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RESEARCH ARTICLE

A STUDY ON RELATION BETWEEN MATERNAL VITAMIN D LEVEL IN EARLY PREGNANCY (LESS THAN 16 WEEKS) & THE RISK OF DEVELOPMENT OF GESTATIONAL DIABETES AND PREGNANCY INDUCED HYPERTENSION

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ABSTRACT

Background and Purpose: Maternal Vitamin D deficiency is a global public health problem. The main objective of this prospective cohort study is to assess the independent effect of maternal 25-hydroxyvitamin D levels in early pregnancy on the risk of GDM and PIH.

Methods: 574 nulliparous mothers with singleton pregnancies attended the antenatal out-patient department of our institution during the study period from July 2013 to June 2014 and were selected after taking proper history and investigations. In the first visit, Vitamin D estimation was done by HPLC method along with other routine investigations. Vitamin D deficient mothers, i.e. serum 25-hydroxyvitamin D<50 nmol/L(20 ng/ml) were taken as exposed and other mothers as non-exposed and followed upto term to study the development of GDM and PIH in the index pregnancy. Only mothers attending OPD on Wednesday were included in our study.

Results: Out of 574 mothers, 375(65.3%) were vitamin D insufficient, 47(8.2%) developed GDM and DGGT, and 57(9.9%) developed PIH in third trimester. A significant positive association between low 25(OH) D levels and an increased risk of GDM(p=.01) and PIH(p=.049) was found in our study. A correlation between obesity, maternal vitamin D status and subsequent development of GDM and PIH was also illustrated but were statistically insignificant.

Conclusion: Hypovitaminosis D during pregnancy and its undesirable health outcomes is an area of growing concern. As maternal obesity rates increase and incidence of GDM also rises, it is becoming increasingly important to understand modifiable risk factors as vitamin D status. Ultimately, RCTs will be needed to test if vitamin D supplement affects GDM and PIH risk and improves maternal and perinatal outcomes.

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INTRODUCTION

Maternal Vitamin D deficiency is a widespread public health problem (Bodnar *et al*., 2007a) with high rates of poor maternal vitamin D status, particularly among those with deeply pigmented skin(Grover and Morley, 2001; Sachan *et al*., 2005; Van der Meer *et al*., 2006). The vitamin D deficiency epidemic during pregnancy is caused by lack of adequate sunlight exposure needed for vitamin D synthesisin the skin, coupled with oral intakes that are too low to meet the increased demands of pregnancy, even with regular use of prenatal vitamins containing 400 IU vitamin D_3 (Bodnar *et al*., 2007a, Lee *et al*., 2007). Gestational diabetes mellitus (GDM) markedly increases risk of type 2 diabetes in later life (Bellamy *et al*., 2009). Lower vitamin D concentrations are inversely associated with maternal glycemia (Clifton-Bligh *et al*., 2008), insulin resistance (Maghbooli *et al*., 2008), and increased risk of GDM (Zhang *et al*., 2008). However, further studies are needed to examine the relevant associations between the variables. This is even more so the case given that the nonpregnant arena results of observational studies and trials of vitamin D in diabetes conflict, with eight trials showing no effect of vitamin D supplementation on glycemia

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or incident diabetes (Pittas *et al*., 2010). Preeclampsia is a pregnancy-specific syndrome that affects approximately 3–7% of primigravidas. The racial disparity in preeclampsia suggests vitamin D may be relevant, with Black women being more likely to develop severe preeclampsia and suffer greater morbidity associated with the disease than white women (MacKay *et al*., 2001). Moreover, the pathogenesis of preeclampsia involves a number of biological processes that may be directly or indirectly affected by vitamin D, including immune dysfunction, placental implantation, abnormal angiogenesis, excessive inflammation, and hypertension (Cardus *et al*., 2006; Hewison, 2010; Li *et al*., 2002). Vitamin D has been known to influence preeclampsia risk (Hypponen, 2005), but we need further studies examining the relation between maternal vitamin D status before the clinical onset of preeclampsia and the risk of disease.

