Trace Elements Homeostasis in Preeclampsia

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Abstract

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

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

Subject and methods: the present study is a cross-sectional case-control study included measurement of minerals (calcium and magnesium) in 60 patients with preeclampsia. They were classified into two groups according to the 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 pregnant controls. They were classified according to the 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, serum magnesium and serum zinc were significantly reduced accompanied by significant high serum copper in pre-eclamptics when compared with that of normal pregnants.

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

All preeclamptics had certain factors that reduce vasodilation, enhance vasospasm and trigger oxidative stress supported by the finding of low level of ionized magnesium (which is essential for maintenance of vascular tone), low ionized calcium level (which is essential for the synthesis of endothelial-derived NO), high copper level (which generates highly reactive oxygen species) and low zinc level (which is essential for antioxidant function).

Key words: preeclampsia, calcium, magnesium, zinc, copper.

IRAQI J MED SCI, 2009; VOL.7 (2):116-123

Introduction

Preeclampsia (PE) 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].

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Received: 18th January 2009, Accepted: 3rd June 2009.

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 complication[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 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[6]. However; the status of ionized magnesium during pregnancy and its relation to other important cations such as ionized calcium have not been described adequately [6]. 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[8].

In addition, endothelium-derived relaxing factor (EDRF) is rapidly inactivated by free radicals. Therefore the hypothetical decrease in EDRF release in preeclampsia might be caused by the increase in oxygen free radical. Because of their biological role as catalyst for endogenous antioxidant enzymes, trace elements have been assessed in many researches to find whether they contribute to the etiology of preeclampsia or not.[9].

Subjects & Methods

A-Subjects

The study was a cross-sectional, case-control study conducted on sixty 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 hrs 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):

Included thirty Preeclamptics in their second trimester of pregnancy. Age range was from 18 to 37 years. The gestational age range was from 20 to 28 weeks.

2.Preeclamptics in the third trimester (G2):

Included thirty preeclamptics in their third trimester of pregnancy. Age range was from 18 to 40 year. Gestational age range was from 29 to 40 weeks.

Controls:

Sixty apparently healthy pregnants 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. Gestational age range was from 20 to 28 weeks.

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. Gestational age range was from 29 to 40 weeks.

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 and trace elements (Ca, Mg, Cu, and Zn). The tubes were stored at -20^{o} C until analysis, which was done within one month after collection [10,11].

Random urine specimens were obtained from each subject in the study to quantify urinary calcium, magnesium, zinc, copper that were expressed as a ratio to urinary creatinine. 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[10,11].

C-Methods

The assay for calcium and magnesium estimation was carried out using *atomic absorption spectrophotometer* (Buck Scientific 210 JVP)[10, 11].

Corrected serum calcium was calculated according to the formula used by Gowenlock-AH [12]:

Instead of obtaining a crude correction for measured calcium, the same data was used to calculate the *ionized calcium(Cai)* according to the formula used by Gowenlock-AH [12]:

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 used by Willis-MJ and Sunderman-FW[13].

Results

It was found that *the serum corrected and ionized calcium concentrations* were low in the preeclamptic women in third trimester G2 group as compared to healthy controls in the third trimester G4 [P< 0.001 for both parameters] and even when compared to the preeclamptics in the second trimester G1 [P< 0.001 for both parameters] as it can be 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 greater than 0.05 for ionized calcium] as it can be 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 parameters].

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 from that of preeclamptics in the second and third

trimester ,G1 and G2 , [P > 0.05] as well as healthy pregnants in the same gestational periods , G3 and G4 , [P > 0.05].

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

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 parameters]. 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].

Serum zinc was significantly lower in preeclamptics (G1 &G2) compared with normal pregnants (G3 & G4) [P < 0.05 for both]. Also serum zinc was significantly lower in G2 compared with G1 [P < 0.05], but there was no significant difference between G3 & G4 [P > 0.05].

Urinary excretion of zinc expressed as zinc: creatinine ratio was significantly increased in preeclamptic in the third trimester G2 when compared to corresponding control G4 [P < 0.05], although this increase excretion of zinc was seen when second trimester groups (G1 & G3) were compared with each other; it failed to reach to a statistically significant level. Moreover, the increment in zinc excretion failed to reach statistically significant level when preeclamptic and pregnant groups were compared with each other [P>0.05].

Serum copper level was significantly higher in preeclamptics (G1 &G2) compared with normal pregnants (G3 & G4) [P < 0.05 for both]. Also serum copper level was significantly higher in G2 compared with G1 [P < 0.05 for both], but there is no significant difference between G3 & G4 [P > 0.05].

According to urinary copper: creatinine ratio, there was a significant retention of copper in preeclamptic in the third trimester G2 when compared with respective control G4 and even with preeclamptic in the second trimester [P < 0.05 for both]. This retention is not significant when preeclamptic in the second trimester G1 was compared with corresponding control G3 [P >0.05] and also among pregnant controls when compared with each other [P > 0.05].

