Research Article

Study of serum calcium in maternal and cord blood of women with preeclampsia and normotensive pregnancies

Kumar Saurabh, Veena Ghalaut, Jyoti Bala
Department of Biochemistry, PGIMS Rohtak, Haryana, India

*Corresponding author
Jyoti Bala
Email: jyotibala5018@gmail.com

Abstract: The aim of this study was to assess Serum Calcium (SCa) levels in the women with preeclamptic and normotensive pregnancies and to estimate its levels in cord blood with a view to relate the levels in pregnant mothers with their new born babies. This observational study was carried out in the Department of Biochemistry in PGIMS, Rohtak from February 2015 to July 2015. Thirty apparently normal pregnant women and 30 preeclamptic pregnant mothers were enrolled in the study. Subjects were excluded if they had chronic medical disease or were taking medications known to interfere with Ca metabolism such as corticosteroids, thyroxine and heparin. Total SCa, Ionized calcium (Ca+2), corrected total Ca and serum albumin (Alb) were estimated in pregnant females. There was a highly significant reduction in total SCa and corrected total Ca in preeclamptic pregnancies when compared to normotensive pregnancies (P<0.001, P<0.001 respectively). Serum Ca+2 also showed significant difference in these two groups (P<0.02). The significant difference in serum Alb levels were noticed in normotensive and preeclamptic subjects. (P<0.001).Total Ca level in cord blood of normotensive was statistically highly significant when compared to cord blood total Ca levels inpreeclamptic pregnancies (P<0.001). In our study, there was a strong positive correlation in total SCa levels in cord blood of new-born and in serum of both healthy and preeclamptic mothers and was statistically highly significant (r=0.562, r=0.680 and p<0.001, p<0.001 respectively). We conclude from our study that reduction in maternal total SCa with consequent decrease inCa^{2+}as a fraction of total calcium may have role in development of preeclampsia. Low SCa inpreeclamptic mothers and in cord blood of their babies could be a useful indicator of the maternal and fetal complications. This biochemical marker would allow early identification of patients at risk of preeclampsia and thus help in providing adequate prenatal care and reduce the maternal mortality.
Keywords: Calcium, Pregnancy, Preeclampsia, Cord Blood.

INTRODUCTION

Pregnancy is a normal physiological phenomenon with many biochemical changes including calcium (Ca) metabolism [1]. Ca is the most abundant mineral in the human bodyand is needed for muscle contraction, blood vessel contraction and expansion, secretion of hormones and enzymes, and sending messages through the nervous system so that these vital body processes function efficiently [2, 3].

Body Ca exists in two major compartments: skeleton (99%) and extracellular fluid (1%). Total blood Ca in the extracellular fluid is present in three forms in equilibrium with one another; ionized Ca (Ca+2) represents about 50-65% of total Ca, Ca bound to plasma protein mainly albumin (Alb) represents about 30-45% of total Ca and Ca complex with an ion as citrate represents about 5-10% of total Ca [2].

A constant level of Ca is maintained in body fluid and tissues within a narrow limit for normal physiological functioning so that when blood Ca decreases it stimulates the secretion of parathyroid hormone (PTH) which stimulates the conversion of vitamin D to its active form (calcitriol) in the kidneys [4, 5]. Calcitriol increases intestinal Ca absorption, which in turn stimulates bone Ca release by activating osteoclasts and decreasing urinary Ca excretion. On the other hand, when blood Ca rises to normal level, the parathyroid glands stop secreting PTH and the kidneys begin to excrete any excess Ca in the urine [2, 5, 6].

Measurement of the total SCa concentration alone can be misleading because the relationship between total and Ca+2 is not always linear. Correlation is poor when the serum Alb concentration is low or, with disturbances in acid-base status. With hypoalbuminemia, the total SCa concentration will be low while the ionized fraction will be normal unless some other factor is affecting Ca metabolism. More so, falsely lowCa+2 levels may be recorded in alkalosis and with heparin use. In general, the plasma Ca concentration falls by 0.8 mg/dL(0.2 mmol/L) for every 1.0 g/dL fall in the plasma Alb concentration [7]. It is

important to identify those with altered Ca+2 since the pathological levels of Ca+2 may be life threatening and the conditions themselves are amenable to treatment[2]. Therefore, estimation of total SCa levels is a poor substitute for measuring the ionized levels.