Vitamin D deficiency during pregnancy has been linked with a number of serious short- and long-term health problems in offspring, including impaired growth, skeletal problems, type 1 diabetes, asthma, and schizophrenia (Holick, 2006). Yet few investigators have explored the role of maternal vitamin D status in adverse pregnancy outcomes. So, we found it worth conducting a prospective cohort study to assess the independent effect of maternal 25-hydroxyvitamin D (25-OH-D) levels in early pregnancy on the risk of gestational diabetes and pregnancy induced hypertension in the present pregnancy.

In the first visit (booking visit), Vitamin D estimation was done by High Performance Liquid Chromatography(HPLC) method. Vitamin D insufficient mothers, i.e. serum 25-hydroxyvitamin D (25(OH)D) of less than 20 ng/ml were taken as exposed and other mothers as non-exposed and followed upto term to study the development of gestational diabetes and PIH. This value was considered because although it is currently suggested that vitamin D depletion is defined by concentrations below 70 or 75 nmol/l(30 ng/ml) (Vieth *et al*., 2007), concentrations are known to be lower in pregnancy.(Calculation: 25-OH Vitamin D3 (ng/mL) x 2.5 = 25-OH Vitamin D3 (nmol/L))In our study settings, apart from routine investigations, according to recommendations from the Diabetes In Pregnancy Study group India (DIPSI) (Seshiah *et al*., 2006), pregnant women were screened at first trimester using a 75 gram 2-hour oral glucose challenge test. Those patients who failed this screening test (glucose≥140 gm/dl) were then followed-up within 1–2 weeks with a 75 g, 2-h oral glucosetolerance test (OGTT)(WHO).If found negative at this time, the screening test was performed again around 24–28 weeks and finally around 32–34 weeks. With 75 gm OGTT (WHO criteria);

Pregnant women having new arterial hypertension(BP≥140/90 mm Hgmeasured on two separate occasions, more than 6 hours apart) after 20 weeks gestation were included in the PIH group(Williams obstetrics, 24th ed.). The mode of delivery and birth weight of the babies were also noted after delivery.

2 hr plasma Glucose	In Pregnancy	Outside Pregnancy
> 200 mg/ dl	Diabetes	Diabetes
> 140 - 199 mg/ dl	Gestational Diabetes Mellitus (GDM)	Impaired Glucose Tolerance (IGT)
120- 139 mg/ dl*	Decreased Gestational Glucose Tolerance (DGGT)	--
< 120 mg/ dl	Normal	Normal
	* Needs follow up	
The term IGT should not be used to indicate any glucose intolerancein pregnancy(as this terminology is used outside pregnancy)		

METHODS

4371 antenatal mothers attended the out-patient department of Gynaecology & Obstetrics of Burdwan Medical College& Hospital, Burdwan (a tertiary care centre of rural India) during the study period of one year from July 2013 to June 2014. Only pregnant women attending OPD on Wednesday were included in our study. Initially, 588 nulliparous antenatal mothers with singleton intrauterine pregnancies attending OPD in early pregnancy (less than 16 weeks),gestational ages determined by the last menstrual period (LMP) or by a first trimester ultrasound scan if the LMP was uncertain, were selected after taking proper history, clinical examination and doing routine radiological and laboratory investigations. We obtained information of covariates including maternal age, educational attainment, occupation, socioeconomic status, height, pre-pregnancy weight, reproductive and medical histories, and medical histories of first-degree family members. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Of them, 14 antenatal mothers had spontaneous abortion. So, we followed rest 574 antenatal mothers up to term to study the development of gestational diabetes and pregnancy induced hypertension (PIH) in the index pregnancy.

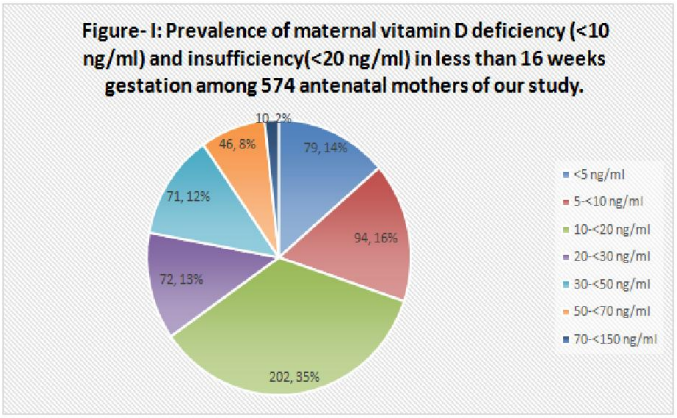
The Vitamin D deficient mothers were treated with regular use of 400 IU vitamin D3 /day although it has been studied previously that such intervention does not alter the incidence of diseases under study in the antenatal mothers. Ethical permission for the study was granted by Ethics Committee, Burdwan Medical College, Burdwan, West Bengaland informed consent was obtained. Statistical methods like Chi-square test, Odds ratio, T Test, Wilcoxon test, Fisher exact test, Analysis of Variance (ANOVA) were employed in the present study. Chi-square test was primarily applied by the statistician.

