

Levels of Serum Calcium and Magnesium in Pre-eclamptic and Normal Pregnancy: A Study from Coastal India

DEEPA V KANAGAL¹, APARNA RAJESH², KAVYARASHMI RAO³, ULLAL HARSHINI DEVI⁴, HARISH SHETTY⁵, SUCHETA KUMAR⁶, PRASANNA KUMAR SHETTY⁷

ABSTRACT

Background: Pre-eclampsia is one of the major causes of maternal and fetal morbidity and mortality. Though the aetiology is obscure, recent studies indicate that serum levels of calcium and magnesium may have a role in pre-eclampsia.

Aim: The aim of this study was to find out the relationship of serum levels of calcium and magnesium in pre-eclamptic pregnancies compared to normal pregnancies in women from southern coastal India.

Settings and Design: This study was done in a medical college hospital in southern coastal India.

Materials and Methods: The blood samples from 60 pre-eclamptic women and an equal number of controls were analysed for calcium and magnesium levels. Data on Body Mass Index, maternal and gestational ages, serum calcium and magnesium were compared between the two groups. Outcome of pregnancy was analysed in both the groups and compared.

Statistical Analysis: Data was expressed as Mean \pm Standard Deviation. Data analysis was done by SPSS version 20.

Comparison of serum levels of the elements between the two groups was performed by Independent t-test and Chi-square test and P-value of < 0.05 was considered as statistically significant.

Results: The serum calcium concentration was significantly lower in the pre-eclamptic group compared to normotensives (7.84 ± 0.87 mg/dl Vs 8.97 ± 0.69 mg/dl, $p < 0.001$) whereas the levels of serum magnesium showed a marginal difference in both the groups. (1.43 ± 0.55 mg/dl Vs, 1.57 ± 0.72 mg/dl P 0.257) The study also showed that pre-eclamptic women were older, their BMI was higher and birth weight of babies lower compared to normotensives.

Conclusion: According to the results of our research, intake of supplements, mainly calcium may help in the reduction of incidence of pre-eclampsia especially in a population of a developing country like ours where the nutrition is poor. Not many studies have been done in developing countries to assess the role of these elements in pre-eclampsia. The actual role of magnesium and calcium supplements needs further investigation.

Keywords: Calcium, Magnesium, Nutrition, Pre-eclampsia

INTRODUCTION

Pre-eclampsia is one of the most common causes of maternal and fetal morbidity and mortality [1]. It is a systemic disease that affects about 5 – 7 % of all pregnancies and is the most common, yet least understood disorder of pregnancy [2]. It is a rapidly progressive condition characterized by high blood pressure, platelet aggregation, swelling of the lower extremities and protein in urine [3]. Sudden weight gain, headaches and changes in vision are important symptoms. Typically blood pressure elevations and pre-eclampsia occur in the late second trimester or third trimester [4]. The pathophysiological mechanism is characterized by a failure of the trophoblastic invasion of the spiral arteries which may be associated with an increased vascular resistance of the uterine artery and a decreased perfusion of the placenta [1]. The incidence is about 6% in primigravid women [5]. Clinically pre-eclampsia is characterized by persistently elevated blood pressure of greater than 140/90 mmHg, proteinuria and oedema [6]. It may be associated with complications like visual disturbances, oliguria, eclampsia, hemolysis, elevated liver enzymes, thrombocytopenia, pulmonary oedema and fetal growth restriction [7]. Early detection and prompt management helps in reducing the complications of this condition. Despite its prevalence and severity, the patho physiology of this multisystem disorder is still poorly understood and its aetiology has not yet been fully elucidated [8]. Environmental and nutritional factors may play a role in the aetiology of pre-eclampsia.

The importance of proper nutrition prior to and throughout pregnancy has long been known for optimizing the health and well being of both mother and baby. Pregnancy is a period of increasing metabolic demands with changes in a women's physiology and the requirements of a growing fetus. The greatest impact of pre-eclampsia is in developing countries where it accounts for 20 – 80% of the strikingly increase maternal mortality [9]. Pregnant women in developing countries have been reported to consume diets with lesser amounts of essential minerals and vitamins. An inadequate intake might be harmful not only for the mother but also for the growing fetus [10].

Hypertensive disorders account for 40,000 maternal deaths annually [11]. Due to this, methods to reduce the risk of hypertensive disorders in pregnancy have received considerable attention. Research is focusing on prevention rather than treatment. There is evidence that indicates a role for micronutrients supplementation in preventing some pregnancy disorders. Among these, increasing calcium and magnesium intake can reduce the risk of pregnancy induced hypertensive disorders [12].