Pre-eclampsia is an idiopathic multisystem disorder specific to human pregnancy. Its incidence is 8-10% of total pregnancies in India [8]. The syndrome of pre-eclamptictoxemia is characterized by hypertension, proteinuria, oedema and abnormal clotting, all of which can be explained by generalized vascular endothelial cell dysfunction [9].

On physiological basis, Cais an essential but controversial nutrient [10, 11]. In pre-eclampsia many factors affect Ca regulation that leads to disturbed Ca balance [12],on the other hand the modification of plasma Ca concentration leads to alteration of blood pressure(BP), the aberrations in Ca homeostasis have been recognized in hypertension in general and specifically in pre-eclampsia [13, 14].

Pre-eclampsia is associated with abnormal Ca metabolism and placental dysfunction. During pregnancy, Ca is transferred actively from the maternal circulation to the fetus by a transplacentalCa pump regulated by parathyroid hormone related peptide (PTHrP) [15]. PTH and calcitonin (CT) do not cross the placental barrier. The majority of fetalCa accretion occurs in the third trimester. This process results in higher plasma Ca concentrations in the fetus than in the mother and leads to fetal Hypercalcemia [16]. SCa in the fetus is 10-11 mg/dL at term (1-2 mg higher as compared to mother) [7].

After the abrupt cessation of placental transfer of Ca at birth, total SCa concentration falls to 8 to 9 mg/dL (2 to 2.25 mmol/L) and Ca+2 to as low as 4.4 to 5.4 mg/dL (1.1 to 1.35 mmol/L) at 24 hours [17, 18]. SCa concentration subsequently rises, reaching levels seen in older children and adults by two weeks of age [19]. After birth the SCa levels in new-borns depend on the PTH secretion, dietary Ca intake, renal Careabsorption, skeletal Ca stores, and vitamin D status [7].

The objective of our study was to assess SCa levels in the women with preeclamptic and normotensive pregnancies and to estimate its levels in cord blood with a view to compare the levels in pregnant mothers with their new born babies.The intended functions as a screening procedure in preeclamptic pregnant and healthy pregnant women.

MATERIALS AND METHODS

This observational study was carried out in the Department of Biochemistry in PGIMS, Rohtak from February 2015 to July 2015. Thirty apparently normal pregnant women and 30 preeclamptic pregnant mothers were enrolled in the study. Subjects were excluded if they had chronic medical disease or were taking medications known to interfere with Ca metabolism such as corticosteroids, thyroxine and heparin.

Total SCa was analysed by autoanalyzer by enzymatic method [20]. Serum Alb was measured by autoanalyzer. Ca+2 were estimated by Ion selective electrode method.

As Ca is predominantly transported bound to serum proteins, so total SCa level are greatly influenced by protein concentration especially for obtaining corrected total Ca value according to the following formula [21]:
Corrected total Ca(mg/dl)=total Ca(mg/dl)+0.8(4-albumin g/dl)

RESULT
The present study enrolled 60 pregnant women.

Table 1: Comparisons of serum total calcium, ionized, and corrected total calcium and serum albumin in normotensive and preeclamptic pregnancies

Parameters	Normotensive pregnancies (n=30)	Preeclamptic pregnancies (n=30)	Significance
Total serum Ca (mg/dl)	9.19$\pm$ 0.60	7.55$\pm$ 0.63	0.000
Serum ionized Ca (mg/dl)	3.97$\pm$ 0.68	3.55$\pm$ 0.62	0.02
Serum corrected total Ca (mg/dl)	9.81$\pm$ 0.64	8.99 $\pm$ 0.54	0.000
Serum albumin (g / dl)	3.22$\pm$ 0.42	2.21 $\pm$ 0.43	0.000

