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RESEARCH ARTICLE

COMPARISON OF SERUM CALCIUM AND MAGNESIUM LEVELS BETWEEN PREECLAMPTIC AND NORMOTENSIVE HEALTHY PREGNANT WOMEN

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ABSTRACT

Introduction: Pregnancy-induced hypertension is developed due to pregnancy which is affected after delivery. Assessments of serum levels of calcium and magnesium in normal pregnancy and preeclamptic pregnancy may benefit in recognizing whether changes in the levels of these micronutrients are contributory to the interconnection of preeclampsia. Our study was evaluated role of serum calcium and magnesium in the pathogenesis of preeclampsia patients also was calculated the value of this biomarker as indicators in the etiopathogenesis and assessing the severity of pre-eclampsia in Eastern India.
Materials and Methods: This hospital based cross sectional study includes total 100 pregnant women during January 2015 to December 2016. Simple random sampling was done based on inclusion and exclusion criteria at Obstetrics ward of SSKM Hospital, Kolkata. Group I (Control)--includes 50 normotensive healthy pregnant women, Group II (Case)--includes 25 mild pre-eclamptic patients and Group III (Case)--includes 25 severe pre-eclamptic patients. Serum calcium and magnesium levels were measured using atomic absorption spectrophotometer with standard methods. Statistical analysis was done by SPSS 20.0.1and Graph Pad Prism version 5.
Results and Analysis: Difference of mean age and gestational age in two groups was not statistically significant. Thus age was matched in two groups. Mean BMI was significantly higher in case compare to control ($p<0.0001$). Mean serum calcium and magnesium level was significantly lower in group-III then group-II. When serum calcium and magnesium were correlated independently with systolic and diastolic blood pressure in preeclampsia patient, a significant negative correlation was obtained.
Discussion: In my study shows that both the serum calcium and magnesium are significantly reduced in severe preeclamptic groups as compared to mild preeclamptic and normotensive healthy pregnant woman. Further when serum calcium and magnesium was correlated systolic and/or diastolic blood pressure a significant negative correlation was obtained.
Conclusion: These findings support the hypothesis that hypocalcaemia and hypomagnesaemia are possible etiologies of preeclampsia. As the severity of preeclampsia increases more and more reduction in the levels of these electrolytes is seen in the maternal blood. Hence adjuvant supplementation of calcium and magnesium may prevent further progression of preeclampsia.

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INTRODUCTION

Pregnancy is a biological stress associated with many complex and interrelated biochemical, physiological and anatomical alterations happening in the body. Biochemical parameters reflect these adaptive changes and are obviously distinct from the non-pregnant state (James and Nelson-Piercy, 2004). Preeclampsia is an immunological multisystem disorder, a pregnancy specific syndrome, is one of the most common causes of maternal and foetal morbidity and mortality in developing countries. The incidence rate of preeclampsia stands 5-15% in India and globally 3-10% (Magee *et al*., 2014; Kosch *et al*., 2000; Osungbade and Ige, 2011). Pregnancy-induced hypertension is progressed due to pregnancy and regresses after delivery. It is a known cause of premature delivery, intrauterine growth restriction, placental abruption, foetal death and numerous adverse pregnancy outcomes. Maternal complications like oliguria, eclampsia, haemolysis, thrombocytopenia, elevated liver enzymes, pulmonary edema and even death. Previous history of preeclampsia, pre-existing diabetes, obesity, multiple pregnancies has been reported to increase the risk of pregnancy induced hypertension and preeclampsia (Duckitt and Harrington, 2005; Owiredu *et al*., 2012). Deficiency of nitric oxide contributes to the development of hypertension. Pre-eclampsia is a multifactorial polygenic disorder. There is interaction of multiple genes