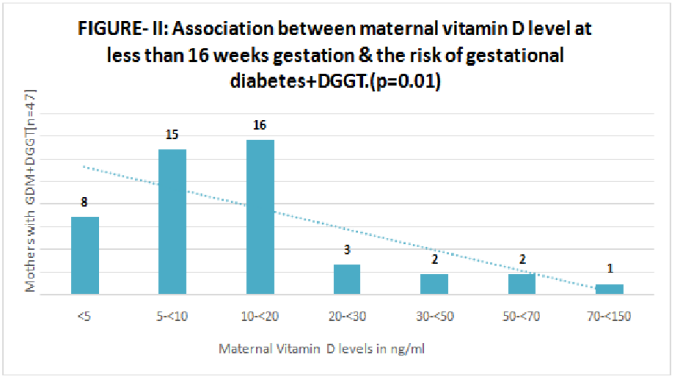
We included nulliparous pregnant women with live singleton intrauterine pregnancy of gestational age less than 16 weeks in our study. Multigravida**,** multiple pregnancy, ectopic pregnancy, hydatidiform mole, previous history of impaired fasting glucose, impaired glucose tolerance, gestational diabetes and hypertension, family history of diabetes and chronic hypertension, risk factors and etiology of diabetes and hypertension were excluded from our study.

RESULTS

Out of 574 antenatal mothers, 173(30.1%) were vitamin D deficient (< 10 ng/ml) and 375(65.3%) were vitamin D insufficient (<20 ng/ml) (Fig. I). 47(8.2%) developed

gestational diabetes (GDM) and decreased gestational glucose tolerance (DGGT) (Table-I) and 57(9.9%) developed pregnancy induced hypertension (PIH) (Table- II) in their third trimester. (Table- I/Fig. II) shows an increased risk of gestational diabetes and DGGT with decremental levels of vitamin D in our study and this is statistically significant. (p=0.01) (Table- II/Fig. III) shows an increased risk of pregnancy induced hypertension with decremental level of vitamin D in our study which is statistically significant.





(p=0.049) In (Table-III), vitamin D insufficiency (<20 ng/ml) is more prevalent among underweight antenatal mothers (32.80% in Vitamin D insufficient mothers vs 27.64% in Vitamin D sufficient mothers) but this is statistically insignificant. (p=0.56) In (Table- IV), overweight (25.53% vs 19.54%) and obese (10.64% vs 8.16%) antenatal mothers are at more risk of GDM+DGGT which is statistically insignificant. (p=0.64) Normal weight (42.11% vs 40.43%), overweight (22.81% vs 19.73%) and obese (12.28% vs 7.93%) antenatal mothers are at more risk of PIH but it is not statistically significant. (p=0.42) (Table- V) In Table- VI, women who developed GDM had higher systolic blood pressure (p=0.003) and birth weight (p=0.081), positively associated with total cholesterol (p=0.033) and triglyceride (p=0.002) and negatively associated with HDL cholesterol (p=0.001).

DISCUSSION

In the present study out of 574 cases, 65.3 % mothers were vitamin D insufficient which is consistent with Farrant *et al*. (2009) study. (Table- VII) The high incidence of maternal hypovitaminosis D, assessed by vitamin D values <20 ng/ml is consistent with findings in other studies (Sachan *et al*., 2005). South Asians have lower serum 25(OH)D concentrations than white Caucasians (Goswami *et al*., 2000; Hamson *et al*., 2003) due to skin pigmentation, covered-up clothing (especially common in women)and low dietary vitamin D intake. There may also be differences in vitamin D metabolism in South Asians. Many studies have reported on the vitamin D status of pregnant women, in a variety of environments. As expected, it was derived that vitamin D deficiency is particularly common in dark pigmented mothers, particularly among those who have migrated to regions with lower ambient UVR than their evolutionary heritage. Nevertheless, low vitamin D status is also prevalent in pregnant Caucasian populations, particularly those residing in regions with suboptimal UVR and not receiving supplementation.

