Serum Calcium and Magnesium Levels in Normal Ghanaian Pregnant Women: A Comparative Cross-Sectional Study

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Abstract

BACKGROUND: Pregnancy is described as a normal physiologic state associated with various biochemical changes. Magnesium and calcium are essential macronutrients required for foetal growth. Complications associated with their deficiency during normal pregnancy include; low neonatal birth weight, pre-eclampsia, eclampsia and preterm labour. Changes in serum levels of magnesium and calcium in normal pregnancy have not been extensively studied among Ghanaian women.

AIM: To determine the variation in serum magnesium and calcium levels with gestational age in normal pregnancy in Ghanaian women.

METHODS: A hospital-based comparative cross-sectional study was conducted among 32 normal non-pregnant women (Group A) and 100 normal pregnant women (Group B) attending the clinic at the Korle-Bu Teaching hospital. The group B pregnant women were further divided into Group B1 (n = 33), Group B2 (n = 37) and Group B3 (n = 30) based on their pregnancy gestation as first, second and third trimester respectively. Blood samples were obtained from the antecubital vein of subjects and total serum calcium, magnesium, protein and albumin were estimated. Data obtained were analysed using SPSS for windows version 20. Analysis of variance (ANOVA) was employed to determine the statistical differences between the groups. A p-value of ≤ 0.05 was considered significant.

RESULTS: Mean serum total calcium and magnesium in first, second and third trimester normal pregnant women were 2.14 ± 0.16, 2.13 ± 0.44, 2.13 ± 0.35 mmol/L and 0.77 ± 0.11, 0.77 ± 0.16 and 0.76 ± 0.14 mmol/L respectively. Mean serum total calcium and magnesium levels in non-pregnant women were 2.20 ± 0.16 and 0.80 ± 0.10 mmol/L respectively. There was a statistically non-significant difference in serum total calcium and magnesium between non-pregnant and normal pregnant women, with p-values of 0.779 and 0.566 respectively. Mean total serum protein and albumin in first, second and third-trimester normal pregnant women were 68.42 ± 10.37, 70.46 ± 6.84, 66.70 ± 7.83 g/L and 39.92 ± 3.22, 40.75 ± 8.06, 38.26 ± 3.02 g/L respectively. Mean total serum protein and albumin in non-pregnant women were 73.13 ± 7.02 and 42.94 ± 3.03 g/L respectively. Mean total serum protein and albumin levels were lower in pregnant women as compared to non-pregnant women with the difference being significant in the third trimester (p-values of 0.012 and 0.002).

CONCLUSION: Total serum calcium and magnesium levels in normal pregnancy were non-significantly lower compared to non-pregnant women in Ghana. There was a reduction in total serum protein, and albumin levels during pregnancy with a significant reduction noticed during the third trimester compared to the non-pregnant state.

Introduction

Magnesium and calcium are essential for foetal growth. Various physiological changes occur during pregnancy for maternal adaptation as well as to meet the nutritional demands of the developing foetus [1]. Maternal nutritional status during pregnancy has been reported to influence the development of the foetus [2], [3]. Imbalances in maternal nutritional status and foetal demands can produce catastrophic complications [4], [5] especially in the third trimester where there is a rapid development of the foetus [6]. Hormonal alterations during pregnancy may alter serum nutritional status including macronutrient levels [7].

