



## VITAMIN D DEFICIENCY AND ITS ASSOCIATION WITH DEVELOPEMENT OF PREECLAMPSIA IN PRIMIGRAVIDA

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### ABSTRACT

**AIM:** To study the association between Vitamin D deficiency and development of preeclampsia in primigravida and thereby natural and fetal outcome

**SUBJECTS & METHODS:** This is a prospective randomised control study at govt Kasturba Gandhi Hospital, MMC.

Vitamin D2 level were taken from all selected patients between the gestational age 12 to 20 weeks.

Predictive range of Vitamin D2 level for development of preeclampsia was identified based on parameter like age, body mass index and gestational age. The statistical significance of their correlations was assessed using two sided p value. A p value of <0.05 was considered stastically significant.

**RESULT:** In this study out of 200 patients 27 developed preeclampsia, 49 developed gestational hypertension, 124 were normal. According to chi square test there is associations between development of Vitamin D levels and association of preeclampsia there is significant correlation with development of preeclampsia when Vitamin D levels lees than 12

**CONCLUSION:** This study shows there is significant association between Vitamin D deficiency and development of preeclampsia. Also Vitamin D supplementation in pregnancy will be a effective means of preventing preeclampsia.

**KEYWORDS :** Vitamin D ,GHT (gestational hypertension), Preeclampsia

### INTRODUCTION

Hypertension represents the most common medical complication of pregnancy affecting around 10% of women. It is a leading cause of maternal and perinatal morbidity. Hence it is important to screen for development of hypertension in pregnancy and prevent the complications.

Abnormal placentation and impaired trophoblastic invasion is the trigerring factor. Vitamin 'D' has immunomodulatory property on placental tissue and its deficiency plays a important role in the pathogenesis of preeclampsia.

In normal pregnancy there is a increase in cardiac output and the systemic vascular resistance decreases due to peripheral vasodilatation. These changes are mediated by prostacyclin, nitric oxide and progesterone.

Diastolic blood pressure drops more than systolic, beginning around 8-10 weeks and reaches a nadir by 22-24 weeks and attains pre pregnancy level by term. Immediately after delivery, BP falls and then rises transiently for first few days due to vasomotor instability.

Preeclampsia is not only associated with maternal mortality and morbidity but also is strongly associated with fetal mortality and morbidity like FGR, LBW, preterm delivery, RDS and admission to NICU.

Hence diagnosis of the disease process early and timely correction helps to prevent disease progression and its complication. Screening and predictor of GHT / preeclampsia at early gestation helps to prevent mortality and morbidity significantly in both mother and fetus.

### Primary Prevention

Primary prevention is modification of risk factors and identify medical disease like chronic HT, DM, Renal disease, obesity (BMI 35Kg/m<sup>2</sup>) PCOS, APLA.

The early diagnosis of preeclampsia with timely referral for higher centre using PRECOG guidelines will help in active management and prevent the complication.

### Secondary Prevention

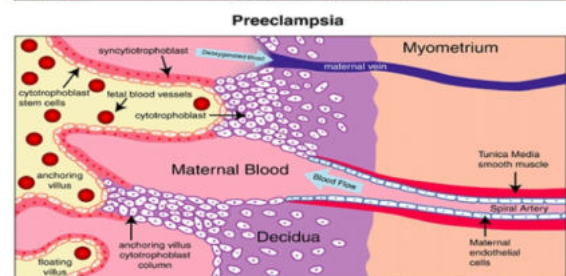
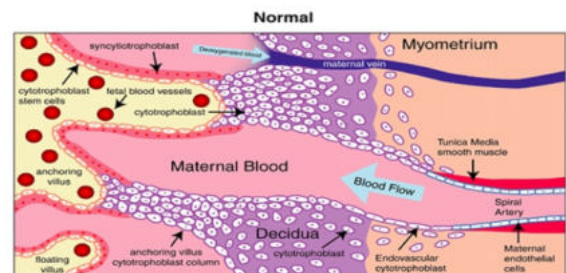
Early Identification and intervention and modification of the disease involve secondary prevention.

Screening methods with criteria including proper history, clinical evaluation and investigation have been used in secondary prevention of preeclampsia.

Vitamin D deficiency in early pregnancy affects placement by its immune modulatory effect.

Vitamin D helps is stimulating the activity of T-regulatory cells which are vital in supporting placental implantation through immune tolerance.

