Serum calcium level and some physiological markers during Pre-eclampsia and normal pregnancy in Babylon province women

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Abstract
Background: preeclampsia (PE) is a pregnancy-related hypertensive disorder occurring in the second half of the pregnancy. The calcium mineral has been proposed to play important role in the pathogenesis of preeclampsia.

Subjects and methods: The data was collected from 50 normal pregnant women, 35 mild preeclamptic women and 30 severe preeclamptic women who were admitted in the Babylon maternity and pediatric teaching hospital between February 2012 and December 2012. The blood samples were collected and analyzed for serum total calcium, serum ionized calcium, serum albumin, The data on body mass index (BMI), a maternal and gestational ages, blood pressure (BP) values were collected and the data were analyzed using analysis of variances (ANOVA).

Results: The (BMI) and (BP) values in the preeclamptic women were significantly higher than those of individuals with normal pregnancy (p < 0.001), the serum total calcium (Ca) and serum albumin (Alb) levels were highly significant lower in hypertensive pregnant in comparison with healthy ones (p < 0.001), while ionized calcium levels had no significant difference between mild preeclamptic women (p > 0.05) as comparison with normal pregnancy, but were significant lower (p < 0.05) in the severe preeclamptic women as compared with control and mild preeclamptic groups.

Conclusion: single estimation of total and ionized calcium, and serum albumin levels late in the second and third trimesters of pregnancy have a little value in the prediction of preeclampsia when the disease process was usually manifest, our study is recommended to properly performed not just these biochemical markers, additionally we must investigated the related vitamins that may be contribute in this disorder, and these tests must be early analyzed in pregnancy for prediction of the subsequent development of preeclampsia.

Key word: total calcium, ionized calcium, serum albumine, pre-eclampsia

Introduction
Pre-eclampsia (PE) is a transient but potentially dangerous complication of pregnancy that affect 3-5% of pregnancies[1] It has been dubbed the “disease of theories” because of the multiple hypothesis have been proposed to explain its occurrence[2].

The syndrome of pre-eclamptic toxemia is characterized by hypertension, proteinuria, oedema and abnormal clotting, all of which can be explained by generalized vascular endothelial cell dysfunction[3].
This maternal hypertension develops after 20 weeks gestation and is associated with >300 mg/day urinary protein excretion[4], the clinical spectrum of preeclampsia ranges from mild to severe depending on the level of the blood pressure(BP) and degree of protein urine[5], in most cases progression through this spectrum is low, and the disorder may never proceed beyond mild preeclampsia. In other cases the disease progress more rapidly changing from mild to severe within days or weeks[6].

Epidemiological and clinical studies have shown that an inverse relationship exists between calcium (Ca) intake and development of hypertension in pregnancy[7,8], as regard that malnutrition is a risk factor in the etiology of preeclampsia and implicate it by deficit intake of calcium[9]. On physiological basis, calcium is the bodies most abundant divalent cation, is an essential but controversial nutrient[10,11], in pre-eclampsia many factor affect calcium regulation and consider the causes of disturbed calcium balance[12],on the other hand the modification of plasma calcium concentration leads to alteration of blood pressure[13]with respect to this, the aberrations in calcium homeostasis have been recognized in hypertension in general and specifically in pre-eclampsia[14]

To facilitate understanding of(Ca) disturbance, it is helpful measure serum total calcium form, but this form sum of the ionized composed of(46.9%) which an alternate metabolic form of calcium with active physiological important and play a key role in the biochemical and metabolic functions[11] and the other, non ionized consist of (39.5% ) protein bound mainly albumin, and (13.6%) diffusible calcium complexes [15] this reveal that calcium bound to albumin and ionized calcium is roughly in equal proportion [16] we conclude from this, the evaluate the total calcium in adjustment based on albumin to be suitable for fulfilled. The intended functions as a screening procedure in preeclamptic pregnant, As a result, our study carry out to estimate the role of these parameters in incidence of preeclampsia.

