STUDY OF SOME BIOCHEMICAL PARAMETERS FOR PREGNANT WOMEN [+]

دراسة بعض متغيرات الكيمياء الحياتية للحوامل

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Abstract:

The research included measurement of calcium, inorganic phosphate (Pi) and creatinine concentrations in blood and urine samples and the measurement of the activity of alkaline phosphatase(AlP) in blood for pregnant.

Venous blood samples were taken from 12 normal healthy nullipourous women and 41 pregnant women and random urine samples were taken from 37 pregnant women and 12 normal healthy volunteers the effect of gestational period, gravidity, milk nourishment and age on some biochemical parameters were studied.

The results showed a significant increase in AlP activity, small decrease in calcium, phosphorous and creatinine concentrations in blood in the third trimester, while Ca/ Cr, P/ Cr and urinary excretion of phosphorous increased in pregnant when compared to control.

The results also predicted that there is no difference between groups when compared according to gravida. Pregnant women who nourished with milk during gestation period have an increase in calcium and phosphorous concentrations in blood and a decrease in calcium and phosphorous excretion.

Finally, a decrease in calcium and phosphorous with age also was noticed, and no effect of age on calcium and phosphorous excretion in urine.

المستخلص:

يتضمن البحث قياس تركيز الكالسيوم والفوسفات اللاعضوية والكرياتنين في الدم والإدرار وقياس فعالية إنزيم الفوسفاتيز القاعدي في دم النساء الحوامل. اخذت نماذج الدم من ١٢ امراة غير حامل و ٤١ امرأة حامل، كذلك أخذت نماذج إدرار عشوائي من ٣٧ امراة حامل و ١٢ امراة غير حامل متطوعة. ثم درست تأثير فترة الحمل وعدد مرات الحمل وتناول الحليب من قبل المراة الحامل والعمر على بعض متغيرات الكيمياء الحياتية.

أوضحت النتائج زيادة معنوية في فعالية أنزيم الفوسفاتيز القاعدي وانخفاض بسيط في تركيز الكالسيوم والفوسفات اللاعضوية والكرياتنين في الدم في الثلاث اشهر الاخيرة من الحمل، كما لوحظ ارتفاع في تركيز الكالسيوم والفوسفات اللاعضوية نسبة الى الكرياتنين وطرح الفوسفات في الإدرار خلال الثلاث أشهر الأخيرة من الحمل عند مقارنتها مع نماذج السيطرة.

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كذلك أوضحت النتائج انه لا تاثير لعدد مرات الحمل على المتغيرات الحياتية التي درست. ايضا أوضحت النتـــائج ان النساء الذين يتغذون على الحليب خلال فترة الحمل يلاحظ ارتفاع في مستوى الكالسيوم والفوسفات اللاعضوية فـــي الدم وانخفاض في طرح الكالسيوم والفوسفات في الادرار.

أخيرا أوضحت النتائج انخفاض في تركيز الكالسيوم والفوسفات اللاعضوية مع تقدم العمر ولاتاثير للعمر على طـــرح الكالسيوم والفوسفات اللاعضوية في الادرار.

Introduction:

Pregnancy is the carrying of one or more fetuses by female mammals, including humans, inside their bodies. Pregnancy is typically broken into three periods, or trimesters, each of about three months [1].

Pregnancy is a time of increased need for calcium and magnesium. Insufficient calcium supply during pregnancy and lactation could result in maternal bone loss [2].

Calcium and inorganic phosphate are macro nutrient, and very essential for bone formation in fetus. In pregnancy the very high concentrations of estrogen and progesterone alter the concentration of many substances in the maternal blood [3].

Khastigir and Studd have noted that there is an increased demand for calcium and inorganic phosphate for fetal development during pregnancy [4]. Calcium absorption and regulation involve a complex interplay between multiple organ systems and regulatory hormones. The major regulators of calcium levels are parathyroid hormone (PTH) and vitamin D, which target the bones, intestine and kidney to increase serum calcium [5].

There are contracting views on the effect of gestational age on serum calcium and inorganic phosphate levels [6,7].