Currently there are no set guidelines for concentration of 25(OH) D that inform vitamin D deficiency or sufficiency; only recommendations by the Institute of Medicine (IOM).IOM issued a guideline that sufficient levels of 25(OH)D were above 20mg/ml(CDC.gov NCHS Data BriefNo.59 March 2011). Interestingly 84% of the study population used supplement containing vitamin D, yet 65.3% remained vitamin D deficient. Guidelines for vitamin D supplementation are still in debate at this time. In the present study, 8.2% of pregnant women were gestational diabetes+DGGT in the 3rd trimester which is consistent with Farrant *et al*. (2009) study.(Table- VIII)Although, the recent data on the prevalence of GDM in our country was 16.55% by WHO criteria of 2 hr PG≥140 mg/dl) (Seshiah *et al*., 2006). However, our study showed a significant positive association between low vitamin D levels in less than 16 weeks gestation and the subsequent development of gestational diabetes+DGGT in the 3rd trimester which is not consistent with Farrant *et al*. study but consistent with Burris *et al*.(2012) study.

The association between vitamin D levels and GDM in the literature is not entirely clear. Farrant *et al.* studied 559 pregnant women in India and found no association between second trimester vitamin D levels and GDM. However, among a subset of mothers with levels <20 ng/ml, they found an inverse association between 25(OH)D levels and 30-minute glucose concentrations after a glucose load, a finding consistent with our results. A recent study by Makgoba *et al*. (2011) of 90 cases of GDM and 158 controls showed no association between first trimester blood samples and subsequent development of GDM, however, consistent with our findings, they did find an inverse correlation between 25(OH)D levels and glucose measurements after a 2-hour fasting glucose tolerance test. Dietary factors or physical activity were not adjusted in these case-control studies which may confound the association between vitamin D status and GDM. A few more studies have shown a link between low 25(OH)D level and GDM. Soheilykhah *et al*. (2010) found in a matched case-control study of 54 women with GDM and 39 women with impaired glucose tolerance (IGT) compared to 111 non-GDM control women in Iran, that maternal 25(OH)D concentrations at24–28 weeks of gestation were significantly lower than non-GDM controls. They found that 83% of GDM women had 25(OH)D levels <20 ng/ml vs. 71% of controls.

Table I. Association between maternal vitamin D level at less than 16 weeks gestation & the risk of gestational diabetes+DGGT

25(OH)D concentrations (ng/ml)	Mothers with GDM+DGGT	Mothers without GDM+DGGT
<5	8	71
5-<10	15	79
10-<20	16	186
20-<50	5	138
50-<150	3	53
TOTAL	47(8.2%)	527(91.8%)

Table II. Association between maternal vitamin D level at less than 16 weeks gestation & the risk of pregnancy induced hypertension

25(OH)D concentrations (ng/ml)	Mothers with PIH	Mothers without PIH
<5	10	69
5-<10	16	78
10-<20	16	186
20-<50	13	130
50-<150	2	54
TOTAL	57(9.9%)	517(90.1%)

Table III. Association between maternal BMI at less than 16 weeks gestation (kg/m2) & Vitamin D status

Maternal BMI at less than 16 weeks gestation(kg/m2)	Total no of mothers (n=574) %	Vitamin D Insufficient (n=375) %	Vitamin D Sufficient (n=199) %
Underweight(<18.5)	178(31.01%)	123(32.80%)	55(27.64%)
Normal weight(18.5-25)	233(40.59%)	151(40.27%)	82(41.21%)
Overweight(25-30)	115(20.03%)	72(19.20%)	43(21.61%)
Obese(>30)	48(8.36%)	29(7.73%)	19(9.55%)

Table IV. Association between maternal BMI at less than 16 weeks gestation (kg/m2) & the risk of gestational diabetes+DGGT