Discussion

A number of studies have found that ***serum total calcium*** level is not different in non-pregnant controls and healthy pregnant women, whereas other researchers like Pederson et. al.[14], found decreased total serum calcium values in normal pregnancy. Furthermore, the beneficial role of a calcium supplementation in preeclampsia is still controversial[15,16]. 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 the findings of Ingec et.al.[17], Kisters et.al.[7], Hojo & August[18] who concluded that a calcium deficit leads to an increased intracellular ionized calcium concentration during late pregnancy contribute to the pathogenesis of preeclampsia. In contrary, many investigators like [6, 14, 19-22] found that serum calcium did not differ significantly from normal pregnant group.

Regarding the **ionized fraction of calcium** which is crucial for the synthesis of vasoactive substances in the endothelium as prostacyclin and nitric oxide[23]. The finding of significant reduction in this fraction, as seen in Table 1 is consistent with those reported by Seely et.al.[24], who concluded 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. In contradiction to the reported difference in ionized calcium between normal and preeclamptic patients, other authors like [14, 19, 21, 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[25]. Particularly, it has been proposed that hypocalciuria may result from decreased dietary intake of calcium resulting in a low circulating calcium and hence low urinary calcium[25]; or from decrease intestinal absorption as secondary result of decreased 1,25 dihydroxyvitamin D, which enhances intestinal absorption of calcium[25]; or it may be due to increased calcium intake by the growing fetus and placenta[25]; lastly, it may be due to intrinsic renal tubular dysfunction, presumably due to decreased glomerular filtration and increased tubular reabsorption[25].

A decrease in both **total and ionized magnesium** was observed through out the course of pregnancy in both normal and preeclamptic pregnant women as seen in Table 1.

Several studies like [6, 7, 26-30] reported that hypomagnesaemia was associated with pregnancy. The level of magnesium cation studied was found to be within the same ranges reported for corresponding non-pregnants in other studies like Brooks & Fry[31], Richard et. al.[22]. other reseachers like Sanders et. al.[19] reported an increase in serum magnesium level in sever preeclampsia. 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 corrected 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 it can be 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[10]. The technique used for measuring ionized magnesium can also be considered, ideally, it is the ion-selective electrode, instead a mathematical equation was employed[10,12].

The relation between serum total and ionized magnesium with intracellular magnesium has not been defined clearly. In previous study[32], 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].

Signicant changes in serum trace metal concentrations, particularly zinc and copper, have been documented during normal pregnancies[11]. In preeclampsia, decreased maternal and fetal serum zinc levels have been determined. In preeclampsia, the lowered serum zinc concentrations have been suggested to be at least partially due to reduced estrogen and zinc binding protein levels. Serum cortisol level increases during normal pregnancy and it is much higher in preeclampsia, which again reduce maternal zinc levels and subsequent urinary zinc[11]. Several investigators have noted that women with preeclampsia as compared with normotensive pregnant women had lower zinc concentrations[9]. On the other hand, reports on changes in copper levels were conflicting: decreased[33] elevated[34] and unchanged levels[35].The possible causes of these changes are discussed in view of the hormonal, metabolic and enzymatic changes in preeclampsia[9]. The physiological increase in copper concentrations in pregnancy is, in part, associated with estrogen induction of copper carrying protein[9]. In this study serum zinc levels were significantly lower and serum Cu levels were significantly higher in both groups with preeclampsia when compared with normal pregnant groups.

Biochemical changes in preeclampsia appear to involve mineral and trace metal metabolism leading to the appearance of the typical pattern which may cause vasospasm of eclampsia. These changes would include low serum ionized calcium, low serum total and ionized magnesium, low serum zinc with elevated serum copper and imbalance in the urinary excretion of calcium, magnesium and trace elements. Further study of intracellular minerals, oxidant-antioxidant status 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}) & trace elements 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)	0.81 ± 0.04	0.65 ± 0.08	1.02 ± 0.04	0.97 ± 0.05
Serum ionized magnesium (mmol/L)	0.45 ± 0.03	0.32 ± 0.06	0.62 ± 0.04	0.43 ± 0.03
Urinary magnesium: creatinine	0.07 ± 0.003	0.09 ± 0.03	0.0149 ± 0.0101	0.04 ± 0.01
Serum Zinc (mmol/L)	0.20±0.019	0.18±0.022	0.24± 0.045	0.22 ±0.042
Serum copper (mmol/L)	0.23± 0.029	0.35± 0.021	0.19± 0.018	0.2 ± 0.028
urinary zinc: creatinine	0.0026 ± 0.004	0.003 ± 0.002	0.0025 ± 0.003	0.0025 ± 0.003
Urinary copper: creatinine	0.006 ± 0.005	0.004 ± 0.005	0.0092 ± 0.007	0.009 ± 0.001

(G1):Preeclamptics in the second trimester.
(G2): Preeclamptics in the third trimester.
(G3):Control pregnants in the second trimester.
(G4):Control pregnants during the third trimester.

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