There was a highly significant reduction in total SCa and corrected total Ca in preeclamptic pregnancies when compared to normotensive pregnancies (P<0.001, P<0.001 respectively). Serum Ca+2 also showed significant difference in these two groups (P<0.02).The same significant difference in serum Alb levels were noticed in normotensive and preeclamptic subjects (P<0.001) (Table I) (Fig.I)
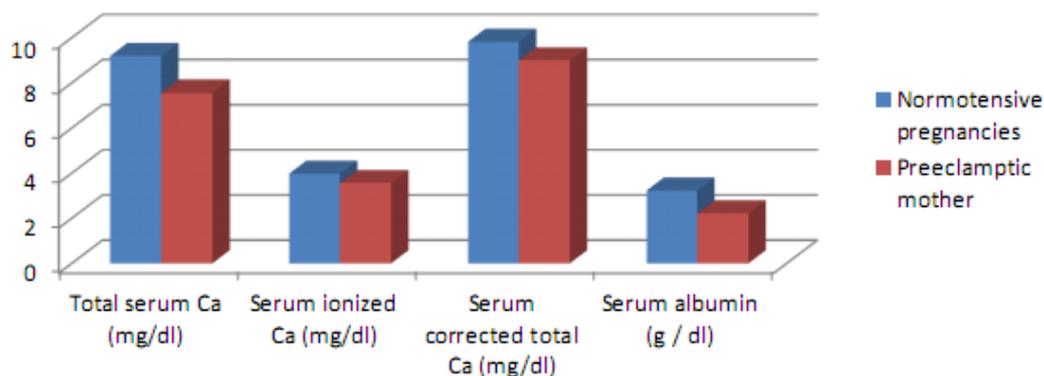
Fig. 1: Comparisons of serum total calcium, ionized, and corrected total calcium and serum albumin in normotensive and preeclamptic pregnancies

Table 2: Correlation between maternal and cord blood total calcium

Total serum calcium (mg/dl)	R value	P value
Healthy mothers and new-born	0.562	0.000
Preeclamptic mothers and new-born	0.680	0.000

In our study, there was a strong positive correlation in total SCa levels in cord blood of newborn and in serum of both healthy and preeclamptic mother and was statistically highly significant.(r=0.562, r=0.680 respectively) (Table II) (Fig. II &Fig.III)
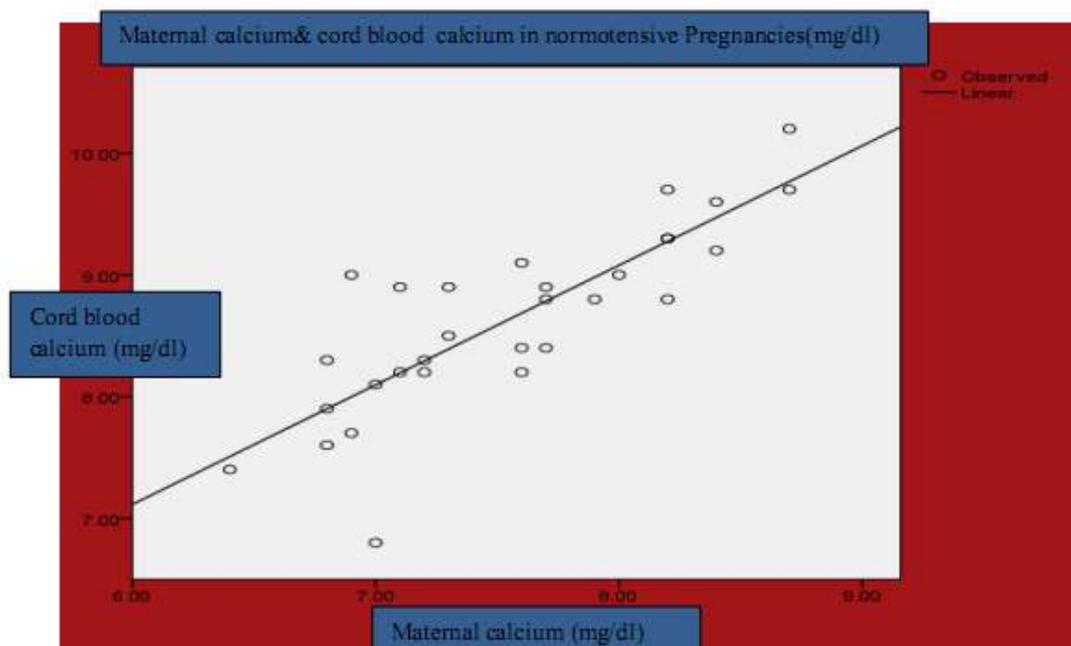