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inherited from both maternal and paternal sites that can support some genetic role (Magee *et al*., 2014; Pedersen *et al*., 1984; Vahidrodsari *et al*., 2008). Deficiency of several essential micronutrients may be a predisposing influence in the development of eclampsia. The result of many studies have documented that the changes in the concentration of minerals like calcium, magnesium may have a role in the etiopathogenesis of preeclampsia (Farzin and Sajadi, 2012; Patel *et al*., 2012). Generally hypocalcaemia and hypomagnesaemia in pregnancy is associated with hemo dilution, altered renal clearance and consumption of minerals by growing foetus (Chanvitya and Boonsri, 2008). Calciumis presentinour body intracellularlyas wellas extracellular. Intracellular calcium is present in two forms firstly a larger fraction which is either stored in the endoplasmic reticulum or bound with proteins like calbindin and troponin. Secondly a smaller fraction, the free calcium. It is the free calcium within the cells which is responsible for the various activities within the cell. The extracellular calcium which includes plasma calcium has different sets of functions. Plasma calcium concentration is normally 9-10mg/100ml. Calcium plays a critical role in the function of vascular smooth muscle and alteration of plasma calcium Concentration leads to raised blood pressure. Low serum calcium may cause high blood pressure by stimulating parathyroid hormone and renin release which in turn increase intracellular calcium in vascular smooth muscle which causes vasoconstriction, increased vascular resistance and rise of blood pressure in preeclamptic other (Indumati *et al*., 2011; Pallavi *et al*., 2012; Robert *et al*., 2003) Magnesium is known to increase the prostacycline release from endothelial cells of blood vessels, which acts as potent vasodialators. In addition magnesium depletion increases the vasoconstrictor effect of angiotensin II and noradrenaline.

Magnesium acts as a cofactor of many enzymes and also plays an important role in neurochemical transmission and peripheral vasodilation. The consequences of low magnesium may lead to a reduction in cerebral blood flow, cerebral vasospasm and increase in neuronal burst (Sanders *et al*., 1999; Chaurasia *et al*., 2012; Aitura *et al*., 1983). It has been found that preeclampsia has more impact in developing countries where pregnant women have been reported to consume diets with lesser amounts of minerals and vitamins. During pregnancy inadequate intake of such minerals and vitamins might be harmful not only to the mother but also to the growing fetuses (Jain *et al*., 2010). Comparisons of serum levels of calcium and magnesium in normal pregnancy and preeclamptic pregnancy may help in identifying whether changes in the levels of these micronutrients are contributory to the causation of preeclampsia. Further the work will initiate a general public awareness over improving the overall health of a pregnant woman by taking food rich in those micronutrients. Despite numerous studies, the etiology of Preeclampsia has not been fully elucidated. Now a day's biochemical approach is implicated in order to prevent the complications in pregnancy and to improve the foetal outcome. This study of serum calcium and serum magnesium in woman with preeclamptic pregnancy compared with healthy normotensive pregnant women in their third trimester. We measured serum levels of Calcium and magnesium in preeclamptic women and to compare those with normotensive healthy pregnant women in third trimester of pregnancy. Our study was evaluated role of serum calcium and magnesium in the pathogenesis of preeclampsia as well as supplementation of these minerals in prevention of preeclampsia also was assessed the value of this biomarker as indicators in the etiopathogenesis and assessing the severity of pre-eclampsia in Eastern India.

MATERIALS AND METHODS

This is a hospital based cross sectional study with comparison groups. Pregnant women attended at Antenatal Clinic or admitted at Obstetrics ward of SSKM Hospital, Kolkata. The biochemical test was conducted at the department of Biochemistry of SSKM Hospital, Kolkata. Study includes total 100 pregnant women during January 2015 to December 2016. Simple random sampling was done based on inclusion and exclusion criteria.

Group I (Control)--includes 50 normotensive healthy pregnant women.
Group II (Case)-- includes 25 mild pre-eclamptic patients (Blood Pressure (BP) ≥140/90).
Group III (Case)-- includes 25 severe pre-eclamptic patients (Blood Pressure (BP) ≥160/110).

Inclusion Criteria

Primigravida, aged 20-35 years with gestational age >28 weeks patients were divided them as cases and control. Participant with good health, normotensive and without proteinuria were enrolled as control. Participant with BP ≥140/90 on two occasions at least 6 hours apart associated with dipstick proteinuria at least 1+ enrolled as case. (I) Mild pre-eclampsia--BP≥140/90 and who had proteinuria at least +1 detected by dip-stick urine analysis. (II) Severe pre-eclampsia-BP≥160/110 and who had proteinuria at least +3 detected by dip-stick urine analysis.