Low serum calcium may cause high blood pressure by stimulating parathyroid hormone and renin release and also by inducing vasoconstriction by increasing its level in vascular smooth muscle [10,1,13]. Calcium might also have an indirect effect on smooth muscle function by increasing magnesium levels [11]. Magnesium plays an important role in peripheral vasodilatation [13]. However,

the role of calcium and magnesium in pregnant women are still being discussed. The aim of this study is to measure serum levels of calcium and magnesium in pre-eclamptic pregnancy and to compare with those in normal pregnancy.

MATERIALS AND METHODS

This was a double blinded case-control study conducted to investigate the levels of calcium and magnesium in pregnant women with and without pre-eclampsia. The study population was pregnant women attending the antenatal clinic and admitted in the obstetric ward in the Department of Obstetrics and Gynaecology in a teaching hospital in coastal southern India, is a developing country where the nutrition of pregnant women is a matter of concern. 120 women were included in the study, of whom 60 were with pre-eclampsia and the other 60 were normal pregnant women without pre-eclampsia who were taken as controls. All participants were in the third trimester of pregnancy with a gestational age of more than 32 weeks, primi or multigravida and with a single fetus. Cases and controls were matched for gestational age, parity, anthropometrics and socioeconomic status. Most of the women were from the lower middle class strata with poor dietary consumption of calcium and magnesium rich food. Pre-eclampsia was diagnosed in a woman who had a blood pressure of 140/90 or more on two occasions each 6 hours apart associated with proteinuria of at least 300 mg per 24 hours or at least 1+ on dipstick testing. Severe pre-eclampsia was defined as a blood pressure of 160/110 mm Hg or above measured on two occasions each 6 hours apart. The repeat measurement of blood pressure was done in the hospital after adequate rest. The women were selected following medical examination and investigations. There were no dropouts in the study. Among the 60 cases, 7 had severe pre-eclampsia. There were no cases of eclampsia.

Informed consent was obtained from each of the subjects before recruiting in to the study. Ethical committee clearance was obtained from the institution.

Patients with history of chronic hypertension, renal disease, cardiovascular disease, liver disease, diabetes, thyroid and other endocrine disorders, multiple gestations, hydatidiform mole and other secondary causes of hypertension, malignancy, hematological disorders etc were excluded from the study. Also women with history of smoking, alcohol and other drug consumption which might affect the blood pressure were excluded from the study. A detailed family and medical history were taken. Thorough clinical examination was done in all the subjects. Systolic and diastolic blood pressure was carefully recorded. Urine analysis was done in all subjects to measure the degree of proteinuria. Blood was taken from the ante cubital vein using a sterile needle and syringe. Blood was collected at 7 am in the morning after overnight fasting. Blood samples were allowed to clot and then centrifuged at 3000 revolutions per minute for 10 minutes. Serum calcium and magnesium level was measured by Colorimetric method. Whenever possible, the analysis was done immediately. When there was a delay, the samples were stored at -20° Celsius till further analysis. Serum calcium was estimated by the O- Cresol Phthalene Complexone method with Erba model Chem 5 plus. Magnesium was estimated by Xylidyl Blue method with Roche Hitachi 902. Data was expressed as Mean \pm Standard Deviation. Data analysis was done by SPSS version 20. Comparison of serum levels of the elements between the two groups was performed by Independent t-test and Chi-square test and p-value of < 0.05 was considered as statistically significant.

RESULTS

The present study enrolled 120 pregnant women. No significant differences in demographic characteristics were present between the pre-eclamptic subjects and the healthy controls. There were no cases of maternal or fetal death or of maternal renal or hepatic insufficiencies. The clinical characteristics of the participants are shown in [Table/Fig-1]. The mean age of women with pre-eclampsia

was higher than normotensive controls. (27.45 ± 4.33 yrs vs 25.87 ± 3.11 yrs p-value 0.023). The mean BMI [Body Mass Index] was significantly higher in pre-eclampsia than normotensives. (27.07 ± 3.07 kg/m² vs 24.9 ± 2.32 kg/m² p < 0.001) The systolic and diastolic blood pressure was significantly higher in cases compared to controls. The period of gestation was significantly higher in the normotensives compared to pre-eclampsia. (36.9 ± 0.9 vs 38.21 ± 0.85 p < 0.001). The fetal birth weight was significantly less in women with pre-eclampsia than normal pregnant women. (2.61 ± 0.53 kg vs 2.98 ± 0.36 kg p-value < 0.001).