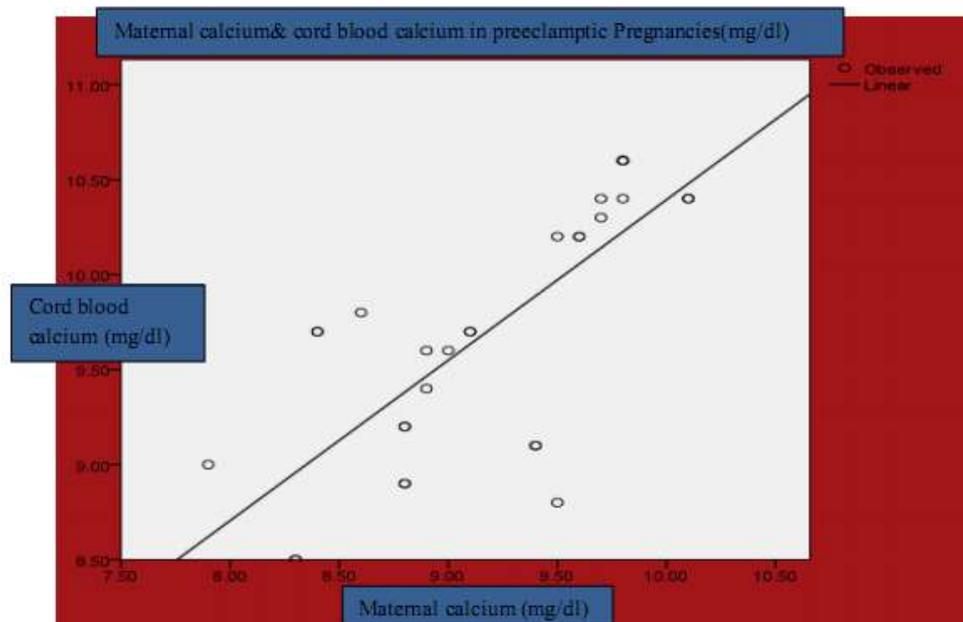**Fig. 2: Correlation between apparently healthy mother and cord blood total calcium**

Fig. 3: Correlation between preeclamptic mothers and cord blood total calcium

Table 3: Total calcium in cord blood of normotensive and preeclamptic pregnancies

Cord blood Ca (mg/dl)	Mean + SD	Significance
Normotensive pregnancy	9.71 + 0.62	P value= 0.000
Preeclamptic pregnancy	8.64 + 0.75	Highly significant

Total Ca level in cord blood of normotensive and preeclamptic pregnancies was 9.71 + 0.62, 8.64 + 0.75 respectively and was statistically highly significant. (P<0.001)
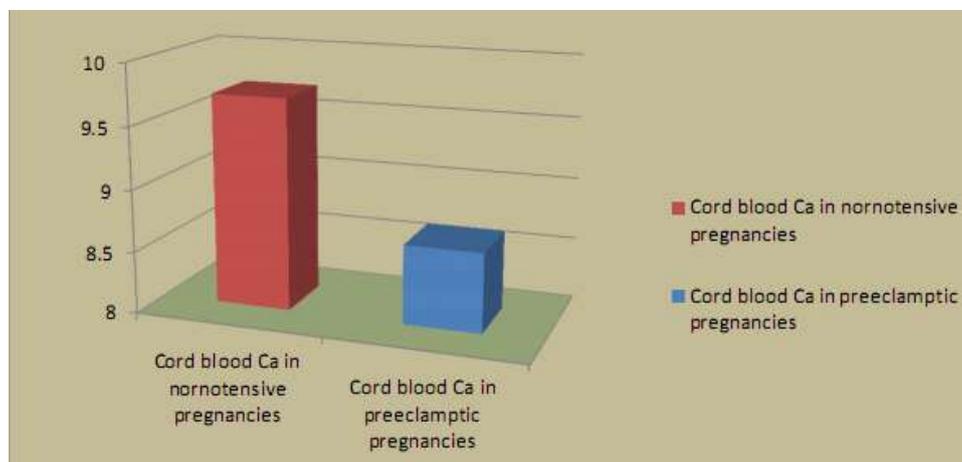

Fig. 4: Total calcium in cord blood of normotensive and preeclamptic pregnancies

DISCUSSION

In our study, hypertensive subjects had significantly lower levels of total SCa and corrected total Ca as compared to the normotensive mothers (p <0.001)(table 1). It has been hypothesized that change in the BP due to disturbances in Ca metabolism includes increased urinary Ca excretion and abundance of calcium-regulating hormones such as PTH and calcitriol.