Exclusion Criteria

Pregnant woman outside 20-35 years and gestational age <28 weeks were excluded in this study. Multigravida, multiple pregnancy, hydatidiform mole, polyhydramnios, history of chronic hypertension, cardiovascular disease, eclampsia, previous history of convulsion, diabetes or history of diabetes & thyroid or other endocrine disorder, hematological disorder, renal disorder, autoimmune disease like SLE, Dyslipidaemia, liver disease and pancreatitis patients were excluded. Woman with history of smoking, alcohol, and other drug consumption which might affect BP and other pre-existing medical condition which alters study parameters was excluded from the study.

Calcium and Magnesium measurement

5ml blood will be collected from anterior cubical veins. Blood will be allowed to clot and separated by centrifugation at 3000rpm for 5 minutes within 30 mins of sample collection and the serum was stored at -20^{o}C until total calcium and magnesium were determined. Calcium and magnesium levels were measured using atomic absorption spectrophotometer with standard methods.

Statistical analysis

For statistical analysis data were entered into a Microsoft excel spread sheet and then analysed by SPSS 20.0.1and Graph Pad Prism version 5. Data have been summarized as mean and standard deviation for numerical variables and count and percentages for categorical variables. The median and the interquartile range have been stated for numerical variables that are not normally distributed. Student's independent sample's t-test was applied to compare normally distributed numerical variables. Correlation was calculated by Pearson correlation analysis. One way ANOVA was applied to numerical variables for more than three groups. Correlation was calculated by Pearson correlation analysis. P-value ≤ 0.05 was considered for statistically significant. P-value ≤ 0.001 was considered for highly statistical significance.

RESULTS AND ANALYSIS

In Case (Group-II and III), the mean age (mean± s.d.) of patients was 25.8200±3.8792years with range 20.00-34.00 years and the median age was 25.50 years. In control (group-I), the mean age (mean± s.d.) of patients was 25.3000±3.3700 years with range 20.00-33.00 years and the median age was 22.00 years. Difference of mean age in two groups was not statistically significant. Thus age was matched in two groups. There was no statistically significant difference in age distribution between the groups. (Numerical variables between groups compared by t-test; (p=0.0858)). In Case (Group-II and III), the mean gestational age (mean± s.d.) of patients was 32.0400±2.3470 weeks with range 29.00-38.00 weeks and the median age was 32.00 weeks. In control (group-I), the mean gestational (mean± s.d.) of patients was 32.3800±2.6021 weeks with range 28.00-38.00 weeks and the median age was 32.00 years. Difference of mean age in two groups was not statistically significant (p=0.4943). In Case (Group-II and III), the mean body mass index (BMI); (mean± s.d.) of patients was 25.3900±3.1579 kg/m2 with range 19.00-32.50 kg/m2 and the median BMI was 25.00 kg/m2. In control (group-I), The mean BMI (mean± s.d.) of patients was 22.8200±2.5868kg/m2 with range 19.00-31.00 kg/m2 and the median BMI was 22.25 kg/m2. Mean BMI was significantly higher in case compare to control (p<0.0001); (Table-1). Mean Systolic blood pressure

Table 1. Distribution of mean Age, Gestational age and BMI in case and control

	Group	Number	Mean	Std Dev	Minimum	Maximum	Median	p-value
Age (Years)	Case (Group-II & III)	50	25.8200	3.8792	20.0000	34.0000	25.5000	0.0858
	Control (Group-I)	50	25.3000	3.3700	20.0000	33.0000	22.0000	
Gestational age (Weeks)	Case (Group-II & III)	50	32.0400	2.3470	29.0000	38.0000	32.0000	0.4943
	Control (Group-I)	50	32.3800	2.6021	28.0000	38.0000	32.0000	
BMI (Kg/Metre2)	Case (Group-II & III)	50	25.3900	3.1579	19.0000	32.5000	25.0000	<0.0001
	Control (Group-I)	50	22.8200	2.5868	19.0000	31.0000	22.2500	

Table 2. Distribution of mean SBP, DBP, Serum Calcium and Magnesium in three groups