Maternal BMI at less than 16 weeks gestation(kg/m2)	Total no of mothers (n=574) %	GDM+DGGT mothers (n=47) %	Mothers without diabetes (n=527) %
Underweight(<18.5)	178(31.01%)	12(25.53%)	166(31.50%)
Normal weight(18.5-25)	233(40.59%)	18(38.30%)	215(40.80%)
Overweight(25-30)	115(20.03%)	12(25.53%)	103(19.54%)
Obese(>30)	48(8.36%)	5(10.64%)	43(8.16%)

Table V. Association between maternal BMI at less than 16 weeks gestation (kg/m2) & the risk of pregnancy induced hypertension

Maternal BMI at less than 16 weeks gestation(kg/m2)	Total no of mothers (n=574) %	Mothers with PIH (n=57) %	Mothers without PIH (n=517) %
Underweight(<18.5)	178(31.01%)	13(22.81%)	165(31.91%)
Normal weight(18.5-25)	233(40.59%)	24(42.11%)	209(40.43%)
Overweight(25-30)	115(20.03%)	13(22.81%)	102(19.73%)
Obese(>30)	48(8.36%)	7(12.28%)	41(7.93%)

Maghbooli *et al*. (2009) found in a study of 741 women in Iran that among the 29 % of participants with 25(OH)D levels <6 ng/ml, the prevalence of GDM was significantly higher compared to women with 25(OH)D levels ≥14 ng/ml.

Clifton-Bligh *et al*. (2008) found in a study of 264 women that among the 32% of women with GDM, 25(OH)D levels were significantly lower than among women without gestational diabetes. Zhang *et al*. (2008)found in a nested case-control study of 57 cases of GDM, that maternal 25(OH)D levels at 16 weeks' gestation were 20% lower among women who later developed GDM. Our findings of an inverse association between glucose and 25(OH)D concentration are consistent with these studies. Although, we understand the clinical insignificance of various glucose levels after the 2-hour glucose load screening test in the absence of a GDM diagnosis and we speculate that the difference in the pattern of associations between 25(OH)D levels and these outcomes may be a function of the different test characteristics between the screening and diagnostic tests.

Obesity is closely associated with both GDM and PIH. However, vitamin D insufficiency (<20 ng/L) is more prevalent among underweight antenatal mothers in our study. Our data were statistically insignificant. The study do not eliminate the likelihood of a persistent association between low 25(OH)D and GDM and PIH among obese women. Mothers with low 25(OH)D levels can have secondary hyperparathyroidism which can increase insulin resistance. Our work raises the possibility that even obese women may benefit from vitamin D supplementation and that such an intervention might decrease their risk of GDM and PIH. Randomized controlled trials are needed to establish the statement. In the present study, 9.9% of pregnant women had pregnancy induced hypertension in the 3rd trimester which is higher than Bodnar *et al*. study (2007b). (Table- IX) Our results showed that maternal vitamin D deficiency at less than 16 weeks gestation was a strong, independent and significant risk factor for PIH with a monotonic dose-response relation between maternal serum 25(OH)D and risk of PIH. As maternal serum 25(OH)D concentrations at less than 16 weeks

increased, the risk of PIH strikingly decreased throughout the 25(OH)D range. Bodnar *et al*. (2007b), in a nested case control study involving 55 nulliparous women, showed that women who ultimately developed hypertension in pregnancy were 2.5 time more likely to have vitamin D deficiency in early pregnancy after adjusting for confounders. Small studies have reported reduced maternal serum 25(OH) D in overt preeclampsia, which we confirmed in our analysis. Baker *et al*. (2010), 2010, in a nested case control study, found a 4-fold increase in severe preeclampsia in women who had <20ng/ml $25(OH)D_3$.Seasonal patterns in preeclampsia show the lowest incidence in summer, when sunlight is plentiful and serum 25(OH)D concentrations are at their peak, and the highest incidence in winter, when synthesis of vitamin D_3 is limited in temperate zones and serum 25(OH)D levels are at their nadir.

A study in British Columbia Canada found that there is no difference in the rates of preeclampsia, gestational hypertension, or adverse pregnancy outcome in a group of 221 pregnant women who were largely vitamin D deficient (Shand *et al*., 2010).

Conclusion

The widespread global prevalence of hypovitaminosis D during pregnancy and its implications for undesirable health outcomes in present and future generations is an area of growing concern. Our results suggest that low first trimester 25(OH)D levels may be associated with increased odds of GDM and PIH, and that 25(OH)D levels were inversely correlated with blood glucose measurement after a 2-hour, 75 gram glucose load.

