). The gestational age range was from 20 to 28 weeks (mean gestational age $\pm$ SD = 26.3 $\pm$ 1.5 week).

2.Preeclamptics in the third trimester (G2):

Includes thirty preeclamptics in their third trimester of pregnancy. Age range was from 18 to 40 year (mean age $\pm$ SD = 25.1 $\pm$ 6.9 year). Gestational age range from 29 to 40 weeks (mean gestational age $\pm$ SD = 35.6 $\pm$ 1.6 week).

Controls:

Sixty apparently healthy pregnant women attending the Antenatal clinic, and Labor Ward at Al-Kadhimiya Teaching Hospital, for re-evaluation of their pregnancy, or for delivery. The control groups were comparable preeclamptic groups regarding the age, gestational age, Depending on the gestational age, the apparently healthy pregnants were divided into two groups:

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

They were thirty apparently healthy pregnants in the second trimester of pregnancy. Age range was from 15 to 38 years (mean age $\pm$ SD = 24.6 + 4.5 year). Gestational age range was from 20 to 28 weeks (mean gestational age $\pm$ SD = 25.5 $\pm$ 1.8 week).

4. Control pregnants during the third trimester (G4):

They were thirty pregnants in the third trimester of pregnancy. Age range was from 18 to 35 year (mean age $\pm$ SD = 24.8 $\pm$ 4.6 year). Gestational age range

was from 29 to 40 weeks (mean gestational age ± SD = 34.6 ± 2.1 week).

B. Blood & urine samples:

Ten milliliters of random venous blood were withdrawn from each patient and control, in supine position, without application of tourniquet. Samples then were transferred into clean new plane tube, left at room temperature for 15 minutes for clotting, centrifuged, and the separated serum was transferred into Eppendrof tube, which was used for measuring minerals (Ca, Mg). The tubes were stored at -20^{o} C until analysis, which was done within one month after collection [9].

Random urine specimens were obtained from each subject in the study to quantify urinary calcium [9], magnesium [9] that were expressed as a ratio to urinary creatinine [9]. As a preservative, 1-2 mls of 6M HCl was added to each random urine specimen; the samples were stored in appropriate containers at -20ºC until analysis [9].

C-Methods

Using atomic *absorption spectrophotometer* (Buck Scientific 210 JVP), the assay for calcium and magnesium estimation was carried out by adding 2.45 ml of (1% lanthanum chloride) solution to 0.05 ml of serum (or urine). These solutions were aspirated directly into air-acetylene flame where the calcium and magnesium hallow cathode lamp were used at wavelength 422.7 and 285.2 nm respectively [9]. *Adjusted serum calcium* can be calculated according to the formula [10]:

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 was used to calculate the ***ionized calcium*** according to the formula [10]:

Ionized calcium (mmol/L) =

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

Where

K' = 0.19 × total protein (g/L) + albumin (g/L).

The concentration of *magnesium ion* in serum was calculated from measurement of concentrations of total serum protein and total serum magnesium according to the equation [11]: $[100.4\ GZ / 100G - P]^2 + (33.77 + 2.42\ f\ P - f\ Mg)\ [100.4\ GZ / 100G - P] - 33.77\ f\ Mg = 0$. Where

P = total protein in gm per 100 ml of serum.

G = specific gravity of serum = 0, 00292 * P + 1.007

f = liters of serum that contain 1 kilo of water = 1000/G (4225.6 - 3225.6 G).

Mg = total magnesium in milli-equivalent per liter of serum.

Z = is Mg^{++} in milli-equivalent per liter of serum.

Results

The serum corrected and ionized calcium concentrations were lower in the preeclamptic women in third trimester G2 group as compared to healthy controls in the third trimester G4 [P < 0.001 for both] and even when compared to the preeclamptics in the second trimester G1 [P < 0.001 for both] as seen in (Table 1). The same significant reduction in *corrected but not ionized calcium* was noticed in the second trimester group G1 when compared to the healthy pregnants in the second trimester group G3 [P < 0.001 for corrected calcium but > 0.05 for ionized calcium] as seen in (Table 1). There was no significant difference in *corrected and ionized serum calcium* values between healthy pregnants in

each group [P > 0.05 for both] as seen in (Table 1).