[Table/Fig-2] shows the comparison of levels of serum calcium and magnesium in both the groups. The serum calcium concentration was significantly lower in the pre-eclamptic cases compared to the normotensive controls (7.84 ± 0.87 mg/dl vs 8.97 ± 0.69 mg/dl, p < 0.001) whereas the levels of serum magnesium showed a marginal difference in both the groups (1.43 ± 0.55 mg/dl vs 1.57 ± 0.72 mg/dl p 0.257) which was statistically not significant.

Clinical characteristics	Cases (n=60)	Controls (n=60)	Significance level (p-value)
Age (years)	27.45 \pm 4.33	25.87 \pm 3.11	0.023*
BMI (kg/m ²)	27.07 \pm 3.07	24.9 \pm 2.32	<0.001*
Gestational age (weeks)	36.9 \pm 0.9	38.21 \pm 0.85	<0.001*
Systolic BP (mm Hg)	155.50 \pm 12.18	108 \pm 6.50	<0.001*
Diastolic BP (mm Hg)	108.18 \pm 10.89	68.69 \pm 8.19	<0.001*
Birth weight (kg)	2.61 \pm 0.53	2.98 \pm 0.36	<0.001*

[Table/Fig-1]: Clinical characteristics of cases and controls

Values are given as mean \pm SD (Standard Deviation); *p < 0.05 - Statistically Significant; BMI - Body Mass Index; BP- Blood Pressure

Parameters	Cases n=60	Controls n=60	Significance (p-value)
Calcium (mg/dl)	7.84 \pm 0.87	8.97 \pm 0.69	<0.001*
Magnesium (mg/dl)	1.43 \pm 0.55	1.57 \pm 0.72	0.257 (NS)

[Table/Fig-2]: Comparison of serum calcium and magnesium levels in cases and controls

Values are expressed as mean \pm SD (Standard Deviation); *p < 0.05 - Statistically Significant; NS - Not Significant

DISCUSSION

Women with a greater BMI in pregnancy are more likely to become hypertensive than those with a lower BMI [14]. In our study, BMI was significantly higher in the hypertensive group compared to the normotensive group (p < .001). Sukonpan and Phupong and Akhtar et al., also found a significantly higher BMI in the hypertensive group [1,15]. Women with pre-eclampsia were significantly older than normotensives in our study. Some studies have confirmed this [16,17] whereas other studies show no relation to age and pre-eclampsia [1,5,15,18]. Lamminpaa et al., in a registry based study in Finland found that women with advanced maternal age exhibited pre-eclampsia (9.4%) than younger women (6.4%) [16]. Another study done in Ghana to analyse the risk factors of pre-eclampsia found that advanced maternal age was a significant risk factor for developing pre-eclampsia [17]. In pre-eclamptic women gestational age was significantly lesser than that of normal pregnant women with p < 0.001. The gestational age was lower in the studies by Harma et al., and Chaurasia et al., [19,20].

The birth weight was significantly lower in the pre-eclamptic babies. This was also found by Sukonpan and Phupong [1]. The study from Ghana to evaluate the effects of early and late onset pre-eclampsia on fetal growth found that development of pre-eclampsia before 37 weeks was associated with significantly lower birth weights [21].

Nutritional deficiencies are common during pregnancy and pregnant women in developing countries have been reported to consume diets that are low in minerals and vitamins [4]. Dietary deficiencies of calcium and magnesium during pregnancy have been implicated in pre-eclampsia, eclampsia, preterm birth and intrauterine growth retardation [10].

Calcium is the micronutrient that has been best studied in relationship to pre-eclampsia. Several epidemiological studies in developing nations indicate an association between reduced calcium intake and pre-eclampsia. These observations led to the hypothesis that the incidence of pre-eclampsia can be reduced in populations of low calcium intake by calcium supplementation [9].

An inverse relationship between calcium intake and hypertensive disorders of pregnancy was first described in 1980 based on the observation that Mayan Indians in Guatemala had a low incidence of pre-eclampsia as their intake of calcium was high due to the traditional method of soaking corn in lime before cooking [22]. Mahomed et al., reported a low intake of calcium during pregnancy in many different parts of the world such as Asia, Latin America, and Africa as well as developed countries such as Canada, USA and the UK. They reported a very low intake of calcium in India. (250mg/d) [23].