Table VI. Characteristics of Mothers with GDM vs. Mothers without GDM

	Mothers without GDM+DGGT	Mothers with GDM+DGGT	P_{value}
n	527	47	
Maternal age(years)	19.5±2	19.3±2	0.104*
Maternal BMI at 12 weeks (kg/m^2)	22.1±3.0	22.1±3.1	0.51+
Systolic Blood Pressure (mm Hg)	112.5±24.0	118.5±20.0	0.003!
Diastolic Blood Pressure (mm Hg)	70.2±17.0	71.7±16.0	0.218*
Birth weight (g)	2700±500	2800±500	0.081*
OGTT			
Fasting/2-h glucose (mmol/L)		5.3±1.8/9.1±1.8	
HbA_{1c} (%)		5.8±0.9	
Total cholesterol (mmol/L)	4.62±0.76	4.86±0.90	0.033*
HDL cholesterol (mmol/L)^	1.74 (0.35)	1.58(0.38)	0.001*
Triglyceride (mmol/L)^	1.20 (0.95-1.56)	1.38 (1.08-2.01)	0.002*
Deficient 25-OH-D (<10 ng/ml)			
No	377(71.5%)	24(51.1%)	0.03!
Yes	150(28.5%)	23(48.9%)	
Insufficient 25-OH-D (<20 ng/ml)			
No	191(36.2%)	7(14.9%)	0.02!
Yes	336(63.8%)	40(85.1%)	
Consumed Prenatal Vitamins	242(45.9%)	20(42.6%)	0.21*
Consumed>=2 servings of fish weekly	300(56.9%)	22(46.8%)	0.23*
Consumed>=1 glass of milk daily	158(30.0%)	14(29.8%)	0.82*
Season of Estimated Time of Conception*			
(March-May) Spring	194(36.8%)	17(36.2%)	1.00*
(June-August) Summer	157(29.8%)	14(29.8%)	
(September-November) Autumn	120(22.8%)	11(23.4%)	
(December-February) Winter	56(10.6%)	5(10.6%)	

Data are means±SD, median (interquartile range), or n (%).*t test. +Wilcoxon test. !Fisher exact test. ^Log-transformed for regression analysis.

Table VII. Comparison of maternal Vitamin D status in pregnancy

Maternal Vitamin D status in pregnancy	Farrant et al.(2009) (n=559)	Bodnar et al.(2007a) (n=1198) Black women	Bodnar et al.(2007a) (n=1198) White women	Present study (n=574)
Vitamin D deficiency(< 10 ng/L)	31%	29.2%	5%	30.1%
Vitamin D insufficiency(< 20 ng/L)	66%	54.1%	42.1%	65.3%
Vitamin D level > 20 ng/L	34%	45.9%	57.9%	34.7%

Table VIII. Comparison of diabetic status in pregnancy

Diabetic status in pregnancy	Jovanovic L et al.(2001)	Farrant et al.(2009) (n=559)	Present study (n=574)
Mothers with GDM+DGGT	14%	7%	8.2%
Mothers without GDM+DGGT	86%	93%	91.8%

Table IX. Comparison of prevalence of PIH in pregnancy

Hypertensive status in pregnancy	Bodnar et al. (2007b) (n=1198)	Griffith et al. (n=803)	Present study (n=574)
Mothers with PIH	4.9%	24%	9.9%
Mothers without PIH	95.1%	76%	90.1%

Numerous maternal outcomes and infant outcomes were reported to have higher incidence as a result of Vitamin D deficiency. As maternal obesity rates increase and the incidence of GDM also rises, it is becoming increasingly important to understand modifiable risk factors such as vitamin D status. Ultimately, randomized controlled trials will be needed to test if vitamin D supplement affects GDM and PIH risk and thereby leads to improved maternal and perinatal outcomes.

Compliance with ethical requirements

(1) Conflict of Interest (CoI) statements

Kousik Seth, Subhash Chandra Biswas, Keya Pal and Rahul Deb Mandaldeclare that they have no conflict of interest.