Subject and methods

This study was conducted in Babylon maternity and pediatric teaching hospital between February 2012 and December 2012 . The studied population consisted of 50 apparently normal pregnant women as a control group , 35 pregnant women with diagnosis of mild preeclampsia and 30 pregnant women with diagnosis of severe preeclampsia, the three groups are included with age rang[20-40] years, and gestational age range [29-39] weeks. those pre-eclamptic pregnant were admitted to hospital for further infestigations,monitoring, ,and/or delivery. subjects were excluded if they had chronic medical disease or were taking medications known to interfere with calcium metabolism such as corticosteroid ,thyroxine,heparin

Study tools and methods:

A- Diagnosis of( PE): All patients must be fulfilling the following inclusion criteria: 1 - Mild preeclampsia is defined as a blood pressure of at least 140/90mm/Hg measured on two occasions each 6 hours apart, accompanied by protein urine of at least 300 mg per 24h,or at least 1± on dipstick testing [6].
2-Severe pre-eclampsia is defined as having one or more of the following criteria blood pressure of at least 160/110mmHg measured on two occasions each 6 hours apart, protein urine of at least 5g per 24h, or at least 3+ on dipstick testing [6]. Two random midstream urine specimens, collected ≥4hours apart taken from each women to avoid error due to false positive tests, the two results must be positive, so the diagnosis of protein urine was significant [17]

**B-Clinical investigation**

Two (Bp) reading were taken five minutes apart, on left arms with a mercury sphygmomanometer [cuff size,12.5  x40cm] The first and fifth phases of korotkoff’s sounds were taken as the criteria for systolic blood pressure(SBP) and diastolic blood. Pressure(DBP) respectively [18] Participants height and weight was measured and recorded. Body mass index [BMI] in kg/m² is calculated by dividing baseline weight in kilograms by height in meters squared[19]

**C-Laboratory Investigation:**

Three milliliters of random venous blood were with drawn from each subject (control and patient) without application of tourniquet. Samples then were transferred into clean new plane tube, left at room temperature for 15 minutes for clotting, followed by centrifugation at 2000xg for 5 minutes ,separated serum was transferred into Eppendorf tube, the tubes were stored at -20c° until analysis, which was done within one week after collection[ 20 ]

The assay for calcium was carried out colometrically using a kit supplied by [biomerieux company]and alb-kit to determine of albumin using kit supplied by [biolabo company]and are preformed using cecil spectrophotometer -1101 in the biochemistry lab[21,22] As calcium is predominantly transported bound to serum proteins, so total serum calcium level are greatly influenced by protein concentration especially albumin[23] for obtaining corrected total calcium value ,the same data was used according to the formula[24]:

**Corrected total Ca(mg/dl)=total Ca(mg/dl)+0.8(4-albumin g/dl)**

As the ionized calcium that is physiological active and with clinically important [13] the same data used to calculate the ionized calcium according to the formula have been based upon plasma albumin[16]:

**Free or ionized Ca+² (mg/dl)= Ca measured in (mg/dl) –[albumin g/dl+4/2]**

**D-Data analysis**

The data was analyzed with( SPSS ) software package version 18.0 and expressed in terms of mean standard deviation(SD) continuous variables of the clinical characteristics, and serum calcium and albumin values for the three group of participants were compared by F-test from analysis variances [ANOVA]. Then scheffe was used for post hoc comparisons if F-test had statistical significance. A( p value <0.05) was considered to be statistically significant.

**The results**
The present study enrolled 115 pregnant women. The clinical characteristic of the participants are shown in Table 1.

Age, gestational age among normal pregnant, mild and severe preeclamptic women were not significantly different.

Table 1. Comparisons of the Clinical characteristics for three groups of participants