In deficiency state, there is increased production of inflammatory cytokines, TNF  $\alpha$ , and play a role in abnormal trophoblastic invasion.



Hence Vitamin D level estimation in early pregnancy and its association with development of preeclampsia is studied. Its role as a predictor for preeclampsia is also studied. Vitamin D levels are measured from gestational age 12 to 20 weeks and followed up until terms and postpartum for prediction of preeclampsia and its

complications.

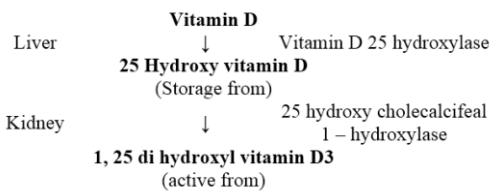
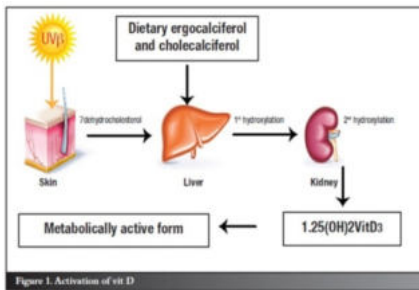
**VITAMIN D**

**The perfect screening test**

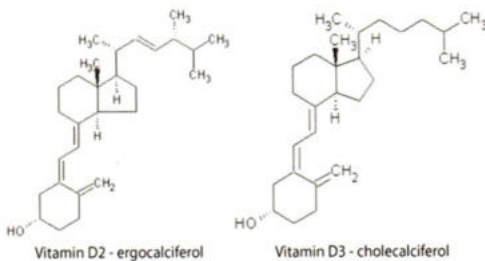
- Simple
- Rapid
- Non invasive
- Can be easily performed
- High sensitivity and predictivity.

Metabolism of Vitamin D

The active form of Vitamin D is 1, 25, dihydroxy vitamin D3, which is formed by two hydroxylation reactions.



**STRUCTURE OF VITAMIN D**



**VITAMIN D IN PREGNANCY**

The human endometrial decidua and the placenta synthesize 1,25 (OH)2D.

**The human placental trophoblast express**

- 1) Vitamin D receptor
  - 2) P450 cytochromes encoded by CYP 27B1 and CYP 24A1 gene.
  - 3) 1 alpha hydroxylase enzyme
- 25, (OH) Vit D is converted into active 1,25 (OH)2 Vit D in placenta by 1 alpha hydroxylase enzyme, which is encoded by CYP 27B1 gene.

**Function:**

- 1) Immunosuppression effect of 1, 25 (OH)2 Vit D allow for proper trophoblast invasion and successful implantation.
- 2) 1,25 (OH)2 D inhibits the IgG production, proliferation and differentiation of B and T lymphocytes.
- 3) It also inhibits the proliferations of Th1 Cell and limits the production of proinflammatory cytokines like interferon gamma, interleukin 2, tumor necrosis factor alpha.
- 4) It induce the cytokines of Th2 cells and produce anti-inflammatory cytokines like IL4, IL5, IL6, IL9, IL10, IL13.

Low Levels of Vit D3 levels impair the normal Th1 to Th2 cytokine

balance, with increased Th1 cytokine expression, affecting the immunological tolerance of embryo implantation and subsequent development of preeclampsia.

**METHODOLOGY**

Tests used to identify the Vitamin D levels measures 25 hydroxy Vitamin D values. It is the best indicator and its represents the summation of total cutaneous production of Vitamin D and the oral ingestion of either Vit D2 or Vit D3.

Half life is 10 days to 3 weeks compared to 1,25 (OH)2D which is only 4 to 6 hours.

Serum samples size of 2 to 4ml is drawn and quantification of Vitamin D is done by chemiluminescent assay.

**REFERENCE VALUES:**

Vitamin D Deficiency -	<12 ng/ml
Insufficiency -	12 to 30 ng/ml
Normal level -	>30 ng/ml

Serum sample are collected from 12 to 20 weeks. Follow up of antenatal mothers are done till third trimester and delivery by monitoring blood pressure and proteinuria.

Association between Vitamin D deficiency and development of pregnancy is studied and prediction of preeclampsia and GHT is done according to Vitamin D levels.

**MATERIALS AND METHODS**

Duration of study:

December 2015 to November 2016

Type:

Prospective randomized control study

Place:

Institute of obstetrics and gynaecology, Kasthurba Gandhi Hospital, Triplicane, Chennai.