(2) Informed Consent in Studies with Human Subjects

Ethical permission for the study was granted by Ethics Committee, Burdwan Medical College, Burdwan, West Bengal, India. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5). Informed consent was obtained from all patients for being included in the study.

(3) This article does not contain any studies with animal subjects

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REFERENCES

Baker, A.M., Haeri, S., Camargo, C.A. Jr, Espinola, J.A. and Stuebe, A.M. 2010. A nested case-control study of midgestation vitamin D deficiency and risk of severe preeclampsia. *The Journal of Clinical Endocrinology and Metabolism,* 95(11):5105-5109.

Bellamy, L., Casas, J.P., Hingorani, A.D., Williams, D. 2009. Type 2 diabetes mellitus after gestational diabetes: a systematic review and meta-analysis. The Lancet, 373: 1773–1779.

Bodnar, L.M., Catov, J.M., Simhan, H.N., Holick, M.F., Powers, R.W. and Roberts, J.M. 2007b. Maternal Vitamin D Deficiency Increases the Risk of Preeclampsia. *The Journal of Clinical Endocrinology & Metabolism,* 92(9):3517–3522.

Bodnar, L.M., Simhan, H.N., Powers, R.W., Frank, M.P., Cooperstein, E. and Roberts, J.M. 2007a. High prevalence of vitamin D insufficiency in black and white pregnant women residing in the northern United States and their neonates. *Journal of Nutrition,* 137:447–452.

Burris, H.H., Rifas-Shiman, S.L., Kleinman, K., Litonjua, A.A., Huh, S.Y., Rich-Edwards, J.W., Camargo, C.A. Jr., Gillman, M.W. 2012. Vitamin D Deficiency in Pregnancy and Gestational Diabetes. *American Journal of Obstetrics & Gynecology,* 207(3):182.e1–182.e8.

Cardus, A., Parisi, E., Gallego, C., Aldea, M., Fernandez, E. and Valdivielso, J.M. 2006. *1,25*-Dihydroxyvitamin D3 stimulates vascular smooth muscle cell proliferation through a VEGF-mediated pathway. *Kidney International,* 69:1377–1384.

Clifton-Bligh, R.J., McElduff, P. and McElduff, A. 2008. Maternal vitamin D deficiency, ethnicity and gestational diabetes. *Diabetic Medicine,* 25:678–684.

Cunningham, F.G., Leveno, K.J., Bloom, S.L., Spong, C.Y., Dashe, J.S., Hoffman, B.L., Casey, B.M. and Sheffield, J.S. 2014. Williams obstetrics, 24:728-779. McGraw-Hill Professional.

Farrant, J.W.H., Krishnaveni, G.V., Hill, J.C., Boucher, B.J., Fisher, D.J., Noonan, K., Osmond, C., Veena, S.R. and Fall, C.H.D. 2009. Vitamin D insufficiency is common in Indian mothers but is not associated with gestational diabetes or variation in newborn size. *European Journal of Clinical Nutrition,* 63(5):646–652.

Goswami, R., Gupta, N., Goswami, D., Marwaha, R.K., Tandon, N. and Kochupillai, N. 2000. Prevalence and significance of low 25-hydroxyvitamin D concentrations in healthy subjects in Delhi. *The American Journal of Clinical Nutrition,* 72:472–475.

Grover, S.R. and Morley, R. 2001. Vitamin D deficiency in veiled or dark-skinned pregnant women. *The Medical Journal of Australia,* 175:251–252.

Hamson, C., Goh, L., Sheldon, P. and Samanta, A. 2003. Comparative study of bone mineral density, calcium, and vitamin D status in the Gujarati and white populations of Leicester. *Postgraduate Medical Journal,* 79:279–283.

Hewison, M. 2010. Vitamin D and the immune system: new perspectives on an old theme. *Endocrinology Metabolism Clinics of North America, 39*(2):365–379.

Holick, M.F. 2006. Resurrection of vitamin D deficiency and rickets. *The Journal of Clinical Investigation,* 116:2062–2072.

Hypponen, E. 2005. Vitamin D for the prevention of preeclampsia? A hypothesis. *Nutrition Reviews,* 63:225–232.

Jovanovic, L. and Pettitt, D.J. 2001. Gestational diabetes mellitus. *Journal of the American Medical Association,* 286:2516–2518.

