

Study of Alkaline Phosphatase Activity and Calcium Levels with Follow of Glucose in Second Trimester Pregnant Women Sera

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Abstract

The aim of this study to measured alkaline phosphatase activity and calcium level with follow glucose in sera of (22) samples in healthy pregnant at second trimester and (20) samples for marriage women non pregnant as control group. This study was done in department of chemistry, college of science, Al-Mustansiriya University in Iraq -Baghdad from December 2013 to February 2014. The result demonstrate that non-significant variation in alkaline phosphatase activity ($P > 0.05$) whereas calcium level in sera of pregnant was decreased highly significant variation ($p < 0.002$) in relationship with that of control group, but there is stay of glucose in normal range.

Keywords: alkaline phosphatase, calcium, glucose, pregnant women.

Introduction

Alkaline phosphatase and calcium are the most important parameters in the pregnant, which belonged to the large change in physiological states and some hormone affect in these parameters in the body of pregnant, also the building of the fetus body related with calcium. Conception associated with multiple physiological variations in the serum calcium and alkaline phosphatase. Biochemical investigations throughout pregnancy might be varying from normal range thus they may be wrongly expressed leading to incorrect therapy [1, 2]. The maternal physiologically changes of pregnancy are normal adaptation that a woman undergoes to better accommodate the embryo or fetus during pregnancy [3].

Calcium, the most abundant mineral in the human body, has several important functions [4]. More than 99% of total body Ca^{+2} is stored in the bones and teeth supporting their structure [5]. The remaining 1% is found throughout the body in blood, muscle, and the fluid between cells needed for muscle contraction, contraction of blood vessel and expansion, secretion of enzymes and hormones, and sending messages through the nervous system so that these vital processes of body function efficiently [6]. A constant level of Ca^{+2} is maintained in body fluid and tissues within a narrow limit for normal physiological functioning so that when blood Ca^{+2} decreases it stimulates the secretion of parathyroid hormone (PTH) which stimulates the conversion of vitamin D to its active form (Calcitriol) in the kidneys [7, 8]. Calcitriol increases intestinal Ca^{+2} absorption, which in turn stimulates bone Ca release by activating osteoclasts and decreasing urinary Ca^{+2} excretions [9].

On the other hand, when blood Ca^{+2} returns to normal level, the parathyroid glands stop secreting PTH and the kidneys begin to excrete any excess Ca^{+2} in the urine [4, 8, and 9]. Although this complex system allows for rapid and tight control of blood Ca^{+2} levels, it does so at the expense of the skeleton [7]. Progesterone and estrogen level rise continuously throughout pregnancy. Which the estrogen preservative on the bone from lyses. Also increase the calcitonin which prevent triggers of calcium from a bone [10, 11, 12].

Alkaline phosphatases are dimeric enzymes present in most, if not all, organisms [13]. They catalyze the hydrolysis or splitting of phosphomonoesters with release of inorganic phosphate [14] at an alkaline pH. It exhibit maximum activity at alkaline pH in the region of 9.0-9.6 [15]. Some divalent ions, such as Mg^{2+} , Co^{2+} , and Mn^{2+} are activators of the enzyme, and Zn^{2+} is a constituent metal ion. Phosphate, borate, oxalate, and cyanide ions are inhibitors of all forms of the enzyme [16].

Among the bone diseases and pregnancy, the highest levels of serum ALP activity are encountered in Paget's disease. Values from 10 to 25 times the upper limit of the reference interval are not unusual [17] and secondary bone disease [18, 19, and 20]. Only moderate rises are observed in osteomalacia, the levels slowly declining in response to vitamin D therapy. Levels are generally normal in osteoporosis. In rickets, levels two to four times normal may be observed, and these falls slowly to normal treatment with vitamin D. Slight to moderate elevation occur in Fanconi's syndrome [16]. Primary hyperparathyroidism and secondary hyperparathyroidism are associated with slight to moderate elevations of ALP activity in serum. Existence and degree of elevation reflecting the presence and extent of skeletal involvement [21].

ALP levels to increased coronary calcification and cardiovascular risk in persons with chronic kidney disease [22-25]. Also associated with kidney failure [35].

Materials and methods

Subjects

Samples were collected from twenty two pregnant and twenty non pregnant healthy woman as a control group and their ages were around (16-32) years.

Serum Samples

Five milliliters of venous blood samples were collected (22) samples from pregnant and (20) samples from non pregnant women as control group without tornique. Samples were allowed to clot at room temperature, centrifuged at 3000 xg for 5 minutes, and then sera were collected and stored at -15°C .

Assay of Alkaline phosphatase activity

Alkaline phosphatase has optimal activity at pH of about 10. The activity of alkaline phosphatase was measured according to Kind and King Method by using Human Kit.

The liberated phenol was measured in the presence of 4-amino antipyrine and potassium ferricyanide. The presence of sodium arsenate in the reagent stops the enzymatic reaction [38] and by the following equation.

Phenyl phosphate $\rightarrow$ phenol + phosphate.

The spectrum absorption at wave length (400 nm). The unit of the measurement is the international unit of enzyme (IU/100ml).

Assay of calcium in serum

Colorimetric determination of total calcium in sera without deproteinization was carried out using Human kit; calcium ion reacts with the 8-hydroxy quinoline in an alkaline medium.