	Group	Number	Mean	Std Dev	Minimum	Maximum	Median	p-value
SBP	Group- I	50	114.0000	12.0272	94.0000	134.0000	113.0000	<0.0001
	Group-II	25	147.0400	4.5137	140.0000	156.0000	148.0000	
	Group-III	25	166.8000	4.9666	160.0000	176.0000	166.0000	
DBP	Group- I	50	75.2000	6.4902	66.0000	86.0000	74.0000	<0.0001
	Group-II	25	94.6400	5.2827	88.0000	106.0000	94.0000	
	Group-III	25	112.1739	3.0697	106.0000	118.0000	112.0000	
Serum Calcium	Group- I	50	9.2052	.5064	8.1000	10.2000	9.2300	<0.0001
	Group-II	25	9.0600	.6772	7.8400	10.8000	8.9600	
	Group-III	25	8.3384	.3337	7.8400	9.1400	8.3000	
Serum Magnesium	Group-I	50	1.9290	.2239	1.3200	2.3000	1.9300	<0.0001
	Group-II	25	1.8856	.1745	1.4600	2.2400	1.8800	
	Group-III	25	1.4628	.2756	0.9200	2.1000	1.4600	

Table 3. Association of mean BMI, Serum Calcium and Magnesium in three groups

		Group-I	Group-II	Group-III	Chi-square value	p- value
BMI	<25 kg/m2 (Number)	38	8	12	14.6141	0.0007
	Percentage (%)	76.0	32.0	48.0		
	≥25 kg/m2 (Number)	12	17	13		
	Percentage (%)	24.0	68.0	52.0		
Serum Calcium	<8 mg/dl (Number)	0	2	4	24.4045	0.0001
	Percentage (%)	0.0	8.0	16.0		
	8-9 mg/dl (Number)	18	12	19		
	Percentage (%)	36.0	48.0	76.0		
	>9 mg/dl (Number)	32	11	2		
	Percentage (%)	64.0	44.0	8.0		
Serum Magnesium	<1 mg/dl (Number)	0	0	1	41.8398	<0.0001
	Percentage (%)	0.0	0.0	4.0		
	1-1.5 mg/dl (Number)	3	1	15		
	Percentage (%)	6.0	4.0	60.0		
	1.5-2 mg/dl (Number)	32	19	7		
	Percentage (%)	64.0	76.0	28.0		
	>2 mg/dl (Number)	15	5	2		
	Percentage (%)	30.0	20.0	8.0		

Table 4. Correlation of SBP and DBP Serum Calcium and Magnesium in case

Parameter	SBP	DBP
Serum Calcium	r= - 0.6043	r= - 0.6742
	p<0.0001	p<0.0001
Serum Magnesium	r= - 0.6365	r= - 0.6771
	p<0.0001	p<0.0001