Recently it has been suggested that the PTH stimulate the synthesis of 1,25 (OH)2 D3 by blocking tubular reabsorption of phosphate thereby reducing the renal cell level of inorganic phosphorous [8]. Phosphate homeostasis is regulated by the kidney, because intestinal phosphate absorption is nearly complete and renal excretion determine the serum level. Excessive intestinal phosphate absorption causes a fall in serum ionized calcium and a rise in PTH secretion, resulting in phosphateuria, thus lowering serum phosphate and permitting calcium to rise [9].

During pregnancy cardiac output and renal blood flow are increased, this leads to an increased in glomeruler filtration rate (GFR) with resultant decreased in concentration of serum urea, creatinine and uric acid. The GFR can be calculated using serum creatinine [10]. Pregnancy induces a variety of physiologic changes in the urinary tract. Glomeruler filtration rate and renal plasma flow are ordinarily increased during pregnancy [11]. The usefulness of measuring urinary calcium in relation to creatinine excretion in random urine specimens has been demonstrated by some investigators [12].

On the light of studies mentioned above and because there is inadequate information on how pregnancy affect some biochemical parameters during gestational period, and what is the effect of multiple pregnancy, milk nourishment and age, so this study was designed to examine this gap in knowledge.

Patients and methods

Venous blood and random urine sample were taken from 12 normal healthy nulliparous women and selected as control, also 41 venous blood sample and 37 random urine sample were taken from pregnant women in a different gestational age who registered for antenatal care and gave birth in Al- Khansaa hospital between May and June 2005, the age range was

16 to 45 years. Samples were classified into groups according to gestational age, parity, milk nourished and age.
The women were compared for calcium, inorganic phosphate and creatinine concentrations and alkaline phosphatase activity in serum. In urine samples, calcium, inorganic phosphate and creatinine concentrations were assayed to calculate Ca/ Cr, P/ Cr and urinary excretion of phosphorous to compare it with the control.
Urinary excretion of phosphorous was expressed as the fractional PO4 (Cpo4/ Ccr) according to creatinine clearance (C) rate, calculated as urine PO4 concentration x serum creatinine / urine creatinine x serum PO4, the units for measurement the concentrations being mg/dl [13].serum calcium was measured by O-cresolphthalein complexone method [14,15], serum phosphorous by molybdate reaction [16], creatinine by picric acid using manual method [17] and alkaline phosphatase activity was estimated according to method of Kind and King [18].
Chemical kits for assay of calcium and AlP was supplied by BioMeriux and phosphorous obtained from Syrbio.
Standard statistical methods was used to calculate the mean (X), standard deviation (SD) and standard error (SE). Z- test was used to differentiate data obtained from P- value.

Results & Discussion:

1- ***Effect of gestation period.***

Pregnant samples were classified into groups according to gestation month as shown in table 1 & 2 and according to trimester as indicated in table 3.
Table 1 indicate that there is a small decrease in AlP activity in the 2, 3 and 4^{th} month, then the activity increased gradually in the 7, 8 and 9^{th} month of gestation, the increase was significant in the 8 and 9^{th} month. Results for calcium indicate that its level decrease to about 11% in the 9^{th} month. Phosphorous concentration decreased when calcium increased & vise versa. In the 3^{rd} month it was noticed that there is a 20% increase in its level. Finally creatinine concentration increased during 2, 3 & 4^{th} then decreased in the 7, 8 & 9^{th} month.
Results shown in table 3 indicate that AlP activity increased while Ca, phosphorous and creatinine decreased in the 3^{rd} trimester. Creatinine increased in the 3^{rd} and 4^{th} month for unknown reason. Urine results as shown in table 2 and 3 indicate that there is an increase in Ca/ Cr, P/ Cr and (Cpo4/ Ccr) especially in the 3^{rd} trimester. The excretion is maximum in the 8th month for calcium and phosphorous. When pregnant compared to non pregnant with out take into account the gestation period, it was noticed that AlP activity increase significantly, small decrease in calcium and a small increase in creatinine, Ca/ Cr, P/ Cr and (Cpo4/ Ccr) increased as shown in table 4.
All of the above results were found to be compatible with the results shown previously by others, as AlP decreased in early pregnancy and rise to normal or above by term [19]. Serum AlP is elevated due to AlP of placental origin [10] and it is not useful as a marker of bone formation [20]. Ionized calcium and phosphate levels remain near normal throughout pregnancy [21].
Current calcium recommendation for non pregnant are also sufficient for pregnant women because intestinal calcium absorption increases during pregnancy [22]. Pregnant and lactating women have increased need of dietary calcium. Most of the calcium is deposited during the third trimester of pregnancy [23]
Approximately 80% of calcium accumulates during the third trimester, when the fetal skeleton is rapidly mineralizing. Serum phosphate levels are normal throughout pregnancy in human and animals, as is the renal tubular reabsorption of phosphate [19].
During pregnancy, serum total calcium, phosphate and magnesium tend to be low due to the expanded intravascular space. Concentration of calcium is also affected by the reduced