Various biological processes in the body require calcium as it is the most abundant mineral in

the body. Total blood calcium consists of ionic/free (50-65%), protein-bound mainly albumin (30-45%) and calcium complexed to anions (5-10%) mainly bicarbonate, citrate and lactate [8]. These three forms exist in equilibrium with each other maintaining a constant serum level in health. The readily available form of calcium to cells is the ionised form which therefore reflects accurate physiological calcium status. About 50% of maternal serum calcium is bound to proteins in healthy non-pregnant states. Maternal serum calcium levels have been reported to vary with the gestational age of pregnancy [9], [10]. Most studies have reported a significantly reduced total serum calcium levels in normal pregnancy especially the third trimester of pregnancy [11], [12], [13]. This has been explained on account of dietary deficiency, increasing demand of the growing foetus [14], [15] as well as haemodilution from fluid retention during the latter stages of pregnancy [16]. This is compounded by increasing urinary loss of calcium [17] and a fall in serum albumin levels [18], [19]. A normal growing foetus accumulates about 21g of calcium (range 13-33g) [20]. However, 80% of this accumulation occurs rapidly in the third trimester due to skeletal mineralisation [18], [21]. However, other studies have reported no variation in total serum calcium levels with increasing gestational age [22] while others have reported an increase in maternal serum calcium levels compared to the non-pregnant state [23].

Magnesium is the fourth commonest mineral ion as well as the second commonest intracellular cation in the body. About 50% of total body magnesium is located in bones while the other 50% is mainly located intracellularly. Approximately 1% is found intravascularly, and its level is tightly controlled [24] Magnesium acts as a co-factor for various enzymatic processes. It also has a role in peripheral vascular relaxation [25] Serum levels of magnesium has been reported to be between 1.5-2.1 mg/dl [26]. The decrease in serum magnesium levels with increasing gestational age has been reported in some studies and initiation of labour has been attributed by some to myometrial magnesium deficiency [27], [28]. Maternal to foetal transfer (with almost 80% of the transfer occurring during the third trimester) and haemodilution during pregnancy has been proposed as mechanisms for the observed decrease in serum magnesium during pregnancy [16].

Magnesium is an important mineral for foetal growth. Deficiency of magnesium during pregnancy has been associated with pre-eclampsia, eclampsia, preterm labour and delivery of babies with low birth weight [29].

A wide range of serum magnesium levels has been reported among non-pregnant women and pregnant women at all trimesters [30]. Other studies have reported no difference in serum magnesium levels between pregnant and non-pregnant women [31].

Therefore in the serum levels of total calcium and magnesium during normal pregnancy seem inconclusive. The paucity of literature on the subject in our setting, therefore, necessitated this study.

Material and Methods

This is a comparative cross-sectional study conducted between June and August 2016 at the Korle-Bu Teaching Hospital

The site of this study was the Korle-Bu Teaching Hospital which is the largest tertiary referral hospital in Ghana. The obstetrics and gynaecology department has approximately 400 beds and conducts over 11,000 deliveries annually.

All healthy pregnant and non-pregnant women between the ages of 18 and 35 years inclusive who visited the antenatal and gynaecology clinic of the hospital were included in the study.

Pregnant and non-pregnant women with diabetes and hypertension, renal disease, chronic and acute infections, history of thyroid; parathyroid; pancreatic; hepatic disease, and women who were on calcium and magnesium supplements/medications containing these minerals were excluded from the study.

On fulfilling the inclusion criteria and obtaining informed consent, consecutive recruitment of one hundred and thirty-two (132) women was done, consisting of 32 normal non-pregnant women as controls (Group A) and 100 normal pregnant women (Group B).

Normal pregnant women in Group B were further divided into three groups (B1, B2 and B3) based on the gestational ages of their pregnancies. Group B1 (gestational age 1-13 weeks, n = 33), Group B2 (gestational age 14-28 weeks, n = 37) and Group B3 (gestational age 29-40+ weeks, n = 30).

The age, weight, and height of the recruited women were recorded. Their blood pressures were also measured in the sitting position using the right arm on two occasions separated by 15 minutes interval and averaged using mercury sphygmomanometer and a stethoscope.