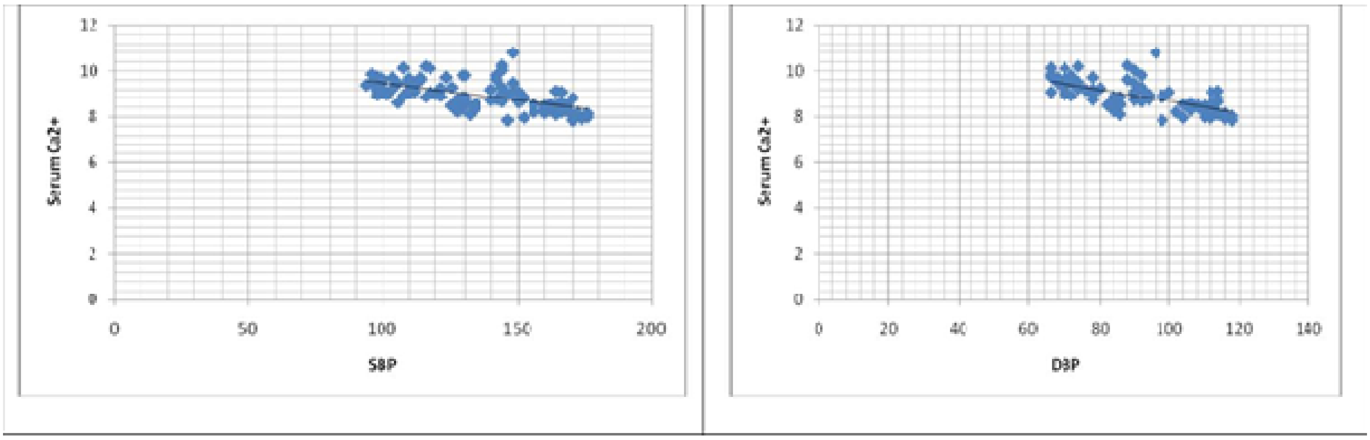

Figure 1. Correlation of SBP and DBP with Serum Calcium in case

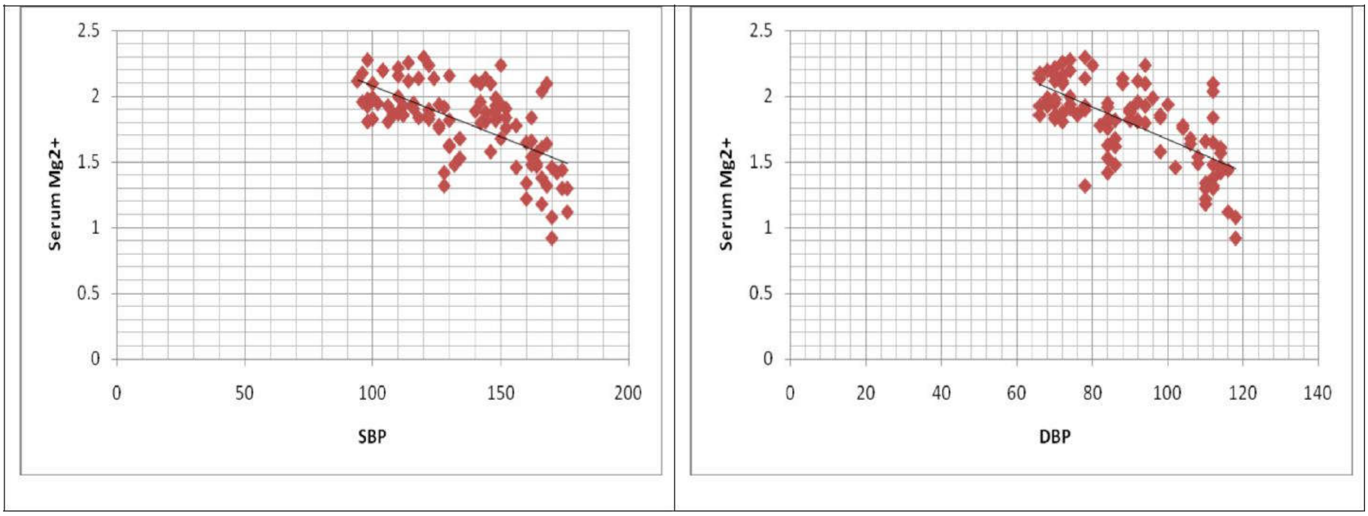

Figure 2. Correlation of SBP and DBP with Serum Magnesium in case

(SBP) of Group-II and Group-III was significantly higher than Group-I ($p<0.0001$). Mean Diastolic blood pressure (DBP) of Group-II and Group-III was significantly higher than Group-I ($p<0.0001$). Difference of mean serum calcium in three groups was statistically significant ($p<0.0001$). Difference of mean serum magnesium in three groups was statistically significant ($p<0.0001$); (Table-2). It was found that 18(36.0%) patients had serum calcium 8-9 mg/dl and 32(64.0%) patients had serum calcium >9 mg/dl in Group-I. 2(8.0%) patients had serum calcium <8 mg/dl, 12(48.0%) patients had serum calcium 8-9 mg/dl and 11(44.0%) patients had serum calcium >9 mg/dl in Group-II. 4(16.0%) patients had serum calcium <8 mg/dl, 19(76.0%) patients had serum calcium 8-9 mg/dl and 2(8.0%) patients had serum calcium >9 mg/dl in Group-III. Association between serum calcium in three groups was statistically significant ($p=0.0007$). Present study found that 3(6.0%) patients had serum magnesium 1-1.5 mg/dl, 32(64.0%) patients had serum magnesium 1.5-2 mg/dl and 15(30.0%) patients had serum magnesium >2 mg/dl in Group-I. 1(4.0%) patients had serum magnesium 1-1.5 mg/dl, 19(76.0%) patients had serum magnesium 1.5-2 mg/dl and 5(20.0%) patients had serum magnesium >2 mg/dl in Group-II. 1(4.0%) patients had serum magnesium <1 mg/dl, 15(60.0%) patients had serum magnesium 1-1.5 mg/dl, 7(28.0%) patients had serum magnesium 1.5-2 mg/dl and 2(8.0%) patients had serum magnesium >2 mg/dl in Group-III. Association between serum magnesium in three groups was statistically significant ($p<0.0001$); (Table-3).