Although the *urinary excretion of calcium (expressed as urinary calcium per creatinine)* was significantly reduced in preeclamptics in both groups G1 [P < 0.01] and G2 [P < 0.05] in comparison with pregnant controls of the same gestational period G3 and G4, the level of *urinary calcium excretion* was not significantly different between preeclamptics in the second and third trimester G1 and G2 [P > 0.05] nor between healthy pregnants in the same gestational periods G3 and G4 [P > 0.05] as seen in (Table 1).

A significant reduction in both *total and ionized serum magnesium* was noticed throughout the course of pregnancy whether among the preeclamptics groups: G1 and G2 [P < 0.001 for both]; or among healthy control pregnant G3 and G4 [P < 0.001 for both]. When preeclamptic groups G1 and G2 were compared with corresponding healthy control pregnant groups G3 and G4, the reduction in *total and ionized serum magnesium* was also significant [P < 0.001 for], as seen in (Table 1).

A significant elevation in *urinary magnesium excretion expressed as a ratio of urinary magnesium to urinary creatinine* was noticed through out the course of pregnancy whether among the preeclamptic groups: G1 and G2 [P < 0.001]; or among healthy control pregnant G3 and G4 [P < 0.01 for both]. When preeclamptic groups G1 and G2 were compared with corresponding healthy control pregnant groups G3 and G4, the significant increase in *magnesium excretion* is also found [P < 0.001], as seen in (Table 1).

A significant elevation in *the ratio between ionized calcium to ionized magnesium* was noticed in preeclamptics in both gestational period groups G1 [P < 0.001] and G2 [P < 0.001] as compared to the healthy pregnants in the same gestational periods G3 and G4. This significant elevation was also present in pregnants in the third trimester groups G2 [P < 0.01] and G4 [P < 0.001] when compared to pregnants in the second trimester groups G1 and G3, as seen in (Table 1).

Discussion

A number of studies have been published finding *serum total calcium* levels not different in non-pregnant controls and healthy pregnant women, whereas other researchers like Pederson et. al. [12], found decreased total serum calcium values in normal pregnancy. Furthermore, the beneficial role of a calcium supplementation in preeclampsia is still controversial [13, 14]. Some investigators reported an increased free erythrocyte and platelets calcium concentration, speculating that transmembrane calcium fluxes re-altered in hypertensive pregnancy, possibly by a specific mechanism probably of placental origin[7]. The finding of low serum total calcium in preeclamptics reported here is in agreement with findings of others [7, 15, 16] who conclude that a calcium deficit leading to an increased intracellular ionized calcium concentration during late pregnancy contribute to the pathogenesis of preeclampsia. In contrary, many investigators [6, 12, 17, 18] found that serum calcium did not differ significantly from normal pregnant group.

Regarding the ionized fraction of calcium, is crucial for the synthesis of vasoactive substances in the endothelium as prostacyclin and nitric oxide [19]. The finding of significant reduction in this

fraction, as seen in Table 1 is consistent with those reported by Seely et.al. [20], who revealed that a low level of active vitamin D (1, 25-$(OH)_2$ D) in preeclamptics, may contribute to suboptimal intestinal absorption of calcium during a time of increased calcium demand resulting in lower ionized calcium, increased PTH, and hypocalciuria in preeclampsia[6]. Abnormalities in calcium homeostasis may contribute to the increased vascular sensitivity documented in preeclampsia [6]. In contradiction to the reported difference in ionized calcium between normal and preeclamptic patients, other authors like Sanders et.al.[17], Siddiqui & Rana[21], Pederson et.al.[12], Richards et. al.[22] found no difference in serum ionized calcium.

Urinary calcium in preeclamptic in this study was observed to be lowered as compared to corresponding control pregnant as seen in (Table 1).

The etiology of hypocalciuria in preeclampsia is unknown. However, different assumptions have been given [23]. Particularly, it has been proposed that hypocalciuria may result from decreased dietary intake of calcium resulting in a low circuating calcium and hence low urinary calcium[23]; or from decrease intestinal absorption as secondary result of decreased 1,25 dihydroxyvitamin D, which enhances intestinal absorption of calcium[23]; or it may be due to increased calcium intake by the growing fetus and placenta[23]; lastly, it may be due to intrinsic renal tubular dysfunction, presumably due to decreased glomerular filtration and increased tubular reabsorption[23].