Without the application of a tourniquet, four (4) millilitres of venous blood was obtained from the cubital vein of the left arm with the patient in the sitting position using a 19G hypodermic needle and a syringe and the blood sample was immediately transferred into a coded plain test tube. Within two (2) hours of collection, samples were sent to the laboratory and centrifuged at 4,000 rpm for 10 minutes to obtain the serum which was subsequently stored at -20°C before analysis within 24 hours of

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sample collection. Serum magnesium and total serum calcium were determined using an atomic absorption spectrometer in acetylene-air flame (Variant 240FS; Varian Australia Pty Ltd, VIC, Australia) with reference ranges of 0.74–1.03 mmol/L and 2.12–2.62 mmol/L, respectively.

Data collected was handled confidentially with anonymous code identifiers for each subject and stored in Microsoft access data base 2010. Analysis of the data was done using the SPSS version 20. Comparison of means of total serum calcium and magnesium between the groups were done using analysis of variance (ANOVA). Statistical significance was considered at a p-value < 0.05.

Approval for this study was obtained from the ethical and protocol review committee of the University of Ghana School of Medicine and Dentistry.

Results

There was no significant difference in age, and systolic blood pressure in the groups of women studied. There was, however, a significant difference in BMI, and diastolic blood pressure of the groups of women studied.

Table 1: General characteristics of subjects

Characteristic	Non-pregnant	First trimester	Second trimester	Third trimester	
	n = 32	n = 33	n = 37	n = 30	
			Mean (SD)		p-value
Age (years)	31 (5.11)	29 (3.76)	30 (3.97)	30 (2.60)	0.291
BMI (kg/m^2)	29 (6.23)	25 (6.25)	28 (5.52)	30 (5.50)	0.001*
Systolic BP (mmHg)	118 (16.08)	111 (14.70)	112 (12.11)	116 (13.38)	0.118
Diastolic BP (mmHg)	77 (9.35)	71 (10.46)	66 (7.68)	68 (8.54)	< 0.001*

*significant at p ≤ 0.05, kg = kilogram, m = metres, mmHg = millimetres of mercury, g = gram, dl = decilitre SD = standard deviation.

The median (ranges) of parity of subjects were; non-pregnant 1(0-5), first trimester 1(1-2), second trimester 1(0-3) and third trimester 1(0-7). There was no significant difference (p-value = 0.965) in parity.

Table 2: Total serum calcium and magnesium levels of subjects

Characteristic	Non-pregnant	First trimester	Second trimester	Third trimester	
	n = 32	n = 33	n = 37	n = 30	
			Mean (SD)		p-value
Total serum calcium (mmol/l)	2.20 (0.16)	2.14 (0.16)	2.13 (0.44)	2.13 (0.35)	0.779
Serum magnesium (mmol/l)	0.80 (0.10)	0.77 (0.11)	0.77 (0.16)	0.76 (0.14)	0.566
Serum albumin (g/l)	42.94 (3.03)	39.92 (3.22)	40.75 (8.06)	38.26 (3.02)	0.004*
Total serum protein (g/l)	73.13 (7.02)	68.42 (10.37)	70.46 (6.84)	66.70 (7.83)	0.014*

*significant at p ≤ 0.05, mmol/l = millimole per litre, g/l = gram per litre, SD = standard deviation.

There was no significant change in total serum calcium and magnesium among the groups of women studied. There was, however, a significant

difference in serum albumin and total serum protein between the groups of women studied.

Table 3: Serum electrolytes range of values in normal pregnancy and non-pregnant women

Characteristics	Non-pregnant	First trimester	Second trimester	Third trimester
	n = 32	n = 33	n = 37	n = 30
	Range			
Total serum calcium (mmol/l)	1.95-2.66	1.58-2.52	1.21-3.70	1.33-2.83
Serum magnesium (mmol/l)	0.62-1.07	0.45-1.04	0.46-1.09	0.49-1.02

There was a wide range of total serum calcium in normal pregnancy (1.21-3.70 mmol/l) and non-pregnant women (1.95-2.66 mmol/l).

There was a wide range of serum magnesium in normal pregnancy (0.45-1.09 mmol/l) and non-pregnant women (0.62-1.07mmol/l).