The value of correlation coefficient (r) is -0.6043. The negative correlation was found between SBP vs. serum calcium. The value of R2, the coefficient of determination, is 0.3652. The P-Value is <0.00001. The result is significant at $p < 0.05$.The value of R is -0.6742. The negative correlation was found between DBP vs. serum calcium. The value of R2, the coefficient of determination, is 0.4545.The P-Value is < 0.00001. The result is significant at $p < 0.05$. When serum calcium was correlated independently with systolic and diastolic blood pressure in preeclampsia patient, a significant negative correlation was obtained (Table-4). The value of R is -0.6365. The negative correlation was found between SBP vs. serum magnesium. The value of R2, the coefficient of determination, is 0.4051.The P-Value is < 0.00001. The result is significant at $p < 0.05$.The value of R is -0.6771. The negative correlation was found between DBP vs. serum magnesium. The value of R2, the coefficient of determination, is 0.4585.The P-Value is < 0.00001. The result is significant at $p < 0.05$. Serum magnesium were correlated independently with systolic and diastolic blood pressure in preeclampsia patient, a significant negative correlation was obtained (Table-4).

DISCUSSION

Preeclampsia has been labelled as a dreaded disease affecting women and their pregnancy right from ancient times. The numerous complications associated with it have triggered a phobia in pregnant women and aroused the interest of obstetricians everywhere. Preeclampsia is a multi-factorial process and multi-organ dysfunction with no individual factor strictly essential or sufficient for causing it. Estimation of electrolytes in preeclampsia provides a very useful index for the study of physiology and pathological changes during pregnancy (Mojo and August, 1997; Prathad *et al*., 1993; Abdelimarouf *et al*., 2007; Bera *et al*., 2011). Calcium plays an important role in muscle contraction and regulation of water balance in cells. Decreased serum calcium leads to increase in parathyroid hormone level and increase in the membrane permeability which leads shift of serum calcium intracellular. The lowering of serum calcium and the increase in cellular calcium can cause an elevation of blood pressure in preeclamptic mother. The increase in cellular calcium concentration causes constriction of smooth muscle in the blood vessels and increase of vascular resistance (Adamova *et al*., 2009; Malas and Shurideh, 2001). Low calcium in the serum also elicits 1, 25-dihydroxy cholecalciferol response which stimulates calcium influx into vascular endothelial cells thus increasing the blood pressure (Mohieldein *et al*., 2007; Lopez-Jaramillo *et al*., 1989; Villar and Belizan, 2000; Hayashi *et al*., 2002). The concentrations of extracellular ionized Calcium are crucial for the synthesis in the endothelium of vasoactive substances, such as prostacyclin and nitric oxide (29, 30). Magnesium acts as a potent vasodilator by increasing the prostacyclin release from endothelium of blood vessels. Like Calcium lowered Magnesium levels are thought to potentiate contractile response of vascular smooth muscle to vasopressor (Sendhav *et al*., 2013; Konijnenberg *et al*., 1999). Reduction in the level of extracellular magnesium causes partial membrane depolarization and decreased repolarization along with opening of membrane calcium channels leading to shift of calcium intracellular. This phenomenon produces vasoconstriction and rise in the blood pressure (Korner *et al*., 1998). Further it has been said that low serum magnesium increases endothelin-1 mediated smooth muscle contraction and hampers the release of prostacyclins from the endothelial cells of the arteries again manifesting as increase in the blood pressure (Maksana *et al*., 2001; Idegun *et al*., 2007). So serum calcium and magnesium are very important for metabolism at cellular levels and are vital for muscle contraction, cell death and neuronal activity.