<table>
<thead>
<tr>
<th></th>
<th>G1/normal pregnant N=50</th>
<th>G2/mild Preeclampsia N=35</th>
<th>G3/severe Preeclampsia N=30</th>
<th>Sig.</th>
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<td>(1vs.2) (2vs.3) (1vs.3)</td>
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<tr>
<td>Age(years)</td>
<td>32.95±4.86</td>
<td>33.15±6.23</td>
<td>32.7±4.18</td>
<td>0.19</td>
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<td>0.86</td>
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<td></td>
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<td>0.15</td>
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<tr>
<td>Gestational age(weeks)</td>
<td>33.55±3.03</td>
<td>33.75±3.29</td>
<td>33.9±3.32</td>
<td>0.85</td>
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<td>0.88</td>
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<td></td>
<td>0.73</td>
</tr>
<tr>
<td>DBP(mm/Hg)</td>
<td>71.50±7.45</td>
<td>95.00±5.13</td>
<td>115±5.13</td>
<td>0.000</td>
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</tr>
<tr>
<td>SBP(mm/Hg)</td>
<td>115.00±6.88</td>
<td>146.00±5.02</td>
<td>170±8.58</td>
<td>0.000</td>
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<td>0.000</td>
</tr>
<tr>
<td>BMI(kg/m²)</td>
<td>32.33±5.21</td>
<td>36.06±4.45</td>
<td>35.66±3.90</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD ; N=number

The mean difference is Significant at the 0.05 level

There was no significant difference in the BMI between mild and severe preeclamptic women ,but the BMI for mild and severe preeclamptic women were more than normal pregnant significantly (36.06±4.45,35.66±3.90kg/m² vs.32.33±5.21kg/m² ,p=0.01,p=0.02 respectively).

There was a highly significant difference between normal pregnant and mild and severe preeclamptic women in blood pressure values, normal pregnant women had mean systolic blood pressure less than both mild and severe preeclamptic women(115.00±6.88mm/Hg vs.146.00±5.02 mm/Hg ,p=0.00;and 170±8.58mm/Hg ,p=0.00 Respectively) .

Systolic blood pressure of severe preeclamptic women were more than mild preeclamptic women(170±8.58mm/Hg vs.146.00±5.02mm/Hg, p=0.00 Respectively).

The results of our study show also that normal pregnant women had mean diastolic pressure less than mild and severe preeclamptic women(71.50±7.45 mm/Hg vs. 95.00±5.13mm/Hg ,p=0.00 and 175±5.13mm/Hg, p=0.00 respectively) .

As regard the laboratory results of maternal serum calcium and albumin level among the preeclamptic and control women explain in [table2],the serum total Ca and corrected total Ca were lower in the preeclamptic women G2 and G3 groups as compared to healthy control group G1 [p<0.001 for both] and even when compared between mild and severe preeclampsia group[p<0.001for both]
Table 2. Comparisons of serum total calcium, ionized, and corrected total calcium and serum albumin for the three groups of participants

<table>
<thead>
<tr>
<th></th>
<th>G1/normal pregnancy N=50</th>
<th>G2/mild preeclampsia N=35</th>
<th>G3/severe preeclampsia N=30</th>
<th>Sig (1vs.2)</th>
<th>(2vs.3)</th>
<th>(1vs.3)</th>
</tr>
</thead>
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<tr>
<td>Total serum Ca (mg/dl)</td>
<td>8.16±0.34</td>
<td>7.81±0.24</td>
<td>6.854±0.28</td>
<td>0.000</td>
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<tr>
<td>Serum ionized Ca (mg/dl)</td>
<td>4.52±0.16</td>
<td>4.52±0.19</td>
<td>4.20±0.19</td>
<td>0.95</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Serum corrected total Ca (mg/dl)</td>
<td>8.89±0.34</td>
<td>8.02±0.25</td>
<td>7.04±0.31</td>
<td>0.001</td>
<td>0.000</td>
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<tr>
<td>Serum albumin (g / dl)</td>
<td>3.10±0.23</td>
<td>2.75±0.25</td>
<td>2.13±0.48</td>
<td>0.000</td>
<td>0.000</td>
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</tr>
</tbody>
</table>

Values are given as mean ± SD ; N=number
The mean difference is Significant at the 0.05 level

As seen in (table 2) the same significant reduction in ionized calcium was noticed in the severe preeclampsia group G3 when compared to the healthy pregnant G1, and when compared to mild preeclampsia group G2 (p<0.05, p<0.05 respectively). But there were no significant difference in ionized serum calcium values between mild preeclampsia group G2 and healthy pregnant group G1 [p>0.05]. Serum albumin level showed a decrease in the sera of the mild and severe preeclampsia groups (p<0.001, p<0.001 respectively) as compared with normal pregnancy group. The same significant difference in serum albumin levels were noticed between mild and severe preeclampsia groups (p<0.001).