Calcium plays an important role in muscle contraction and regulation of water balance in cells. Modification of plasma calcium concentration leads to the alteration of blood pressure. The lowering of serum calcium and the increase of cellular calcium can cause an elevation of blood pressure in pre-eclamptic mothers. The increase of cellular calcium concentration when serum calcium went lower led to constriction of smooth muscles in blood vessels and increase of vascular resistance [1].

Magnesium has been known as an essential cofactor for many enzyme systems. It also plays an important role in neurochemical transmission and peripheral vasodilatation [13]. Magnesium sulfate has been used as the drug of choice in severe pre-eclampsia and eclampsia treatment. The modification of calcium and magnesium metabolism during pregnancy could be a potential cause of pre-eclampsia. Magnesium promotes vascular muscle relaxation [1]. Many women, especially those from disadvantage backgrounds, have intake of magnesium below recommended levels. Magnesium supplementation during pregnancy may be able to reduce fetal growth retardation and pre-eclampsia and increase birth weight [24]. The success of magnesium therapy as a treatment for eclamptic seizures and the known effect of magnesium on vascular responses in vitro suggested that magnesium might be deficient in women with pre-eclampsia [9].

Jain et al., Sukonpan and Phupong found a decrease in both serum calcium and magnesium in pre-eclamptic pregnant women as compared to normal pregnant women in their study thus supporting the hypothesis that hypocalcemia and hypomagnesemia are possible aetiologies of pre-eclampsia [10,1] whereas a research work by Golmohammad and Yazdian in Iran did not show significant difference in the levels of calcium and magnesium in women with pre-eclampsia and normotensives [18].

Punthumapol and Kittichotpanich showed that serum calcium in severe pre-eclamptic women was lower than normal pregnant women and mild pre-eclamptic women respectively. There was no difference in serum magnesium among normal pregnancy and both mild and severe pre-eclampsia [13]. Similarly, Kumru et al., in their study found significantly lower levels of calcium (10%) in pre-eclamptic women compared to healthy controls, whereas magnesium concentration showed nonsignificant differences between the two groups. They concluded that measurement of these elements may be useful for the early diagnosis of a pre-eclamptic condition [5]. Akhtar S et al., in a study from Bangladesh showed significantly lower levels of calcium in pre-eclamptic women [15]. They gave an opinion that early detection and supplementation to treat this deficiency may reduce the incidence of pre-eclampsia. Akinloye et al., in a study from Nigeria reported a decrease in serum magnesium in pregnant women with pre-eclampsia [4].

A Cochrane review analysed 12 high quality trials after excluding 24 trials on supplementation of calcium to prevent pre-eclampsia in normotensive pregnant women. 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 pre-eclampsia. The reduction was greatest for women at high risk of developing pre-eclampsia and for those with low baseline dietary calcium. The Relative Risk of having the composite outcome maternal death or serious morbidity was reduced for women allocated calcium supplementation compared with placebo. The review commented that adequate dietary calcium before and in early pregnancy may be needed to prevent the underlying pathology responsible for pre-eclampsia. They also suggested that the research agenda should be redirected towards calcium supplementation at a community level [25].

Another Cochrane review selected randomised and quasi randomised trials of dietary magnesium supplementation during pregnancy. Seven trials involving 2689 women were included in the review. There was no apparent effect of magnesium treatment on maternal systolic or diastolic blood pressure during pregnancy. There was no difference in the risk of pre-eclampsia or pregnancy induced hypertension between the magnesium and placebo groups. It also showed that magnesium treatment was associated with a reduced risk of low birth weight and of small for gestational age babies compared with placebo. They concluded that there is no high quality evidence to show that dietary magnesium supplementation during pregnancy is beneficial [24].