Cahomeostasis needs to be maintained in pregnancy because its deficiency leads to a state of convulsions [22]. It has been suggested that there is an association between Ca intake and pregnancy-induced hypertension (PIH). Women with low Ca intake have an increase in mean blood pressure which predisposes them to the development of this process [23].Pregnant women who develop severe pre-eclampsia have significant lower dietary Ca intake when compared to normotensive women [24].

Preeclamptic women showed markedly lower levels of daily urinary Ca excretion. Primary deficiencies in Ca intake and serum calcitriol levels

decrease serum Ca+2 causing hypocalciuria. This is due to action of increased levels of PTH at kidney which inturn leads to increased Ca reabsorption in distal tubules. The lowering of SCa or the increase of intracellular Ca can cause an elevation of BP in preeclamptic women due to the constriction of smooth muscles in blood vessels and subsequent increase in vascular resistance. This effect of Ca on BP is explained by the influence of calcitrophic hormones on intracellular Ca.Calcitriol stimulates Ca influx in vascular smooth muscle cells and exerts a repressor effect , promotes contraction and increases peripheral vascular resistance and thus contribute to vascular damage and hypertension [22].

Low SCa levels and elevated PTH and calcitrol may also affect BP control by the central nervous system by stimulating the release of nor epinephrine (a potent vasoconstrictor) and its post-synaptic effect [25]. Thus intracellular Ca serves as a second messenger in excitation-contraction coupling for vascular smooth muscle cells, causing constriction and increased BP [26].

Deregulations of Ca homeostasis may contribute to obesity by the lack ofCa influx in human adipocytes, resulting in stimulation of lipogenesis, inhibition of lipolysis and expansion of triglyceride stores [27]. Thisobesity is responsible for the physiological alterations of vasoactive mediators leading to hypertension during pregnancy [28].

In our study, there was a strong positive correlation in total SCa levels in cord blood of newborn and in serum of both healthy and preeclamptic mothers and was statistically highly significant.(r=0.562, r=0.680 respectively)(table-II).

Therefore the role played by Ca in the pathogenesis of pregnancy induced hypertension is nowadays receiving growing interest, and the Ca supplementation during pregnancy can possibly be explained by reduction in parathyroid Ca release and intracellular Ca concentration, thereby reducing smooth muscle contractility and promoting vasodilatation [29].

Our study showed similar results aswith previous studies [30, 31]. In contrary, many investigators [32] found that SCa in preeclamptic group did not differ significantly from normal pregnant group. This difference can be attributed to the different dietary habits and the different genetic pools of the population in which the study has been carried out as compared with our population.

The significant difference in serum Alb levels were noticed in normotensive and preeclamptic subjects. (P<0.001) (table 1).The adjustment of total SCa concentration for Alb is essential to detect abnormal values andalso to assess changes in a value [31].

Our findings are in accordance with those reported by other authors [33, 34],while Gojnic et al, concluded 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 [31] and proposed that serum Alb levels may serve as indicator of the severity of preeclampsia. However our findings disagree with investigators that found serum Alb level was higher in preeclampsia group [35, 36]. This contrast may be caused due to mislead between underlying chronic hypertension or renal disease during pregnancy and preeclampsia condition.

Serum Ca+2 also showed significant decrease in hypertensive pregnancies(p<0.02) (table 1). Ca+2 which is crucial for the synthesis of vasoactive substance in the endothelium. Significant reduction in Ca+2 is also reported by another study which confirmed that lower $1,25\text{-}(OH)_2D$ levels in preeclamptic pregnant women may contribute to the suboptimal intestinal absorption of Ca during a time of increased Ca demand, thus resulting in lower Ca+2 levels, increased PTH and hypocalciuria in preeclampsia [31].

Total SCa level in cord blood of normotensive and preeclamptic pregnancies was 9.71 $\pm$0.62, 8.64 $\pm$ 0.75 respectively and was statistically highly significant (P<0.001)(table-III).

Hypocalcemiain term neonates are defined as a total SCa concentration of less than 2 mmol/L (8 mg/dL). Two type of hypocalcaemia can occur in newborn infants. The first develops early, during the first 3 days of life and isattributed to parathyroid immaturity and maternal hyperparathyroidism (or both) resulting in neonatal parathyroid suppression and often resolves within first week of life. Hypocalcemia in infants with 'late-onset' approximately 1 week after birth can be caused by feeding them milk with a high phosphorus level leading to hyperphosphatemia and then to hypocalcemia [37]. Measurement of SCa in cord blood may be helpful in evaluating neonatal hypocalcemia, and for monitoring hypo- or hypercalcemia associated with malignancy, pancreatitis and other conditions.