The color intensity of the Ca-8-HQ complex, read at (540 nm), is proportional to the quantity of calcium present in the sample. 8-hydroxyquinoline reacts with calcium to form color complex. Cresophthalein eliminates interferences from proteins.

Assay of glucose in serum

Colorimetric method to determination of glucose in the sera was carried out using Human kit. The measurement of glucose done by enzymatic oxidation of glucose via glucose oxidase to produce hydrogen peroxide, and then react with 4-amino phenazone to produce color compound quinoneimine read at (500 nm).

Results

Serum calcium level was determined calorimetrically by using Human kit, which the table showed comparison between tow groups pregnant with control group as marriage women and non pregnant.

The results in table (1) reveal that serum calcium level in the sera of pregnant group decreased highly significantly ($P < 0.002$) when compared with that of the control group.

Table (1): Calcium level in the sera of pregnant group and control group with statistical analysis value.

Groups	N (Numbers)	Age/year	Range (mg/dl)	Mean (mg/dl)	SD	P-Value
Pregnants	22	16-32	4-13.5	7.06	2.8	$P < 0.002$
Non Pregnants	20	18-28	8-12	9.51	1.56	

The alkaline phosphatase activity was determined calorimetrically. The activity of alkaline phosphatase was measured according to Kind method by using Human Kit, which the table showed comparison between tow groups pregnant with control group as marriage women and non pregnant.

The results in table (2) reveal that alkaline phosphatase activity in the sera of the pregnant group non significantly variation when compared with that of the control group ($P > 0.05$).

Table (2): Alkaline Phosphatase activity in the sera of pregnant group and control group with statistical analysis value.

Groups	N (Numbers)	Age/year	Range (I.U/100ml)	Mean (I.U/100ml)	SD	P- Value
Pregnants	22	16-32	25-91	47.3	18.8	(P > 0.05)
Non Pregnants	20	18-28	20-63	45.19	13.97	

Serum glucose concentration was determined calorimetrically by using human kit, which the table showed comparison between two groups pregnant with control group as marriage women and non pregnant

The results in table (3) reveal that serum glucose concentration in the pregnant group none significantly variation when compared with that of the control group ($P > 0.05$).

Table (3): Glucose concentration in sera of pregnant group and control group with statistical analysis value.

Groups	N (Numbers)	Age/year	Range (mg/dl)	Mean (mg/dl)	S.D	P-Value
Pregnants	22	16-32	78-128	105	28	Non Significant
Non Pregnants	20	18-28	85-113	98	19	

Discussion

The results in the table (1) refer to the significant variations in the sera calcium level in the pregnant group in comparison with that of control group, which the result refer to decrease in the calcium level in the pregnant as comparison with control.

Our result agrees with many studies [26, 27]. Which these studies refer to drop in calcium levels in the pregnant. Maternal bone loose during pregnancy might lead to

osteoporosis or fracture by reducing bone mass peak this can be recognized but rare complication of pregnancy [28]. This disorder in pregnancy is related to disturbances in metabolism of calcium, which the suggest for these changes due to the insufficient dietary calcium supply [27].

Calcium absorption is increased in pregnant women [29-31]. In urinary tract calcium excretion is also increased [30]. Therefore the decrease in calcium level in our result due to the change in calcium metabolism in pregnancy in advance of the increased calcium requirement for fetal growth [30, 32, 33]. This may be response to the expanding extracellular fluid volume and the increased glomerular filtration rate in order to maintain plasma ionized calcium concentration and calcitonin are raised [34], and serum calcium decrease in line with changes albumin concentration with no changes in ionized calcium [27].

The results in the table (2) refer to the non significant variations in alkaline phosphatase activity in the pregnant group in comparison with that of control group. But these results non accord the result in many studies [36]. Zinc is an essential constituent of several enzymes such as alkaline phosphatase some divalent ions, such as Mg^{+2} , Co^{+2} , and Mn^{+2} , are activators of the enzyme, and Zn^{+2} is constituent metal ion [37]. Therefore none elevated due to deficient one of these metals in the sera of pregnants.

Conclusion

The calcium level in this study is decrease in pregnants sera due to the consumption to build fetus body, but alkaline phosphatase activity stay in normal value or there is not variation in the pregnants group when compared with control group, which belonged to other parameters such as phosphorus, and these result accompanied with non diabetic symptoms.

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دراسة فعالية الفوسفاتيز القاعدي ومستوى الكالسيوم بمتابعة الكلوكوز في مصول النساء الحوامل في الفصل الثاني من فترة الحمل

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الخلاصة

الهدف من هذه لدراسة قياس فعالية انزيم الفوسفاتيز القاعدي ومستوى الكالسيوم مع متابعة نسبة السكر في مصول 22 نموذج لنساء حامل اصحاء في الفصل الثاني من فترة الحمل وكذلك 20 نموذج لنساء متزوجات غير حوامل كمجموعة سيطرة. اجريت هذه الدراسة في قسم الكيمياء، كلية العلوم، الجامعة المستنصرية وذلك للفترة من كانون الاول 2013- شباط 2014. اوضحت النتائج ان لا فروقات معنوية في فعالية انزيم الفوسفاتيز القاعدي ($P > 0.05$)، بينما هناك انخفاض معنوي كبير في مستوى الكالسيوم في مصول النساء الحوامل باحتمالية ($p < 0.002$) مع بقاء نسبة السكر ضمن الحدود الطبيعية عند المقارنة مع مجموعة السيطرة.