Table 4: Pairwise comparison analysis of differences in diastolic blood pressure

Comparison	p-value
First trimester *vrs* Second trimester	0.137
First trimester *vrs* Third trimester	0.445
Second trimester *vrs* Third trimester	0.937
First-trimester *vrs* Non-pregnant	0.068
Second trimester *vrs* Non-pregnant	< 0.001*
Third-trimester vrs Non-pregnant	0.001*

*significant at p ≤ 0.05, *vrs* = versus.

There was no significant change in diastolic blood pressure with gestational age among the normal pregnant women. The diastolic blood pressures were however significantly lower among women in the second and third trimester of pregnancy compared to the non-pregnant state.

Table 5: Pairwise comparison analysis of differences in total protein

Comparison	p-value
First trimester *vrs* Second trimester	0.719
First trimester *vrs* Third trimester	0.836
Second trimester *vrs* Third trimester	0.239
First-trimester *vrs* Non-pregnant	0.094
Second trimester *vrs* Non-pregnant	0.526
Third-trimester vrs Non-pregnant	0.012*

*significant at p ≤ 0.05, *vrs* = versus.

There was a reduction in total serum protein levels during pregnancy with a significant reduction noticed during the third trimester compared to the non-pregnant state. There was no significant inter-trimester change in total serum protein.

Table 6: Pairwise comparison analysis of differences in Serum albumin

Comparison	p-value
First trimester *vrs* Second trimester	0.900
First trimester *vrs* Third trimester	0.557
Second trimester *vrs* Third trimester	0.186
First-trimester *vrs* Non-pregnant	0.078
Second trimester *vrs* Non-pregnant	0.275
Third-trimester vrs Non-pregnant	0.002*

*significant at p ≤ 0.05, *vrs* = versus.

There was a reduction in serum albumin levels during normal pregnancy with a significant

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reduction noticed during the third trimester compared to the non-pregnant state. Serum albumin levels showed no significant inter-trimester change.

Discussion

Normal pregnancy is associated with a reduction in systemic vascular resistance secondary to vasodilatation from reproductive hormones such as oestrogen and progesterone [32], [33]. Both systolic and diastolic blood pressures (DBP) have been reported to decrease in normal pregnancy. The reduction in DBP is reportedly greater than the reduction in systolic blood pressure (SBP). We observed a non-significant reduction in systolic blood pressures but a significant reduction in diastolic blood pressure especially in second and third trimesters compared to the non-pregnant state, with no significant inter-trimester change in DBP, a finding consistent with those of other similar studies [32], [34], [35]. The reduction in blood pressure noted in these studies starts in the first trimester and nadirs in the second trimester, with a rise towards the pre-pregnancy levels in the third trimester just as noted in our study. The DBP in the third trimester in this study was still significantly lower compared to the non-pregnant state.

Other studies have reported a non-significant change in blood pressures [36] while others have also noted a progressive rise in blood pressure throughout pregnancy [34], [37], [38]. These studies analysed the blood pressures based on patients' weight before pregnancy or total weight gained during pregnancy, factors which were not considered in this study and may, therefore, account for the differences in our results

We observed a non-significant reduction in total serum calcium levels during normal pregnancy (p = 0.779). This observation is supported by those of Standley et al., [22] and Olatunbosum et al., [39] but contrast those of Bassam Hanna [40] and Sultana et al., [41]. Studies by Bassam Hanna and Sultana et al., argued that the low serum total calcium observed in late normal pregnancy was due to increased foetal demand and physiological haemodilution secondary to increased intravascular volume as a result of significantly reduced serum albumin levels (which is the main calcium binding plasma protein) [40], [41]. In this study, we observed significantly reduced total serum proteins (p = 0.012) and albumin (p = 0.002) in the third trimester compared to the non-pregnant state as observed by Sultana et al.,