**Inclusion Criteria:**

All normotensive, non-proteinuric primigravida attending antenatal clinic, between gestational age of 12 to 20 weeks are selected randomly.

**Exclusion Criteria:**

- Women with multiple pregnancy
- Essential hypertension
- Diabetes mellitus
- Chronic renal disease
- Molar pregnancy

The study started after getting ethical committee approval and after getting informed consent from all patients. Details of the patients were recorded as per the proforma. (Appendix A). Complete history of the patient and detailed clinical examination was done. Vitamin D2 level were taken from all selected patients between the gestational age 12 to 20 weeks.

All the patients were followed upto their third trimester and blood pressure and proteinuria evaluated and were allocated into normotensive, gestational hypertension and preeclampsia.

- Vitamin D2 level were estimated by automated CLIA method.
- Association between the development of preeclampsia and Vit D2 deficiency levels was studied.
- GHT and preeclampsia was classified based on low level of Vitamin D.
- Predictive range of Vitamin D2 level for development of preeclampsia was identified.
- Prediction of preeclampsia based on parameter like age, body

mass index and gestational age was also studied.

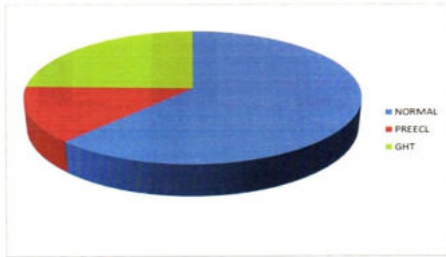
**Statistical Analysis**

Descriptive statistics were used to illustrate the study population. The statistical significance of these correlations was assessed using a two sided p-value.

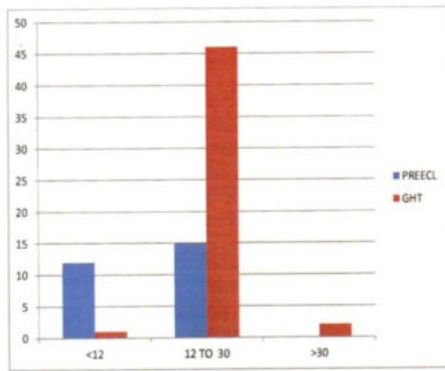
A p-value of <0.05 was considered as statistically significant. The chi square test was used to assess the statistical significance. Paired 'T' test was also used for the comparison of various descriptive within the groups. A commercially available computer softer were package, (Statistical package for the social sciences SPSS) version 17) was used for statistical analysis.

**RESULTS**

In this study, out of 200 patients, 27 patients developed preeclampsia, 49 developed GHT and 124 patients were normal.



**VITAMIN D LEVELS AND HYERTENSIVE DISORDERS**



In patient with VIT D level less than 12, 12 patients developed preeclampsia and one developed GHT.

In VIT D level between 12 and 30, 15 patients developed preeclampsia and 46 patients developed GHT.

In VIT D level more than 30, two patients developed GHT.

**AGE GROUP \* PREECL**

**GROUP 1 : AGE <20, GROUP 2 : 20-25, GROUP 3 : >25**

		Crosstab			
		PREECL		Total	
		0	1		
	Count	40	7	47	
AGE GROUP	1	% within AGE GROUP	85.1%	14.9%	100.0%
		% within PREECL	23.1%	25.9%	23.5%
		% of Total	20.0%	3.5%	23.5%
	Count	94	15	109	
	% within AGE GROUP	86.2%	13.8%	100.0%	
	% within PREECL	54.3%	55.6%	54.5%	
	% of Total	47.0%	7.5%	54.5%	
	Count	39	5	44	
	% within AGE GROUP	88.6%	11.4%	100.0%	
	% within PREECL	22.5%	18.5%	22.0%	
	% of Total	19.5%	2.5%	22.0%	

Total	Count	173	27	200
	% within AGE GROUP	86.5%	13.5%	100.0%
	% within PREECL	100.0%	100.0%	100.0%
	% of Total	86.5%	13.5%	100.0%

Chi Square = 0.257 p = 0.880 not significant

Out of 200 patients, 47 patients belong to age less than 20, 40 patients (85.1%) had no preeclampsia, 7 patients (14.9%) developed preeclampsia.