albumin concentration. During pregnancy cardiac output and renal blood flow are increased, this leads to an increased GFR with resultant decreased concentration of serum urea, uric acid and creatinine [10]. Urinary excretion of calcium increased in gestation secondary to an increased calcium load filtered by the kidneys and increased GFR of pregnancy [24, 10]. Hypercalciuria was seen as a concomitant of pregnancy due to increased GFR, calcium filtration and intestinal calcium absorption related to high levels of plasma calcitriol [25].
Parathyroid hormone fall to (10-30) % of the mean non pregnant range in the 1st trimester, but increase again by term [26] phosphate excretion by the kidneys is greatly increased by PTH, thereby playing important role in the control of plasma phosphate concentration [27]

2- ***Effect of gravidity***
Pregnant women were divided into three groups according to gravidity. Group 1 are those which are pregnant for a first time, group 2 are those women with their pregnancy number ranging between (2-5) times, group 3 are those which are become pregnant for more than 5 times.
Results as shown in table 5 indicated that AlP activity increase in all groups without any difference between each other, a small decrease in serum calcium in all groups and no alteration in serum phosphorous was noticed except those of group 1 was increased for unknown reason. Also an increase in Ca/Cr, P/ Cr and (Cpo4/ Ccr) was noticed in urine samples of all groups especially in those of group 1.
All of the above results were in direct support with the results obtained previously, as AlP activity increased due to AlP of placental origin [10], the decrease in serum calcium was due to a high parity as it was reported by other investigators [28]. Other studies showed that maternal serum calcium fell with each succeeding pregnancy [29] and this seemed likely to be related to the higher frequency of hypocalcemic convulsions in infants born to mothers of higher parity and age [28]. On the other hand it was seen that no correlation could be detected between changes in mineral content and number of previous pregnancies [30].

3- ***Effect of milk nourishment***
Milk and milk products are the major sources of calcium, phosphorous is abundant in natural foods and renal conservation of it is quite efficient [23] . during pregnancy a mother has to supply the fetus with up to 30gm of calcium, if her diet is deficient in calcium, she draws upon the reserves in her bones. Repeated pregnancies with long lactation in women whose diet contains little or no milk and so is low in calcium leads to demineralization and softening of her bones (Osteomalacia) [31].
On the other hand the changes in calcium absorption that occur during pregnancy suggest that the physiologic adjustments are likely to cover the increased requirement of the mother without a need for her to increase her calcium intake [32].finally, no correlation could be detected between changes in mineral content and milk consumption [30].
The results shown in table 6 was in complete agreement with the results obtained by others, pregnant women were divided into two groups, group 1 was those which their diet lack a milk and group 2 was those milk nourished women, it was seen that calcium concentration increased to about 5% in the 2nd group, small increase in phosphorous was noticed and a decrease in Ca/Cr and a high decrease in (Cpo4/ Ccr).