However, total serum calcium levels were not significantly reduced. Thus changes in total serum calcium levels in pregnancy may be unrelated to alterations in serum protein levels as noted by Oberst and Plass [42]. The differences in results may be attributed to factors that were not considered in this study but which are known to influence total serum calcium levels including pre-analytical factors such as alterations in blood pH, exercise, postural changes, increased ventilation and diurnal variation, [8], [43], [44] and dietary habits [8], [45], [46]. Another factor that could have affected the total serum protein and albumin levels and therefore total serum calcium levels which were not considered in this study is proteinuria [47], [48]. Differences in seasons and climate (differences in exposure to sunlight and therefore vitamin D synthesis), as well as racial differences, could also account for the differences in results [49], [50], [51].

We also observed a non-significant reduction (p = 0.566) in serum magnesium during the progression of normal pregnancy compared to non-pregnant women as noted by Zohreh and Sara [52] and Deeper V Kanagal et al., [53]. Although a lot of studies have reported a fall in serum magnesium levels during normal pregnancy [3], [28], [54], [55] and has attributed this to foetal demand, haemodilution especially in late pregnancy and urinary loss, Newman (1957) and Archari et al., (1961) reported a non-significant change in serum magnesium levels in both pregnant and non-pregnant women as a result of a wider range of serum magnesium levels in both pregnant and non-pregnant women [30], [31]. In this study, there was a wider range of serum magnesium levels in both non-pregnant and pregnant women. This could, therefore, account for our finding of a non-significant difference in serum magnesium levels in non-pregnant and pregnant women. The dietary history to estimate calcium and magnesium intake of the women in our study was not done. Genetic differences concerning dietary absorption, utilisation and demand by the foetuses, as well as urinary excretion of calcium and magnesium may account for differences between our findings and those of others.

In conclusion, we observed a non-significant reduction in systolic blood pressures but a significant reduction in diastolic blood pressure especially in second and third trimester compared to the non-pregnant state, with no significant inter-trimester change in DBP. There was no significant difference in total serum calcium and magnesium levels in normal pregnancy compared to non-pregnant women in Ghana. There was a reduction in total serum protein, and albumin levels during pregnancy with a significant reduction noticed during the third trimester compared to the non-pregnant state.

Further longitudinal research is however needed to determine the normal ranges of serum magnesium and total calcium levels as well as the influence of dietary intake and the urinary excretion of these electrolytes on serum levels of both pregnant and non-pregnant Ghanaian women.

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Acknowledgement

References

1. Dutta DC. Textbook of Obstetrics,; New Central Book agency (P) Ltd., 2011; 81:7381-142.

2. Allen LH. Introduction (please, complete the reference).

3. Luke B. Nutritional influences on fetal growth. Clinical obstetrics and gynecology. 1994; 37(3):538-49. https://doi.org/10.1097/00003081-199409000-00007 PMid:7955642

4. King JC. Determinants of maternal zinc status during pregnancy–. The American journal of clinical nutrition. 2000; 71(5):1334S-43S. https://doi.org/10.1093/ajcn/71.5.1334s PMid:10799411

5. Scholl TO, Reilly T. Anemia, iron and pregnancy outcome. The Journal of nutrition. 2000; 130(2):443S-7S. https://doi.org/10.1093/jn/130.2.443S PMid:10721924

6. Brar HS, Rutherford SE. Classification of intrauterine growth retardation. In Seminars in perinatology. 1988; 12(1):2-10. PMid:3287628

7. Zilva JF, Pannall PR, Mayne PD. Clinical chemistry in diagnosis and treatment. London: Lloyd-Luke, 1975. PMid:15637929 PMCid:PMC475734

8. Beckett G, Walker S, Rae P. Peter Ashby Clinical biochemistry (Lecture notes), 2005.

9. Pitkin RM, Reynolds WA, Williams GA, Hargis GK. Calcium metabolism in normal pregnancy: a longitudinal study. American Journal of Obstetrics & Gynecology. 1979; 133(7):781-90. https://doi.org/10.1016/0002-9378(79)90115-7