CONCLUSION

We conclude from our study that reduction in maternal total SCa with consequent decrease inCa+2as a fraction of total SCa, may have role in pregnant women in subjects, as well as in development of preeclampsia. Low SCa preeclamptic mothers and in cord blood of their babies could be a useful indicator of the maternal and fetal complications. This biochemical marker would allow early identification of patients at risk of preeclampsia and thus help in providing

adequate prenatal care and reduce the maternal mortality

In addition, to have better understanding of calcium homeostasis in preeclamptic women, we not just need to measure total and ionized calcium, and serum albumin, but similarly need to have a better understanding of vitamin related disorders. As a result of that other studies are needed to confirm whether the supplementation of these mineral and other vitamins has favourable or adverse effects on fetal and neonatal outcomes as well as maternal outcomes. Our results also emphasize the need for further studies on the calcium status of infants born to mothers with preeclampsia with larger sample size.

REFERENCES
1. Tran HA; Biochemical tests in pregnancy. AustPrescr., 2005;28:98-101.
2. Beckett G, Walker S, Rae P, Ashby P; Lecture notes clinical biochemistry. 4th ed. Blackwell Publishing 2005; 55, 72, 74, 82, 83, 87.
3. Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine. Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D and Fluoride.Washington DC: The National Academies Press 1997.
4. Weaver CM, Heaney RP. Calcium. In Modern Nutrition in Health and Disease (Shils M, Olson JA, Shike M, Ross AC, eds). 9th ed. Baltimore: Williams & Wilkins, 1999; 141-55.
5. Pearce SH, Thakker RV; The calcium-sensing receptor: insights into extracellular calcium homeostasis in health and disease. J Endocrinol., 1997;154: 371-8.
6. Robert K, Murray RK, Daryl K, Granner DK, Rodwell VW; Harper's illustrated biochemistry. 27th ed. McGraw-Hill Companies 2006; Chapter 41.
7. Jain A, Agarwal R, SankarMJ,Deorari A, Paul VK;Hypocalcemia in the Newborn. Indian JPediatr., 2010;77:1123-8.
8. Sarsam DS, Shmden M, Alwazan R; Expectant versus aggressive management in severe preeclampsia remote from term. Singapore Med J., 2008;149:698.
9. Mittal S,Shaikh MKS, Thakur R, Jain D;Comparison of serum calcium and magnesium levels between preeclamptic and normotensive healthy pregnant women. Int J ReprodContraceptObstetGynecol., 2014;3(4):959-62.
10. CougheyAB, Stotland NE, Washington AE,Escobar GJ; Maternal ethnicity, paternal ethnicity, and parental ethnic discordance: predictors of preeclampsia.ObstetGynecol., 2005;106:156-61.
11. Bongard FS, Sue DY, Vintch JR; Current diagnosis and treatment, critical care(3rd ed)2008.Newyork:McGraw-Hill/lange,p.51.
12. Rajarman S, SeLvanayagam S, Chidambaram R; Correlation study between total calcium, ionized calcium, serum albumin and their significance with vitamin D.Journal of Experimental sciences, 2011;2(12):17-21.
13. Jones and Bartlett learning,LLC.Not for SALE or Distribution.chapter6,calcium Imbalances.
14. Dunthumapol C, Kittichot PB; Serum calcium, maganesium and uric acid in preeclampsia and normal pregnancy. Med assoc Thai., 2008;91:968-73.
15. Belizan JM, Villar J; The Relation between Ca intake and edema, proteinuria and hypertension gestosis: An hypothesis. Am J ClinNutr.,1980;33:2202-10.
16. Kovacs CS, Lanske B, Hunzelman JL, Guo J, Karaplis AC, Kronenberg HM; Parathyroid hormone-related peptide (PTHrP) regulates fetal-placental calcium transport through a receptor distinct from the PTH/PTHrP receptor. ProcNatlAcadSci USA, 1996; 93:15233-8.
17. Rubin LP, Posillico JT, Anast CS, Brown EM;Circulating levels of biologically active and immunoreactive intact parathyroid hormone in human newborns. Pediatr Res., 1991;29:201.
18. Loughead JL, Mimouni F, Tsang RC; Serum ionized calcium concentrations in normal neonates. Am J Dis Child., 1988;142:516.
19. Wandrup J, Kroner J, Pryds O, Kastrup KW; Age-related reference values for ionized calcium in the first week of life in premature and full-term neonates. Scand J Clin Lab Invest., 1988;48:255.
20. Rubin LP; Disorders of calcium and phosporus metabolism. In: Avery's Diseases of the Newborn, 7th ed, Taeusch HW, Ballard RA (Eds), WB Saunders, Philadelphia 1998. p.1189.
21. Carl AB, Edward RA; Tietz text book of clinical chemistry.W.B.saunders(Ed 3rd)1999,1400.
22. Sridevi M, KusumaKumari P, Harikrishna K, Manohari AL; A Case Control Study of Changes in Levels of Serum Calcium and Uric Acid in Preeclamptic Women in Visakhapatnam Region, Andhra Pradesh-India Journal of Dental and Medical Sciences,2015:14;7-9 .
23. Malas NO, Shurideh ZM; Does serum calcium in pre-eclampsia and normal pregnancy differ? Saudi Med J 2001; 22(10): 868-71.
24. Mohieldein AH, Dokem AA, Osman YHM, Idris HMA; Serum calcium levels as a marker of pregnancy-induced hypertension.Sudan Journal of Medical Sciences,2007;2:245-8.
25. Oshima T, Young EW; Systemic and cellular calcium metabolism and hypertension. Seminars in Nephrology 1995;15(6):496-503.
26. Jain S, Sharma P, Kulshreshtha S, Mohan G, Singh S; The role of calcium,magnesium, and zinc in pre-