Lee, J.M., Smith, J.R., Philipp, B.L., Chen, T.C., Mathieu, J. and Holick, M.F. 2007. Vitamin D deficiency in a healthy group of mothers and newborn infants. *Clinical Pediatrics,* 46:42–44.

Li, Y.C., Kong, J., Wei, M., Chen, Z.F., Liu, S.Q. and Cao, L.P. 2002. 1,25- Dihydroxyvitamin D_3 is a negative endocrine regulator of the renin-angiotensin system. *The Journal of Clinical Investigation,* 110:229–238.

Looker, A.C., Johnson, C.L., Lacher, D.A., Pfeiffer, C.M., Schleicher, R.L. and Sempos, C.T. 2001–2006. Vitamin D Status: United States. CDC.gov NCHS Data BriefNo.59 March 2011.

MacKay, A.P., Berg, C.J. and Atrash, H.K. 2001. Pregnancy-related mortality from preeclampsia and eclampsia. *Obstetrics & Gynecology,* 97:533–538.

Maghbooli, Z., Hossein-Nezhad, A., Karimi, F., Shafei, A.R. and Larijani, B. 2008. Correlation between vitamin D3

deficiency and insulin resistance in pregnancy. Diabetes/Metabolism Research and Reviews, 24:27–32.

Maghbooli, Z., Hossein-Nezhad, A., Mirzaei, K., Besharati, A., Omidfar, K. and Larijani, B. 2009. Association between retinol-binding protein 4 concentrations and gestational diabetes mellitus and risk of developing metabolic syndrome after pregnancy. *Reproductive Sciences,* 17:196–201.

Makgoba, M., Nelson, S.M., Savvidou, M., Messow, C.M., Nicolaides, K. and Sattar, N. 2011. First-Trimester Circulating 25-Hydroxyvitamin D Levels and Development of Gestational Diabetes Mellitus. *Diabetes Care,* 34:1091–1093.

Pittas, A.G., Chung, M., Trikalinos, T., Mitri, J., Brendel, M., Patel, K., Lichtenstein, A.H. and Balk, E.M. 2010. Systematic review: vitamin D and cardiometabolic outcomes. *Annals of Internal Medicine,* 152:307–314.

Sachan, A., Gupta, R., Das, V., Agarwal, A., Awasthi, P.K. and Bhatia, V. 2005. High prevalence of vitamin D deficiency among pregnant women and their newborns in northern India. *The American Journal of Clinical Nutrition,* 81:1060–1064.

Seshiah, V., Das, A.K., Balaji, V., Joshi, S.R., Parikh, M.N. and Gupta, S. 2006. Gestational Diabetes Mellitus – Guidelines for Diabetes In Pregnancy Study Group (DIPSI). *Journal of the Association of Physicians of India,* 54:622-628.

Shand, A.W., Nassar, N., Von Dadelszen, P., Innis, S.M., Green, T.J. 2010. Maternal vitamin D status in pregnancy and adverse pregnancy outcomes in a group at high risk for pre-eclampsia. BJOG: *An International Journal of Obstetrics and Gynaecology,* 117(13):1593-1598.

Soheilykhah, S., Mojibian, M., Rashidi, M., Rahimi-Saghand, S. and Jafari, F. 2010. Maternal vitamin D status in gestational diabetes mellitus. *Nutrition in Clinical Practice,* 25:524–527.

Van der Meer, I.M., Karamali, N.S., Boeke, A.J.P., Lips, P., Middelkoop, B.J.C., Verhoeven, I. and Wuister, J.D. 2006. High prevalence of vitamin D deficiency in pregnant non-Western women in The Hague, Netherlands. *The American Journal of Clinical Nutrition***,** 84:350–353.

Vieth, R., Bischoff-Ferrari, H., Boucher, B.J,. Dawson-Hughes, B., Garland, C. and Heaney, R.P. 2007. The urgent need to recommend an intake of vitamin D that is effective.The *American Journal of Clinical Nutrition,* 85:649–50.

Zhang, C., Qiu, C., Hu, F.B., David, R.M., vanDam, R.M., Bralley, A. and Williams, M.A. 2008. Maternal plasma 25-hydroxyvitamin D concentrations and the risk for gestational diabetes mellitus. Public Library of Science ONE, 3(11):e3753.

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