10. Dahlman T, Sjöberg HE, Bucht E. Calcium homeostasis in normal pregnancy and puerperium: a longitudinal study. Acta obstetricia et gynecologica Scandinavica. 1994; 73(5):393-8. https://doi.org/10.3109/00016349409006250 PMid:8009970

12. Black AJ, Topping J, Durham B, Farquharson RG, Fraser WD. A detailed assessment of alterations in bone turnover, calcium homeostasis, and bone density in normal pregnancy. Journal of Bone and Mineral Research. 2000; 15(3):557-63. https://doi.org/10.1359/jbmr.2000.15.3.557 PMid:10750571

13. Naylor KE, Iqbal P, Fledelius C, Fraser RB, Eastell R. The effect of pregnancy on bone density and bone turnover. Journal of Bone and Mineral Research. 2000; 15(1):129-37. https://doi.org/10.1359/jbmr.2000.15.1.129 PMid:10646122

14. Bidlack WR. Interrelationships of food, nutrition, diet and health: the National Association of State Universities and Land Grant Colleges White Paper. Journal of the American College of Nutrition. 1996; 15(5):422-33. https://doi.org/10.1080/07315724.1996.10718620 PMid:8892167

15. Khastgir G, Studd JW, King H, Abdaila H, Jones J, Carter G, Alaghband-Zadeh J. Changes in bone density and biochemical markers of bone turnover in pregnancy-associated osteoporosis. BJOG: An International Journal of Obstetrics & Gynaecology. 1996; 103(7):716-8. https://doi.org/10.1111/j.1471-0528.1996.tb09845.x

16. Pitkin RM, Gebhardt MP. Serum calcium concentrations in human pregnancy. American Journal of Obstetrics & Gynecology. 1977; 127(7):775-8. https://doi.org/10.1016/0002-9378(77)90256-3

17. Cunningham FG, Gant NF, Leveno KJ, Larry C. Williams obstetrics. The Journal of Midwifery & Women's Health. 2003; 48(5):369. https://doi.org/10.1016/S1526-9523(03)00291-5

18. Kovacs CS. Calcium and bone metabolism in pregnancy and lactation. The Journal of Clinical Endocrinology & Metabolism. 2001; 86(6):2344-8. PMid:11397820

19. Kahn DA, Koos BJ. Maternal physiology during pregnancy. Current Diagnosis and Treatment Obstetrics and Gynecology. 2007; 10:155.

20. Givens MH, Macy IG. The chemical composition of the human fetus. Journal of Biological Chemistry. 1933; 102:7-17.

21. Trotter M, Hixon BB. Sequential changes in weight, density, and percentage ash weight of human skeletons from an early fetal period through old age. The anatomical record. 1974; 179(1):1-8. https://doi.org/10.1002/ar.1091790102 PMid:4821360

22. Standley CA, Whitty JE, Mason BA, Cotton DB. Serum ionized magnesium levels in normal and preeclamptic gestation. Obstetrics & Gynecology. 1997; 89(1):24-7. https://doi.org/10.1016/S0029-7844(96)00380-8

23. Pedersen EB, Johannesen P, Kristensen S, Rasmussen AB, Emmertsen K, Møller J, Lauritsen JG, Wohlert M. Calcium, parathyroid hormone and calcitonin in normal pregnancy and preeclampsia. Gynecologic and obstetric investigation. 1984; 18(3):156-64. https://doi.org/10.1159/000299073 PMid:6489849

24. Shaikh MK, Devrajani BR, Soomro AA, Ali Shah SZ, Devrajani T, Das T. Hypomagnesemia in Patients with Diabetes mellitus. World Applied Sciences Journal. 2011; 12(10):1803-6.

25. Indumati V, Kodliwadmath MV, Sheela MK. The role of serum electrolytes in pregnancy induced hypertension. J Clin Diagnostic Res. 2011; 5(1):66-9.