We conducted this study to find out the serum levels of calcium and magnesium in pre-eclampsia and normal pregnancies and to evaluate the association between these elements and pre-eclampsia. There was a significant reduction in calcium levels and a marginal decrease in magnesium levels in pre-eclamptic women compared to normotensives. We also analysed the serum samples of non pregnant healthy women in the population for the levels of these elements and found the levels to be similar to healthy pregnant women. According to the results of our research, intake of supplements, mainly calcium may help in the reduction of incidence of pre-eclampsia especially in a population of a developing country like ours where the nutrition is poor. Not many studies have been done in developing countries to assess the role of these elements in pre-eclampsia. A study from Northern part of India by Chaurasia et al., found significantly lower levels of serum calcium and magnesium in pre-eclamptic women compared to normal pregnant women [20]. Another study by Sandip et al., found significantly lower levels of calcium and magnesium in severe pre-eclamptic women compared to normotensive and mild pre-eclamptic women. There was no significant difference found between normal and mild pre-eclamptic women [26]. Limited data is available on the role of calcium and magnesium from rural south India where the diet is deficient of the necessary elements. High risk women with low calcium and magnesium intake will be benefited with supplementation. The recommended dietary allowance in the USA recommends that pregnant women should take 1 to 1.5 gms of calcium daily for pre-eclamptic complication prevention [1]. Milk, soy milk, yogurt, cheese and vegetables like cabbage, broccoli, almonds, sardine and salmon with bones and calcium fortified orange juice are good sources of calcium. The daily requirement of magnesium is about 350mg/day [27]. Foods rich in magnesium include whole grains, nuts and green vegetables. Green leafy vegetables are particularly good sources of magnesium. The limitation of our study was that a detailed dietary assessment of the subjects was not done. Pregnant women in developing countries should be encouraged to consume food rich in calcium and magnesium. If the intake is less than the recommended dose, a supplement can be given.

CONCLUSION

To conclude, our study shows a significant reduction of serum calcium and only a marginal reduction in serum magnesium levels in pre-eclamptics compared to normotensives. This supports the hypothesis that hypocalcemia may have a role in the aetiology of

pre-eclampsia. Further trials are needed regarding the role of calcium and magnesium supplementation in prevention of pre-eclampsia.

