



**ORIGINAL RESEARCH PAPER**

**Biochemistry**

**VITAMIN D STATUS AND ITS ASSOCIATION WITH SERUM BETA HCG AND SERUM AFP IN SECOND TRIMESTER OF PREGNANCY FOR PREDICTING PREECLAMPSIA**

**KEY WORDS:** SERUM  $\beta$  HCG, AFP AND VIT D, PRE-ECLAMPSIA

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**ABSTRACT**

**Objectives:** Investigate the association of second trimester -HCG, AFP, Vitamin D concentration with subsequent development of Preeclampsia.

**Material & Methods:**  $\beta$ -HCG, AFP, Vitamin D, Waist circumference were measured in 200 Pregnant women in 13th-20th wks. of gestation fulfilling the inclusions criteria These tests were measured by CLIA method by Advia Centaur XP machine.

**Results:** Serum vitamin D levels (second trimester) in pregnancies with preeclampsia are found to be significantly lower than those in pregnancies with normal outcome (p value <0.001). Percentage of cases with high serum AFP levels in second trimester (MOM >2) is found to be significantly higher in the pregnancies with preeclampsia than those in pregnancies with normal outcome (p value <0.001) Percentage of preeclampsia (as outcome) is significantly higher in cases with high serum  $\beta$ HCG (MOM >2) than those with low serum  $\beta$ HCG (MOM <2) in second trimester (p value <0.001). There is significant positive correlation of serum  $\beta$ HCG with serum AFP levels (second trimester) in pregnancies with preeclampsia.

**Conclusion:** Our study shows that serum  $\beta$ -HCG and AFP levels in second trimester of pregnancy has predictive value for developing Preeclampsia. Also, vitamin D may have a decisive role in predicting pre-eclampsia and has a potential to emerge as an independent marker of developing pre-eclampsia.

**INTRODUCTION**

Pregnancy induced hypertension (PIH) is a unique disease seen only in pregnancy affecting 5-10% of all pregnant woman (1). In spite of improvement in maternal and neonatal care, PIH and its sequel are a dreaded complication of pregnancy. If prediction becomes possible, prevention will follow easily. World health organisation (WHO) systemically reviews maternal mortality worldwide and as high as 16% of maternal death were reported due to maternal hypertensive disorders. This proportion is greater than the three leading causes of maternal death that include haemorrhage, abortion and sepsis. Most of these hypertensive related deaths are preventable (2,3). Related maternal complications are pre-eclampsia, eclampsia, disseminated intravascular coagulation, intracranial bleed, pulmonary oedema, renal failure, heart failure, abruption placentae, and death (4,5,6) and foetal complications are preterm birth and low birth weight, IUGR. So PIH increases perinatal mortality and morbidity (8).

Preeclampsia is defined as new onset hypertension  $\geq 140/90$  mm Hg after 20 weeks of pregnancy with proteinuria  $\geq 300$ mg/24 hours or  $\geq 1+$  dipstick. It is a pregnancy specific syndrome that can affect virtually all organ of the body (1,8). Pre-eclampsia is responsible in 25% of all foetal growth retardation and 15% preterm birth in developed countries. The incidence of pre-eclampsia in India is about 8-10%, and maternal mortality about 8%. Pre-eclampsia is more common below the age of 25 years. (9) Pre-eclampsia complicates about 2-8% of all pregnancies and the syndrome results in more than 63,000 maternal deaths every year worldwide (10,11). The maternal mortality rate is highest in low- and middle-income countries but pre-eclampsia is also a potentially life-threatening condition in high income countries. (12)

In normal pregnancy uterine spiral arterioles undergo extensive remodelling as they are invaded by endovascular trophoblasts which transform them into low resistance high flow system (13). Etiopathogenesis though not very clear, in

pre-eclampsia incomplete trophoblastic invasion (up to decidua) leads to high resistance low flow uteroplacental circulation (ischaemia). As there is reactive hyperplasia of the trophoblastic cells in pre-eclampsia which secretes the glycoprotein  $\beta$ -HCG and as also there is trophoblastic dysfunction in pre-eclampsia, which is expected to influence the serum AFP levels (secreted from foetal gastrointestinal tract. (14). But till date the results are inconsistent and contradictory.

Hsu C-D, Chan DW, Sablok at al. (16) showed that elevated serum human chorionic gonadotropin as evidence of secretory response in severe preeclampsia. Kebbuck et al. studied 610 pregnant women in second trimester, grouping them according to the multiple of median (MOM) of beta HCG and found that women with elevated second trimester human chorionic gonadotropin levels (>2 MOM) are at increased risk for preeclampsia (Odds ratio 5.93, 95 % confidence interval 1.97 to 15.88).