26. Schouten LJ, Goldbohm RA, van den Brandt PA. Height, weight, weight change, and ovarian cancer risk in the Netherlands cohort study on diet and cancer. American journal of epidemiology. 2003; 157(5):424-33. https://doi.org/10.1093/aje/kwf224 PMid:12615607

27. Hantoushzadeh S, Jafarabadi M, Khazardoust S. Serum magnesium levels, muscle cramps, and preterm labor. International Journal of Gynecology & Obstetrics. 2007; 98(2):153-4. https://doi.org/10.1016/j.ijgo.2007.04.009 PMid:17574257

28. Takaya J, Yamato F, Kaneko K. Possible relationship between low birth weight and magnesium status: from the standpoint of "fetal origin" hypothesis. Magnesium research. 2006; 19(1):63-9. PMid:16846102

29. Makrides M, Crosby DD, Bain E, Crowther CA. Magnesium supplementation in pregnancy. Cochrane Database of Systematic Reviews. 2014(4).

30. Newman RL. Serum electrolytes in pregnancy, parturition, and puerperium. Obstetrics & Gynecology. 1957; 10(1):51-5. PMid:13441219

31. Achari G, Mishra KC, Achari K, Ramkissun R, Upadhyay SN. Serum magnesium in women in the normal state and in certain conditions. Journal of the Indian Medical Association. 1961; 36:93-5. PMid:13681198

32. Mahendru AA, Everett TR, Wilkinson IB, Lees CC, McEniery CM. A longitudinal study of maternal cardiovascular function from preconception to the postpartum period. Journal of hypertension. 2014; 32(4):849-56. https://doi.org/10.1097/HJH.0000000000000090 PMid:24406777

33. Gilligan DM, Badar DM, Panza JA, Quyyumi AA, Cannon RO. Effects of estrogen replacement therapy on peripheral vasomotor function in postmenopausal women. American Journal of Cardiology. 1995; 75(4):264-8. https://doi.org/10.1016/0002-9149(95)80033-O

34. Grindheim G, Estensen ME, Langesaeter E, Rosseland LA, Toska K. Changes in blood pressure during healthy pregnancy: a longitudinal cohort study. Journal of hypertension. 2012; 30(2):342-50. https://doi.org/10.1097/HJH.0b013e32834f0b1c PMid:22179091

35. Sanghavi M, Rutherford JD. Cardiovascular physiology of

_____

pregnancy. Circulation. 2014; 130(12):1003-8. https://doi.org/10.1161/CIRCULATIONAHA.114.009029 PMid:25223771

36. Penaz J, Voigt A, Teichmann W. Contribution to the continuous indirect blood pressure measurement. Zeitschrift fur die gesamte innere Medizin und ihre Grenzgebiete. 1976; 31(24):1030-3. PMid:1020409

37. Nama V, Antonios TF, Onwude J, Manyonda IT. Mid-trimester blood pressure drop in normal pregnancy: myth or reality?. Journal of hypertension. 2011; 29(4):763-8. https://doi.org/10.1097/HJH.0b013e328342cb02 PMid:21178781

38. Gaillard R, Bakker R, Willemsen SP, Hofman A, Steegers EA, Jaddoe VW. Blood pressure tracking during pregnancy and the risk of gestational hypertensive disorders: the Generation R Study. European heart journal. 2011; 32(24):3088-97. https://doi.org/10.1093/eurheartj/ehr275 PMid:21821845

39. Olatunbosun DA, Adeniyi FA, Adadevoh BK. Serum calcium, phosphorus and magnesium levels in pregnant and non-pregnant Nigerians. BJOG: An International Journal of Obstetrics & Gynaecology. 1975; 82(7):568-71. https://doi.org/10.1111/j.1471-0528.1975.tb00688.x