We found also, a decrease in both total and ionized magnesium throughout the 2nd and 3rd trimesters of pregnancy in both normal and preeclamptic pregnant women as seen in Table 1, like several studies [6, 7, 24-28]. The level of the cation studied was found to be within the same ranges reported for corresponding non-pregnants in other studies like [7, 20, 24, 27]. Although the reason for the reduction in total and ionized magnesium is not clear, it is not likely to be due solely to hemodiluton and extracellular fluid volume expansion as serum magnesium levels are still observed to decrease when correcting for protein dilution[6]. An increase in the renal clearance during pregnancy may contribute to the reduction in serum magnesium, since the kidney is the main regulator of the body magnesium [6]. This was supported by the finding of significant increase in magnesium excretion in healthy control and preeclamptic pregnancy with advancing gestational age according to magnesium: creatinine ratio, as seen in (Table 1). Other factors that may contribute to hypomagnesaemia in pregnancy include poor dietary intake[6] which is accompanied by consumption of minerals by the growing fetal skeletal system [6]. Hypoproteinaemia is another contributing factor since extracellular magnesium accounts for about 1% of the total body magnesium content. About 55% of magnesium is free, 30% is associated with proteins (primarily albumin), and 15% is complexed with phosphate, citrate, and other anions [9]. The technique used for measuring ionized magnesium can also be considered, ideally, it is the ion-selective electrode which is not available in Iraqi laboratories, instead a mathematical equation was employed [9, 11].

We also found an increased ionized calcium:ionized magnesium ratio during normal and complicated pregnancy, as seen in (Table 1). In

previous reports [28], the molar ratio of total calcium to total magnesium remained constant throughout pregnancy. However, ionized magnesium can be altered independent of total magnesium concentrations [29]. A high calcium-magnesium ratio has been associated with increasing vasospasm [30]. Increased intracellular calcium and decreased intracellular magnesium have been reported in women with hypertension and diabetes [30]. Thus electrolytes abnormalities may contribute to altered blood pressure [23].

The relation between serum total and ionized magnesium with intracellular magnesium has not been defined clearly. In previous study [30], there was no significant difference in red blood cell magnesium levels in teenagers with pregnancy-induced hypertension, whereas plasma magnesium tended to decrease with increasing gestation in this same group. However, recent evidence suggests that extracellular magnesium may modulate intracellular magnesium in vascular smooth-muscle cells [6].

On the basis of previous experimental data, the mechanisms underlying the *magnesium-induced vasodilation* may be due to a modification of the response to vasopressor hormones [7], and an interaction with cellular calcium handling[7]. These possible mechanisms were discussed by Kisters et. al. 2000 [7].

Further study of intracellular minerals and the membrane Na, K ATPase and calcium pumps to explore their potential role in the pathogenesis of preeclampsia is required for future work.

Table 1: The mean value of minerals (corrected Ca^{+2}, ionized Ca^{+2}, total Mg^{+2}, ionized Mg^{+2}, ratio of ionized Ca^{+2}: ionized Mg^{+2}) in the sera & urine of different preeclamptic and pregnant control groups (presented as mean ± SD).

Variable	G1	G2	G3	G4
Serum corrected calcium (mmol/L)	2.3 ± 0.05	2.2 ± 0.09	2.5 ± 0.1	2.5 ± 0.1
Serum ionized calcium (mmol/L)	1.2 ± 0.08	1.1 ± 0.05	1.2 ± 0.05	1.2 ± 0.05
Urinary calcium : creatinine	0.6 ± 0.27	0.58 ± 0.59	0.94 ± 0.6	0.8 ± 0.19
Serum magnesium (mmol/L)	0.11 ± 0.005	0.08 ± 0.01	0.15 ± 0.007	0.13 ± 0.0006
Serum ionized magnesium (mmol/L)	0.011 ± 0.002	0.006 ± 0.001	0.057 ± 0.0037	0.018 ± 0.0010
Urinary magnesium: creatinine	0.07 ± 0.003	0.09 ± 0.03	0.0149 ± 0.0101	0.04 ± 0.01
Serum ionized calcium: ionized magnesium ratio	172.37 ± 36.36	250.64 ± 134.32	32.25 ± 2.45	101.06 ± 7.1

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