4- ***Effect of age***
Pregnant women were classified into 4 groups according to their ages, group 1 are those women with less than 20 years old, group 2 are those their ages ranging between (21-25), group 3 those their ages ranging between (26-30) and group 4 are those women with ages ranging between (31-35) years old.

It was noticed as indicated in table 7, a decrease in calcium and phosphorous with age, creatinine decrease in group 2 then increase gradually till group 4 to about 14%, also Ca/ Cr, P/ Cr and (Cpo4/ Ccr) decreased in all groups without difference between each other.
All of the above results was compatible with the results obtained by others, as no correlation could be detected between changes in mineral content and age [30]. Some old people have a markedly reduced capacity to absorb calcium from the gut [33] calcium absorption efficiency is increased during pregnancy and lactation, but the elderly in both sexes show a pronounced fall in calcium absorption [34].

Conclusions:

1-AlKPase activity increased significantly in pregnant women in the third trimester.
2-Calcium, phosphorous and creatinine decreased in blood and their excretion in urine increased in the third trimester.
3-No effect of gravidity on calcium and phosphorous concentrations.
4-Milk nourishment during gestation period increase calcium and phosphorous in blood and decrease their excretion in urine.
5-Decrease in calcium and phosphorous level in blood with age.

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Table (1) Effect of gestation period / month on the level of serum parameters assayed

Sample		Parameters assayed											
		AlP (Kind &king U/l)			Ca mg/dl			PO4 mg/dl			Cr mg/dl		
		N	Mean	%change	N	Mean	% change	N	Mean	% change	N	Mean	% change
Control		12	6.29		12	10.02		12	2.91		8	1.739	
Gestation month	2	3	6.03	-4.13	3	10.75	7.28	3	2.51	-13.74	3	1.749	0.57
	3	6	6.31	0.31	6	9.97	-0.49	6	3.51	20.61	5	2.070	19.03
	4	6	4.84	-23.05	6	9.83	-1.89	6	2.98	2.40	6	2.440	40.31
	7	7	8.4	33.54	7	10.77	7.48	7	2.74	-5.84	6	1.388	-20.18
	8	4	10.69	69.95*	4	10.22	1.99	4	2.97	2.06	2	1.194	-31.33
	9	14	15.87	152.3*	14	8.91	-11.07	14	2.84	-2.4	11	1.636	-5.92

* Significant at p≤ 0.05

Table (2) Effect of gestation period/ month on the level of urine parameters assayed

Sample		Parameters assayed								
		Ca/Cr			PO4/Cr			CPO4/Ccr		
		N	mean	% change	N	Mean	% change	N	mean	% change
Control		3	5.74		3	78.63		3	59.692	
Gestation month	2	3	13.12	128.57	3	82.05	4.34	3	68.072	14.03
	3	2	8.77	52.78	2	162.45	106.6	2	109,154	82.92
	4	6	8.33	45.12	6	103.83	32.04	6	93.194	56.12
	7	6	7.36	28.22	6	70.19	-10.73	6	41.970	-29.68
	8	2	59.05	928.74	2	670.39	752.58	2	251.869	321.94
	9	10	7.64	33.10	11	102.99	30.98	11	87.299	46.24

* Significant at p≤ 0.05

Table (3) Effect of trimester on the level on serum and urine parameters assayed

	Parameters assayed	Sample										
		Control		1st trimester			2nd trimester			3rd trimester		
		N	Mean	N	Mean	% change	N	Mean	% change	N	Mean	% change
Serum	AlP (Kind &king U/l)	12	6.29	9	6.22	-1.11	7	5.71	-9.22	25	12.95*	105.88
	Ca mg/dl	12	10.02	9	10.23	2.09	7	9.88	-1.39	25	9.64	-3.79
	PO4 mg/dl	12	2.91	9	3.17	8.93	7	2.98	2.40	25	2.83	-2.74
	Cr mg/dl	8	1.739	8	1.950	12.13	7	2.500	43.76	19	1.511	-13.11
Urine	Ca/Cr	3	5.74	5	11.38	98.25	7	8.30	44.59	18	13.26	131.01
	PO4/Cr	3	78.63	5	114.21	45.24	7	97.61	24.13	19	152.35	93.75
	CPO4/Ccr	3	59.692	5	84.504	41.56	7	88.064	47.53	18	90.475	51.56

* Significant at p≤ 0.05

Table (4) A comparison of serum and urine parameters assayed between pregnant & non pregnant.