40. Hanna B. The role of calcium correction during normal pregnancy at third trimester in Mosul. Oman medical journal. 2009; 24(3):188. https://doi.org/10.5001/omj.2009.37

41. Sultana MS, Begum R, Akhter QS, Lovely NS, Akhter S, Islam MT. Serum calcium and phosphate level in normal pregnant women. Bangladesh Journal of Medical Science. 2012; 11(3):217-20. https://doi.org/10.3329/bjms.v11i3.11732

42. Oberst WF, Plass ED. The variations in serum calcium, protein, and inorganic phosphorus in early and late pregnancy, during parturition and the puerperium, and in non-pregnant women. The Journal of clinical investigation. 1932; 11(1):123-7. https://doi.org/10.1172/JCI100395 PMid:16694022 PMCid:PMC435802

43. Dominiczak MH. Tietz Textbook of Clinical Chemistry. By CA Burtis and ER Ashwood, editors. Clinical Chemistry and Laboratory Medicine. 1999; 37(11-12):1136.

44. Winkel P, Statland BE, Bokelund H. The effects of time of venipuncture on variation of serum constituents: Consideration of within-day and day-to-day changes in a group of healthy young men. American journal of clinical pathology. 1975; 64(4):433-47. https://doi.org/10.1093/ajcp/64.4.433 PMid:1239188

45. Arneson WL, Brickell JM. Clinical Chemistry: a laboratory perspective. FA Davis, 2007.

46. Walker BR, Colledge NR. Davidson's Principles and Practice of Medicine E-Book. Elsevier Health Sciences, 2013.

47. Harewood WJ, Gillin A, Hennessy A, Armitstead J, Horvath JS, Tiller DJ. The effects of the menstrual cycle, pregnancy and early lactation on haematology and plasma biochemistry in the baboon (Papio hamadryas). Journal of medical primatology. 2000; 29(6):415-20. https://doi.org/10.1111/j.1600-0684.2000.290606.x PMid:11168833

48. Arneson WL, Brickell JM. Clinical Chemistry: a laboratory perspective. FA Davis, 2007.

49. Lips PT, Chapuy MC, Dawson-Hughes B, Pols HA, Holick MF. An international comparison of serum 25-hydroxyvitamin D measurements. Osteoporosis International. 1999; 9(5):394-7. https://doi.org/10.1007/s001980050162 PMid:10550457

50. Sayre RM, Dowdy JC, Shepherd J, Sadiq I, Bager A, Kollias N. Vitamin D production by natural and artificial sources. Orlando, Florida, Photo Medical Society Meeting; 1998; 3:1.

51. Mull JW, Bill AH. Variations in serum calcium and phosphorus during pregnancy: I. Normal variations. American Journal of Obstetrics & Gynecology. 1934; 27(4):510-7. https://doi.org/10.1016/S0002-9378(34)90732-8

52. Tavana Z, Hosseinmirzaei S. Comparison of maternal serum magnesium level in pre-eclampsia and normal pregnant women. Iranian Red Crescent Medical Journal. 2013; 15(12). https://doi.org/10.5812/ircmj.10394 PMid:24693379 PMCid:PMC3955494

53. Kanagal DV, Rajesh A, Rao K, Devi UH, Shetty H, Kumari S, Shetty PK. Levels of serum calcium and magnesium in pre-eclamptic and normal pregnancy: A study from Coastal India. Journal of clinical and diagnostic research: JCDR. 2014; 8(7):OC01. https://doi.org/10.7860/JCDR/2014/8872.4537

54. De Swiet M. The respiratory system, in Clinical physiology in obstetrics, G. Chamberlain and F. Broughton Pipkin, Editors. Blackwell Science Ltd: Oxford, 1998:111-128.

55. Susser M. Maternal weight gain, infant birth weight, and diet: causal sequences. The American journal of clinical nutrition. 1991; 53(6):1384-96. https://doi.org/10.1093/ajcn/53.6.1384 PMid:2035466