REFERENCES

- [1] Sukonpan K, Phupong V. Serum calcium and serum magnesium in normal and pre-eclamptic pregnancy. *Arch Gynecol Obstet*. 2005;273: 12-6.
- [2] Ziaei S, Ranjesh F, Faghizadeh S. Evaluation of 24-hour copper in pre-eclamptic vs normotensive pregnant and non-pregnant women. *Int J Fertil Steril*. 2008;2: 9-12.
- [3] Sarsam DS, Shamden M, AlWazan R. Expectant versus aggressive management in severe pre-eclampsia remote from term. *Sing Med J*. 2008; 49: 698.
- [4] Akinloye O, Oyewale OJ, Oguntibeju OO. Evaluation of trace elements in pregnant women with pre-eclampsia. *Afr J Biotechnol*. 2010;9(32):5196-5202.
- [5] Kumru S, Aydin S, Simsek M, Sahin K, Yaman M, Ay G. Comparison of Serum Copper, Zinc, Calcium and Magnesium Levels in Pre-eclamptic and Healthy Pregnant Women. *Biol Trace Elem Res*. 2003;94:105-12.
- [6] American College of Obstetrics and Gynaecology (ACOG) Practice bulletin. Diagnosis and management of pre-eclampsia and eclampsia. *Obstet Gynaecol*. 2002;99:159-67.
- [7] Cunningham FG, Leveno KJ, Bloom SL, Hauth JC, Gilstrap LC III, Wenstrom KD. *Williams Obstetrics*. 22nd ed. New York: McGraw – Hill:761-808
- [8] Bringman J, Gibbs C, Ahokas R. Differences in serum calcium and magnesium between the gravidas with severe pre-eclampsia and normotensive controls. *Am J Obstet Gynecol*. 2006;195:148.
- [9] Roberts JM, Balk JL, Bodnar LM, Belizan JM, Bergel E, Martinez A. Nutrient Involvement in Pre-eclampsia. *J Nutr*. 2003;133:1684-92.
- [10] 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;133:162-70.
- [11] Hofmeyr GJ, Duley L, Atallah A. Dietary calcium supplementation for prevention of pre-eclampsia and related problems: a systematic review and commentary. *BJOG*. 2007;114: 933-43.
- [12] Cetin I, Berti C and Calabrese S. Role of micronutrients in the periconceptual period. *Human Reprod Update*. 2010;16(1):80-95.
- [13] Punthumapol C, Kittichotpanich B. Serum Calcium, Magnesium and Uric Acid in Pre-eclampsia and Normal Pregnancy. *J Med Assoc Thai*. 2008;91(7):968-72.
- [14] Pipkin FB. Risk Factors for Pre-eclampsia. *N Engl J Med*. 2001;344:925-6.
- [15] Akhtar S, Begum S, Ferdousi S. Calcium and Zinc Deficiency in Pre-eclamptic Women. *J Bangladesh Soc Physiol*. 2011;6(2): 94-9.
- [16] Lamminpaa R, Vehvilainen JK, Gissler M, Heinonen S. Pre-eclampsia complicated by advanced maternal age: a registry-based study on primiparous women in Finland 1997-2008. *BMC Pregnancy Childbirth*. 2012;11:12:47.
- [17] Owiredo WKBA, Ahenkorah L, Turpin CA, Amidu N, Laing EF. Putative risk factors of pregnancy-induced hypertension among Ghanaian pregnant women. *JMBS*. 2012;1(3):62-76.
- [18] Golmohammad lou S, Yazdian M, Pashapour N. Evaluation of Serum Calcium, Magnesium, Copper, and Zinc Levels in Women with Pre-eclampsia. *IJMS*. 2008;33(4):231-4.
- [19] Harma M, Harma M, Kocyigit A. Correlation Between Maternal Plasma Homocysteine and Zinc Levels in Pre-eclamptic Women. *Biol Trace Elem Res*. 2005;104:97-105.
- [20] Chaurasia PP, Jadav PA, Jasani JH. Changed in Serum Calcium and Serum Magnesium Level In Pre-eclamptic VS Normal Pregnancy. *IJBAR*. 2012; 3(6):511-3.
- [21] Obed SA, Patience A. Birth Weight and Ponderal Index in Pre-Eclampsia: A Comparative Study. *Ghana Med J*. 2006;40(1):8-13.
- [22] Belizan JM, Villar J. The relationship between calcium intake and oedema, proteinuria and hypertension gestosis: a hypothesis. *Am J Clin Nutr*. 1980;33: 2202-10.
- [23] Mahomed K, Williams MA, Woelk GB, Mudzamiri S, Madzime S, King IB, et al. Leukocyte Selenium, Zinc, and Copper Concentrations in Pre-eclamptic and Normotensive Pregnant Women. *Biol Trace Elem Res*. 2000;75: 107-18.
- [24] Makrides M, Crowther CA. Magnesium supplementation in pregnancy (Review) *Cochrane Database Syst Rev*. 2012;10:CD000937.
- [25] Hofmeyr GJ, Duley L, Atallah AN. Calcium supplementation during pregnancy for preventing hypertensive disorders and related problems. *Cochrane Database Syst Rev*. 2010;8:CD001059.
- [26] Sandip S, Asha K, Paulin G, Hirens S, Gagandeep S, Amit V .A Comparative Study Of Serum Uric Acid, Calcium And Magnesium In Pre-eclampsia And Normal Pregnancy. *JARBS*. 2013; 5(1):55-8.
- [27] Oladapo A Ladipo. Nutrition in pregnancy: mineral and vitamin supplements. *Am J Clin Nutr*. 2000; 72:280S-90S.

PARTICULARS OF CONTRIBUTORS:

1. Associate Professor, Department of Obstetrics and Gynaecology, K.S. Hegde Medical Academy, Mangalore, Karnataka, India.
2. Associate Professor, Department of Obstetrics and Gynaecology, K.S. Hegde Medical Academy, Mangalore, Karnataka, India.
3. Post Graduate, Department of Obstetrics and Gynaecology, K.S. Hegde Medical Academy, Mangalore, Karnataka, India.
4. Research Assistant, Department of Biochemistry, K.S. Hegde Medical Academy, Mangalore, Karnataka, India.
5. Professor and HOD, Department of Obstetrics and Gynaecology, K.S. Hegde Medical Academy, Mangalore, Karnataka, India.
6. Professor, Department of Biochemistry, K.S. Hegde Medical Academy, Mangalore, Karnataka, India.
7. Professor, Department of Obstetrics and Gynaecology, K.S. Hegde Medical Academy, Mangalore, Karnataka, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Deepa V Kanagal,
Associate Professor, Department of Obstetrics and Gynaecology, K.S. Hegde Medical Academy, Deralakatte,
Mangalore, Karnataka, India.
Phone: 09980164615, E-mail: deepakanagal@yahoo.co.in

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Feb 10, 2014**

Date of Peer Review: **Apr 03, 2014**

Date of Acceptance: **May 02, 2014**

Date of Publishing: **Jul 20, 2014**