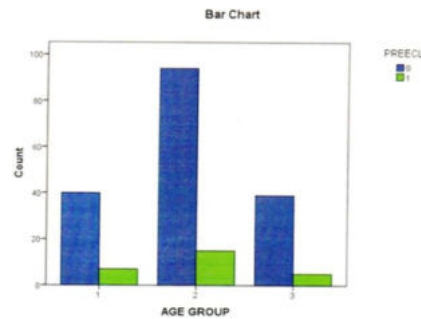
109 patients belong to age group 20 to 25 and 94 patients (86.2%) had no preeclampsia, 15 patients (13.8%) developed preeclampsia.

39 patients belong to age group above 25 and 39 patients (88.6%) has no preeclampsia, 5 patients (11.4%) had preeclampsia.

Chi-Square Tests			
	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.257a	2	.880
Likelihood Ratio	.263	2	.877
Linear-by-Linear Association	.238	1	.625
N of Valid Cases	200		

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 5.94.

According to chi square test correlation between age and development of preeclampsia have no significance.



**VITD2 \* PREECL**

**GROUP 1 : VIT D2 <12, GROUP 2 : 12-30, GROUP 3 : >30**

		Crosstab			
		PREECL		Total	
		0	1		
	Count	1	12	13	
VITD2	1	% within VITD2 C	7.7%	92.3%	100.0%
		% within PREECL	.6%	44.4%	6.5%
		% of Total	.5%	6.0%	6.5%
	Count	75	15	90	
	% within VITD2	83.3%	16.7%	100.0%	
	% within PREECL	43.4%	55.6%	45.0%	
	% of Total	37.5%	7.5%	45.0%	
	Count	97	0	97	
	% within VITD2	100.0%	.0%	100.0%	
	% within PREECL	56.1%	.0%	48.5%	
	% of Total	48.5%	.0%	48.5%	
Total	Count	173	27	200	
	% within VITD2	86.5%	13.5%	100.0%	
	% within PREECL	100.0%	100.0%	100.0%	
	% of Total	86.5%	13.5%	100.0%	

Chi Square = 85.052 p = 0.000 < 0.001 significant.

Out of 200 patients, 27 patient developed preeclampsia, with vit d levels less than 12, 12 patients (92.3%) had preeclampsia, 1 patient (7.7%) had no preeclampsia. With vit d level between 12 to 30, 15 patients (16.7%) developed preeclampsia and 75 patients (83.3%)

had no preeclampsia. With levels more than 30, no preeclampsia developed.

Chi-Square Tests			
	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	85.052 <sup>a</sup>	2	.000
Likelihood Ratio	70.161	2	.000
Linear-by-Linear Association	62.121	1	.000
No of Valid Cases	200		

a. 1 cells (16.7%) have expected count less than 5.  
The minimum expected count is 1.76.

According to chi square tests there is association between development of Vitamin D levels and association of preeclampsia. There is significant correlation (p value < 0.000) with development of preeclampsia when vit D level less than 12.

**VITD2\*GHT**

Out of 200 patients, 46 patients developed GHT. In group 1, out of 13 patients 1 patient (7.7%) developed GHT. In group 2 out of 90 patients 46 patients (51.1%) has GHT and 44 patient (48.9%) had no GHT. In group 3 out of 97 patient, 2 patients (2.1%) developed GHT and 95 (97.9%) had no GHT.

According to chi square test there is significant association between development of GHT and low levels of Vitamin D. p value = 0.000.

**T-Test**

**PREECLAMPSIA AND AGE (C.I. 95)**

**GROUP 1 – PREECLAMPSIA GROUP 2-NO PREECL**

Group Statistics						
	PREECL	N	Mean	Std. Deviation	Std. Error Mean	P
AGE	1	27	23.26	3.046	.586	.928
	0	173	23.32	3.129	.238	.927

Out of 200 patients, 27 belong to group 1. The minimum age for development of preeclampsia is 19 and the maximum age is 30. While in group 2, no preeclampsia, the minimum age is 19 and the maximum age is 31.

The mean age for development of preeclampsia in this study is 23.26 The mean age for no preeclampsia in this study is 23.32. There is no significant association between age and development of preeclampsia in this study.