Discussion

Women with greater body mass index (BMI) in pregnancy are more likely to become hypertensive than those with a lower BMI[25] so, over weight women are more likely to develop disease that are specific to pregnancy, such as preeclampsia[26]. According to these above mention, we found in the present study the mean BMI values in normal healthy pregnant was less than both mild and severe preeclamptic pregnant women. These findings are also supported by other studies in which pregnant women who subsequently developed preeclampsia, had higher body mass index when compared to healthy pregnant control[27,28,29]. Elevated BMI is associated with insulin resistance, which is also associated to preeclampsia, but the exact mechanisms is not known, obesity might also act through increased sympathetic activity, this has been associated with hypertensive disorders of pregnancy[30,31]. In addition, the regulation of intracellular calcium plays a key role in obesity and hypertension[32]. Deregulations of Ca homeostasis in humen may contribute in obesity by the lake calcium influx in human adipocytes, resulting in stimulation of lipogenesis, inhibition of lipolysis and expansion of triglyceride store[33] this obesity, are responsible for the
physiology alterations of vasoactive mediators leading to hypertension during pregnancy[34]thus, in our study has shown that systolic and diastolic blood pressure in maternal preeclamptic women was highly significant as compared with control pregnant, therefore the role played by calcium in the pathogenesis of pregnancy induced hypertension is nowadays receiving growing interest, and the calcium supplementation during pregnancy in reducing hypertensive disorders has been evaluated[35].

In this study, hypertensive subjects had significantly lower levels of serum total Ca as compared to the normotensive(p <0.001)(table2). It has been hypothesized that change in the blood pressure due to disturbances in calcium metabolism include increased urinary calcium excretion and abundance of calcium-regulating hormones such as parathyroid hormone(PTH) and calcitriol.

Low calcium levels and elevated PTH and calcitriol may also affect blood pressure control by the central nervous systems by stimulating the release of nor epinephrine (a potent vasoconstrictor) and its post-synaptic effect[36]thus intracellular calcium serves as a second messenger in excitation-contraction coupling for vascular smooth muscle cells, causing constriction and increased blood pressure[37].

Our finding reported here is in agreement with previous studies[38,39,13], in contrary, many investigators like[40,41,42]found that serum calcium in preeclamptic group did not differ significantly from normal pregnant group, this difference which can be attributed to the different dietary habits and the different genetics pools of the population in which the study had been carried out as compared with our population.

The adjustment of serum total calcium concentration for albumin is essential to detect abnormal values and to assess changes in a value[43]. The results demonstrated that the levels of the serum corrected total calcium and serum albumin were highly significant decreased (p< 0.001) in the preeclamptic women as compared to that in normal pregnant group.

Our finding are in accordance with those reported by other authors [44,45], wherever Gjoinc et al, conclude that hypoalbuminemia in preeclampsia is the result of reduced hepatic blood flow which is secondary to hypovolemia created by higher filtration pressure in the capillaries[46] and propose the serum albumin level may serve as indicator of the severity of preeclampsia. However our findings was disagree with investigators found that serum albumin level was higher in preeclampsia group[47,48], this contrast may be caused from the mislead between underlying chronic hypertension or renal disease during pregnancy and preeclampsia condition.

Regarding the ionized fraction of calcium which is crucial for the synthesis of vasoactive substance in the endothelium[49], the finding of significant reduction in this fraction, as seen in (table2) is consistent with those reported by Seely et al[50] who confirmed that preeclamptic pregnant women had significantly lower 1,25-dihydroxy vitamin D[1,25-(OH)2D] levels, Thus lower 1,25-(OH) 2D levels may contribute to the suboptimal intestinal absorption of calcium during a time of increased calcium demand, thus resulting is lower ionized calcium levels, increased PTH and hypocaliuria in preeclampsia.

Our study coincided with other studies (51,52) reported difference in ionized calcium between normal and preeclamptic groups.