	Parameters assayed	Sample				
		Non pregnant		Pregnant		
		N	Mean	N	Mean	% Change
Serum	AlP (Kind &king U/l)	12	6.29*	41	10.24	62.79
	Ca (mg/dl)	12	10.02	41	9.81	-2.09
	PO4 (mg/dl)	12	2.91	41	2.93	0.68
	Cr(mg/dl)	8	1.739	34	1.818	4.54
Urine	Ca/Cr	3	5.74	30	11.79	105.40
	PO4/Cr	3	78.63	31	133.84	70.21
	CPO4/Ccr	3	59.692	30	88.917	48.95

* Significant at p≤ 0.05

Table (5) Effect of gravidity on serum and urine parameters assayed

Parameters assayed		Pregnancy number										
		Control		1			(2-5)			>5		
		N	Mean	N	Mean	%change	N	Mean	%change	N	Mean	%change
Serum	AlP(Kind&king U/l)	12	6.29	7	11.07	75.99	16	9.96	58.34	17	10.50	66.93
	Ca (mg/dl)	12	10.02	7	9.79	-2.29	16	9.94	-0.79	17	9.63	-3.89
	PO4 (mg/dl)	12	2.91	7	3.30	13.4	16	2.86	-1.71	17	2.95	1.37
Urine	Ca/Cr	3	5.74	6	23.77	314.11	13	7.95	38.50	11	9.79	70.36
	PO4/Cr	3	78.63	6	300.51	282.18	13	95.70	21.71	12	91.82	16.77
	CPO4/Ccr	3	59.692	6	141.297	136.71	13	70.745	18.51	11	81.822	37.07

Table (6) Effect of milk nourishment on the level of serum and urine parameters assayed.

Parameters assayed		Sample				
		Without milk		Milk nourished		
		N	Mean	N	Mean	% Change
Serum	AlP (Kind &king U/l)	33	8.96	8	15.49*	72.87
	Ca (mg/dl)	33	9.72	8	10.17	4.62
	PO4 (mg/dl)	33	2.92	8	2.99	2.39
Urine	Ca/Cr	24	12.06	6	10.72	-11.11
	PO4/Cr	25	147.18	6	78.25	-46.83
	CPO4/Ccr	24	101.082	6	40.256	-60.17

* Significant at p≤ 0.05

Table (7) Effect of age on the level of serum and urine parameters assayed.

Parameters assayed		Group number												
		1		2			3			4			5	
		N	Mean	N	Mean	%change	N	Mean	%change	N	mean	%change	N	mean	%change
Serum	AlP (Kind &king U/l)	7	10.64	12	13.26	24.62	10	8.33	-21.71	5	9.48	-10.9	7	7.92	-25.56
	Ca mg/dl	7	10.07	12	9.92	-1.48	10	9.72	-3.47	5	9.14	-9.23	7	9.96	-1.09
	PO4 mg/dl	7	3.17	12	2.81	-11.35	10	3.00	-5.36	5	2.80	-11.67	7	2.91	-8.20
	Cr mg/dl	7	2.079	9	1.213	-41.65	9	2.071	-0.38	4	2.385	14.71	5	1.632	-21.50
Urine	Ca/Cr	6	24.14	8	6.02	-75.06	8	12.72	-47.3	4	7.18	-70.25	4	7.56	-68.68
	PO4/Cr	6	308.54	8	65.27	-78.84	9	135.05	-56.22	4	90.37	-70.71	4	49.69	-83.89
	CPO4/Ccr	6	150.281	8	30.895	-79.44	8	121.954	-18.84	4	97.138	-35.36	4	38.621	-74.30

Group no: 1< 20 years 2 (21-25) years 3 (26-30) years 4 (31-35) years 5 > 35 years