**T-Test**

**PREECLAMPSIA AND G.A (C.I. 95)**

**GROUP 1 – PREECLAMPSIA GROUP 2 – NO PREECL**

Group Statistics						
	PREECL	N	Mean	Std. Deviation	Std. Error Mean	P
G.A (Weeks)	1	27	15.96	2.328	.448	.392
	0	173	16.40	2.472	.188	.376

Gestational age (wks)

	minimum	Maximum
1	12	20
0	12	20

In both groups, the minimum and maximum gestational age were 12 and 20 weeks respectively.

The mean G.A for development of preeclampsia is 15.96

The mean G.A for no preeclampsia 16.40

In this study there is no significant association between gestational age and preeclampsia.

**T-Test**

**PREECLAMPSIA AND BMI (C.I. 95)**

**GROUP 1 – PREECLAMPSIA GROUP 2 – NO PREECL**

Group Statistics						
	PREECL	N	Mean	Std. Deviation	Std. Error Mean	P
G.A (Weeks)	1	27	26.6493	2.64596	.50922	0.002
	0	173	25.0346	2.39876	.18237	0.005

**BMI**

	minimum	Maximum
1	20	30
0	19	34

Out of 27 preeclampsia patients, the lower level of BMI was 20 and higher level of BMI was 30. In no preeclampsia patient the lower level of BMI was 19 and higher level of BMI was 34.

The mean BMI in preeclampsia group was 26.

The mean BMI in no preeclampsia group was 25.03.

There is a significant association between BMI and preeclampsia.

	minimum	Maximum
1	8	20
0	20	40

The minimum VIT D2 level in preeclampsia group was 8 ng/ml and the maximum level was found to be 20 ng/ml. In no preeclampsia group, the minimum and maximum level of VIT D2 level was 20 and 40 ng/ml respectively.

The mean VIT D2 level was 13.885 in preeclampsia group.

The mean VIT D2 level in no preeclampsia group was 29.466

There is significant association between VIT D2 level and development of preeclampsia.

**T-Test**

**PREECLAMPSIA AND BIRTH WEIGHT (C.I. 95)**

**GROUP 1 – PREECLAMPSIA GROUP 2 – NO PREECL**

Group Statistics						
	PREECL	N	Mean	Std. Deviation	Std. Error Mean	P
B.WT	1	27	2.474	.4280	.0824	.000
	0	173	2.900	.5921	.0450	.000

**BIRTHWEIGHT**

	minimum	Maximum
1	1.8	3.3
0	1.775	4.15

The minimum birth weight in preeclampsia group is 1.8kg and the maximum birth weight is 3.3kg. In no preeclampsia group, the minimum birth weight was 1.775 and maximum birth weight was 4.1kg.

There is a significant association between birth weight and preeclampsia.

**DISCUSSION**

The main aim was to study the association between Vitamin D deficiency and development of preeclampsia in primigravida.

In this study, out of 200 patients, 27 patients developed preeclampsia and 49 developed GHT.

In patient with Vitamin D deficiency (<12ng/ml), 12 patients had preeclampsia and one developed GHT.

In vitamin D insufficient group (12 to 30 ng/ml), 15 patients developed preeclampsia and 46 developed GHT.

In Vitamin D sufficient group (>30 ng/ml) two patients developed



**GHT.**

When the level of Vitamin D and development of preeclampsia was analysed, there is a strong association with 100% sensitivity and 85.5% specificity. The p value being <0.0001, the disease prevalence was found to be 13.5%.

The cut off value for development of preeclampsia based on this study is  $\leq 23.5$  ng/ml.

A prospective cohort study conducted in Canada measuring vitamin D levels at 12 to 18 weeks and at 24 to 26 weeks of gestation and development of preeclampsia. 39% were Vitamin D deficient (<50nmol/L). Vitamin D level at 24 to 26 weeks was significantly lower in women who developed preeclampsia than those who didn't develop. (mena +\_ SD 48.9+\_ 16.8 vs 57.0+\_ 19.1 nmol/L) p value = 0.03. (1 ng = 2.496 nmol/L)

In another nested case control study from two prospective Canadian cohorts, maternal serum Vitamin D in early pregnancy and subsequent development of preeclampsia was studied. Results showed that patient who developed preeclampsia had a significantly lower concentration of vitamin D at 14 weeks compared to those who in control group. P value <0.0001.