On the other hand, physiologically important changes in ionized calcium can be produced without change in the total Ca concentration, by altering the affinity of albumin for calcium, two factors affect on change the amount of calcium bound, the PTH and extra cellular pH[53].
As a result, present study (table2) exhibited that the changes occurred in the total calcium concentration don't correspondence with the changes in ionized calcium levels within preeclampsia groups. Accordingly in our study there were no significant difference (p> 0.05) in serum ionized calcium concentration between mild preeclamptic and healthy pregnant women, our findings indicate adjusted blood pH as is sometimes done for ionized calcium results[54], to avoid any confusion that may occurs during test.

**Conclusion**

From theoretical and clinical points of view, we conclude from our study that reduction in maternal serum total calcium with consequent an ionized calcium as a fraction of total calcium, may have role in pregnant women in subjects to this disorder, as well as development of preeclampsia.

In addition, to have better understanding of calcium homeostasis in preeclamptic women, not just need to measure total and ionized calcium, and serum albumin, but similarly to get a better understanding of vitamins related disorder. As a result of that we needed other studies that confirm whether the supplementation of this mineral and other vitamins has favorable or adverse effects on fetal and neonatal outcomes as well as maternal outcomes.

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مستوى الكالسيوم وبعض المؤشرات الفسيولوجية خلال مرض قبرل الشرنج والحمل الطبيعي في نساء محافظة بابل

1- نوران جميل إبراهيم- دلاليا شاكر عبد
1-قسم علوم الحياة-كلية العلوم للبنات –جامعة بابل2-مستشفى بابل للولادة والأطفال-مختبر الكيمياء الحياتية

الخلاصة

يعد مررض قبرل الشرنج ارطراط اردط الردم المربط بحمرل خرلال الفصرل اليراني منر، وافترارت العديرد مرن الدراسات إن معدن الكالسيوم يلعط دور مهم في أمراايت.

انتقىت 111 امرأة حامل دخلت إلى مستشفى بابل للولادة والأطفال للفترة برين شرباط 2112–2112 كراانون الأول وتراوحت أعمارهن بين (20-40) سنة وتراوح عمر الحمل بين (29-39) أسبوع وتشملت المجموعة 50 امرأة حامل سليمة والتي عدت مجموعة سيطرة وشخصت 35 حامل مصابة بمرض قبل الشنج نعو الحاد و30 حامل مصابة بمرض قبل الشنج نعو المبسط وتتم تحليل مصور أولك النساء(المرضيات والسيطرة) وقيم كالسيوم المصل الكلي والكالسيوم الكلي المصحح والكالسيوم الحر أو المتأين والألوبمين، كما تم جمع قيم مؤشر كثلة الجسم وضغط الدم الإنساطي والاقضائي وتم تحليل النتائج باستعمال النظام الإحصائي وتمقارنة بين نتائج المجمعم قيد الدراسة.

أظهرت النتائج زيادة معنوية (p<0.01) في قيم مؤشر كثلة الجسم وضغط الدم في النساء المصابات بمرض قبل الشنج في طريقة (الحاد والمتوسط) بالمقارنة مع النساء ذوات الحمل الطبيعي، كما أظهرت النتائج وجود نقصان معنوي في مستوى الكالسيوم الكلي والكلي المصحح والألوبمين في النساء المصابات بمرض قبل الشنج بالمقارنة مع الاحوال السليمة، في حين أظهرت النتائج نقصان غير معنوي (0.05<p<0.01) في مستوى الكالسيوم المتين في النساء المصابات بمرض قبل الشنج المبسط مقارنة بمجموعة السيطرة. في حين كان الانخفاض في تركيز الكالسيوم المتين معنوي (p<0.05) في مجموعة مرض قبل الشنج الحاد مقارنة مع النساء في مجموعة السيطرة.

وقد نستنتج من نتائج بحثا هذا بأن إجراء تلك الفحوصات الكيميائية والفسيولوجية متأخرة أثناء الحمل قد تجعل تلك القيم تمكن دور قليل لتبنا حديث مرض قبل الشنج الذي يمكن أن يكون قد تطور ووصول مراحل متأخرة، لذا توصي دراستنا بضرورة إجراء تلك الفحوصات مبكرًا إثناء الحمل كما يجب أن يدرس مستقبلا بعمق الدور الذي تلعبه نفس بعض الفيتامينات في حدوث هذا المرض.