eclampsia. Biol Trace Elem Res., 2010;133(2):162-70.

27. Murai JT, Muzykanskiy E, Taylor RN; Maternal and fetal modulators of lipids metabolism correlate with the development of preeclampsia. Metabolism,1997;46:963-7.

28. Imdad A, Jabeen A, Bhutta ZA; Role of calcium supplementation during pregnancy in reducing risk of developing gestational hypertensive disorders: ameta-analysis of studies from developing countries:BMC Public Health 2011;11(Suppl3):S18.

29. Imdad A, Jabeen A, Bhutta ZA; Role of calcium supplementation during pregnancy in reducing risk of developing gestational hypertensive disorders. BMC Public Health,2011;11(3):18.

30. Kim J, Kim YJ, Lee R, Moon JH, Jo I; Serum levels of zinc, calcium, and iron are associated with the risk of preeclampsia in pregnant women. Nutr Res., 2012;32(10):764-9.

31. Ibraheem NJ, Obiade DS; Serum calcium level and some physiological markers during Pre-eclampsia and normal pregnancy in Babylon province women. Magazin of Al-Kufa University for Biology, 2013;5.

32. Fatemeh VR, Ayaty S, Tourabizadeh A, Ayat-Allahi H, Esmeali H, Shahabian M; Serum calcium and magnesium in preeclamptic and normal pregnancies, comparative stud. Reports Infertil.,2008;9(3):256-62.

33. Seong WJ, Chong GO, Hong DG, Lee TH, Lee YS, Cho YL, Chun SS, Park IS;Clinical significance of serum albumin level in pregnancy-related hypertension. J Obstet Gynaecol Res., 2010; 36(6):1165-73.

34. Alavi A, Jahanshahi KA, Karimia S, Arabzadea N, Fallahi S; Comparison of serum calcium, total protein and uric acid levels between hypertensive and healthy pregnant women in Iranian population. Life Science Journal, 2012;9(4):485-8.

35. Salako BL, Odukogbe ATA, Olayemmi O, Apedapo KS, Aimakhu CO, Alu F E etal; Serum albumin, creatinine, uric acid and hypertensive disorders of pregnancy. East African Medical Journal, 2003;80(8):424-8.

36. Salari Z, Eftekhari N, Tajaddini M; The comparison of total protein, albumin and phosphorous level in preeclamptic pregnant women and women with normal pregnancy. Journal of Kerman University of Medical Sciences, 2006;13(4):209-14.

37. Deshpande N, Patil L, Deshpande S, Chavan S; Study of ionic calcium in maternal and cord blood and baby's blood at 48-h age. Med J DY Patil Univ., 2014;7:152-5.