Jain *et al*, found a decrease in both serum calcium and magnesium in preeclamptic women as compared to normal pregnant women in their study thus, supporting the hypothesis that hypocalcaemia and hypomagnesaemia are possible aetiologies of preeclampsia (Jain *et al*., 2010). A study from northern part of India by Chaurasia *et al* found significantly lower levels of calcium and magnesium in preeclamptic woman compared to normal woman. Sandip *et al* in his study found significantly lower levels of calcium and magnesium in severe preeclamptic women compared to normotensive and mild preeclamptic women (Sandip *et al*., 2013). Punthu mapol & Kittichotpanich showed that serum calcium in preeclamptic women was lower than normal pregnant women but no difference between serum magnesium levels (Punthumapol and Kittichotpanich, 2008). A research work by Golmohammad & Yazdian in Iran did not show significant difference in the level of serum calcium and magnesium in preeclamptic and normotensive pregnancy (Golmohammad *et al*., 2008). Kumru *et al* in their study found significantly lower levels of calcium in preeclamptic women compared to healthy controls, whereas magnesium concentration showed non-significant differences between the two groups (Kumru *et al*., 2003). Akinloy *et al* in a study from Nigeria reported a decrease in serum magnesium in pregnant women with preeclampsia (Akinloye *et al*., 2010). Studies conducted by Aruna Patel *et al*, Selina Akhtar*et al*, Abdelmarouf H.Mohieldein *et al*, Indumati V *et al* also showed significant reduction in serum calcium in the preeclampsia patients. Further when serum calcium was correlated with systolic and diastolic blood pressure, a significant negative correlation was obtained. This suggests a strong relationship between deficiency of calcium and risk of developing preeclampsia (Selina *et al*., 2011). We found that the negative correlation was showed between SBP vs. serum calcium level. There was significantly negative correlation was found between DBP vs. serum calcium level. It was found that SBP and serum magnesium level was significantly negatively correlated. The negative correlation was showed between DBP vs. serum magnesium level. SBP or DBP value was increased with decrease serum calcium and magnesium level. A Cochrane review analysed 12 high quality trials on supplementation of calcium to prevent preeclampsia in normotensive pregnant woman. The dose of calcium evaluated was 1.5 to 2 gm. daily. There was less high blood pressure associated with calcium supplementation rather than placebo. Calcium supplementation appeared to approximately half the risk of preeclampsia. The reduction was greatest for women at high risk of developing preeclampsia and for those with low baseline dietary calcium. The review commented that adequate dietary calcium before and in early pregnancy may be needed to prevent the underlying pathology responsible for preeclampsia (Hofmeyr *et al*., 2012).

The recommended dietary allowance in the USA recommends that pregnant woman should take 1 to 1.5 gms of calcium daily for presentation of preeclamptic complication. Milk, soymilk, cheese, egg vegetables like cabbage, almonds and calcium fortified orange juice are good sources of calcium. The daily requirement of magnesium is about 350 mg per day and food rich in magnesium includes whole grains, nuts, beans, almonds, banana, green leafy vegetables and sea food. Pregnant woman in developing countries should be encouraged to consume food rich in calcium and magnesium. If the intake is less than recommended dose, a supplement can be given (Oladapo A Ladipo, 2000). In my study shows that both the serum calcium and magnesium are significantly reduced in severe preeclamptic groups as compared to mild preeclamptic and normotensive healthy pregnant woman. Further when serum calcium and magnesium was correlated systolic and/or diastolic blood pressure a significant negative correlation was obtained. The limitation of my study is that the dietary pattern of study group related to consumption of calcium and magnesium in the diet is unavailable. Women with greater BMI in pregnancy are more likely to become hypertensive than those with lower BMI. In my study BMI was significantly higher in the hypertensive group compared to the normotensive group.

Conclusion

There was no significant difference in the mean serum calcium and magnesium levels in mild preeclampsia and healthy normotensive pregnant woman but there was significantly reduced both serum calcium and magnesium levels in severe preeclamptic group compared to normotensive counterparts. Women with greater BMI in pregnancy are more likely to become hypertensive than those with lower BMI. In the light of above observation, it can be concluded that preeclamptic pregnant woman have decreased level of serum calcium and magnesium as compared to normotensive pregnant woman in their third trimester. These findings support the hypothesis that hypocalcaemia and hypomagnesaemia are possible etiologies of preeclampsia. As the severity of preeclampsia increases more and more reduction in the levels of these electrolytes is seen in the maternal blood. Hence adjuvant supplementation of calcium and magnesium may prevent further progression of preeclampsia. Still further studies are required to find out if whether estimation of these minerals at an early gestational age can be used as a predictive marker for early diagnosis of preeclampsia as well as role of calcium and magnesium supplementation in prevention of preeclampsia.

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