Study comparing Vitamin D levels and caesarean section rates by alonso et al., showed that no difference in relation to low vitamin D levels. In another study, by Merewood et al., showed a fourfold increase in primary caesarean rate with low vitamin D levels (<37.5 nmol/L). In our study, there is a significant association between low vitamin D levels who developed preeclampsia and caesarean delivery. (preeclampsia group p value 0.002) In GHT group there was no significant association.

When variables like age, gestational age, BMI APGAR was compared, in preeclampsia group there was a significant association with BMI but no association with age, gestational age and apgar.

In GHT group, there was a significant association with age and BMI, while no association with gestational age and apgar.

Recommendation from UK chief medical officer and NICE guidance (48) state that all pregnant women and lactating mothers should take 400 U of Vitamin D women at High risk for preeclampsia are advised to take at least 800 U a day combined with calcium.

Women who are deficient in Vitamin D, 20000 IU of cholecalciferol per week or 10000 IU of ergocalciferol twice a week, for 4 to 6 weeks, followed by supplementation.

Hence measurement of Vitamin D levels in early pregnancy helps in early diagnosis of preeclampsia and GHT and prevents its complications.

**CONCLUSION**

- 1) This study shows that there is a significant association between Vitamin D deficiency and development of preeclampsia. The cut off level of Vitamin D being 23.5 ng/ml.
- 2) Also there is association between low vitamin D levels and development of GHT.
- 3) In preeclampsia group there is a significant association between Vitamin D, BMI, low birth weight whereas in GHT group maternal age also had a significant association.
- 4) Hence when compared to other biochemical parameters, vitamin D levels have more sensitivity and specificity.
- 5) Vitamin D deficiency remains a independent risk factor for development of preeclampsia. Extensive researches are being carried out to know the exact mechanism of action of vitamin D in pathogenesis of preeclampsia.
- 6) Also vitamin D supplementation in pregnancy will be a effective mean of preventing preeclampsia.

**References:**

- 1) Ian Donalds practical problems, 7th edition, 2014, chapter 8, hypertensive disorders.
- 2) McCowan LME, Buist RG, North RA et al. Perinatal morbidity in chronic hypertension. Br. J. OBG 1996;103:123-9
- 3) Brown MA, Reiter L, Smith B, et al, Measuring blood pressure in pregnant women; a comparison of direct and indirect methods. Am. J. OBG 1994; 171; 661-7
- 4) Vitamin D and relation of placental inflammation. Nancy Q Liu, Ambet T Kaplan, Venu lagishetty, journal of immunology.
- 5) Maternal Vitamin D status and risk of preeclampsia, systematic review and meta analysis, Marjan Tabesh, Amir salehi abargoeul, maryam tabesh, and ahmad esmail zadeh. Isfahan university of medical science, iran J clin endocrine metab 98; 3165-73; 2013
- 6) Broughton pipkin F, Risk factors for preeclampsia. N Engl J Med 2001, 344; 925-6. An excellent editorial on the multiple risk factors for development of preeclampsia.
- 7) Roberts J endothelial dysfunction in preeclampsia, Semin reproductive endocrinology 1998 16;5-15
- 8) Chambers JC, Fusi L, Malik S et al, Association of maternal endothelial dysfunction with preeclampsia. JAM 2001;258;1607-12
- 9) Haggerty CL, Seifer Me, Tang G et al, second trimester anti angiogenic proteins and preeclampsia, Pregnancy hypertension 2012 Apr 1;2(2)158-63
- 10) Redman CWG, Sargent L, Immunology of abnormal pregnancy and preeclampsia, Chesley's hypertensive disorders of pregnancy 3rd edition p129
- 11) Roberts JM, hubel CA, Is oxidation stress a link in the two stage model of preeclampsia? Lancer 1999 54;788-9
- 12) Hefmeyer GJ, Lawrence Ta, Atallah AN, duley L., Calcium Supplementation during pregnancy for preventing hypertensive disorders, Cochrane database, syst revv 2010 aug 4(8) CD001059
- 13) Chappell LC, Seed PT, Briley AJ, et al., Effects of antioxidants on the occurrence of preeclampsia in women at increased risk a randomised trial, LANCET 1999, 359; 810-16
- 14) Caritis S, Sibai BM, Hauth J et al, Low dose aspirin to prevent preeclampsia in women at risk, N Engl. J med 1998-338 701-5
- 15) CLASP, a randomised trial of low dose aspirin for the prevention and treatment of preeclampsia among 9364 pregnant women Lancet 1994 mar 12; 343; 619-29