Waller DK, Lustig LS, Cunningham GC, et al studied the mean values for HCG and AFP were significantly higher in women with subsequent preeclampsia (p < 0.0003 and p < 0.03, respectively).

Vitamin D also has been implicated in cases of pre-eclampsia, but results are not consistent. Tabash et al (2013) stated that, systematic review and meta-analysis of eight published observational studies also revealed a significant relationship between Vitamin D deficiency and increased risk of Pre-eclampsia. Sabol at al. (2017) (16)) also stated, Serum samples of 792 high risk women at 12-26 weeks of gestation were assayed for 25-hydroxyvitamin D in a multicentre clinical trial of low-dose aspirin for the prevention of Pre-eclampsia. They drew a conclusion that maternal Vitamin D levels in the second pregnancy trimester correlated with the risk of SGA among all women and in the subgroups of white and non-obese women. Several studies were conducted by various researchers regarding association of serum vit-D, serum  $\beta$ -HCG and serum AFP levels, with pre-eclampsia but results are

still inconsistent and inconclusive. It is believed that early placental dysfunction, which influences maternal serum AFP and -HCG, can lead to placental hypoperfusion and a maternal endothelial reaction which results in foetal growth restriction, preeclampsia, and even foetal death. Attempts have been made to identify early markers of faulty placentation, impaired placental perfusion, endothelial cell activation and dysfunction, and activation of coagulation. For the most, these have resulted in testing strategies with poor sensitivity and with poor positive-predictive value for Preeclampsia (20,21). A combination of two or more marker improves the possibility of prediction. In this study serum -HCG, AFP and Vitamin D are used as markers to predict the disease and its severity. Therefore, this study is designed to evaluate the serum Vit-D, serum -HCG and serum AFP levels to find out any association between these parameters and onset of pre-eclampsia in 2<sup>nd</sup> trimester of pregnancy.

**MATERIALS AND METHODS:**

The study was performed at the Department of Biochemistry, Medical college and Hospital, Kolkata and Department of Obstetrics and Gynaecology, Medical college, Kolkata. All pregnant women attending antenatal clinic during 13<sup>th</sup> -20<sup>th</sup> of pregnancy between 1<sup>st</sup> April 2016 to 30<sup>th</sup> March 2017. This is a hospital based prospective study.

**Inclusion Criteria:** All pregnant women attending antenatal clinic during 13<sup>th</sup> -20<sup>th</sup> wks. of gestation with normal blood pressure

**Exclusion Criteria:** All nonpregnant women, women having chronic HTN, renal disease, GDM, cardiovascular disease, any other medical disorder, multiple pregnancy, obese women, anomalous foetus diagnosed by USG, previous history of preeclampsia or eclampsia etc. It is a hospital based prospective study. Among 200 pregnant women fulfilling inclusion and exclusion criteria are selected as sample.

Serum beta HCG by Chemiluminescence immunoassay (CLIA), AFP and Vitamin D by Chemiluminescence immunoassay (CLIA)

**N.DATA ANALYSIS**

Categorical variables are expressed as number of patients and percentage of patients and compared across the groups (Developed and Not developed preeclampsia) using Pearson's Chi Square test for Independence of Attributes. Continuous variables are expressed as Mean± Standard Deviation and compared across the groups using Unpaired t test. The statistical software SPSS version 20 has been used for the analysis. An alpha level of 5% has been taken, i.e. if any p value is less than 0.05 it has been considered as significant.

**Statistical Analysis:** - Data will be entered in Microsoft Excel software 2007 and statistical analysis will be done in SPSS software version 20 with suitable statistical tests.

**C.ETHICAL CLEARENCE:** This study was cleared by Institutional Ethics committee as per ref no. MC/ KOL/ IEC/ NON-SPON/168/11-2015DT 28/11/15.

**Human Resources:**

1.Faculty 2.Colleagues 3.lab technicians.

**P. FINANCIAL SUPPORT:** No financial aid required as investigation procedures performed within departmental resources.

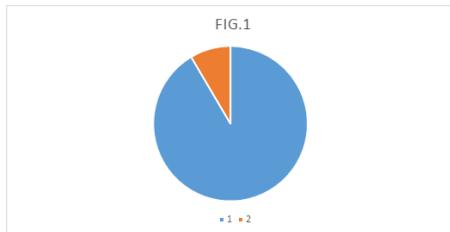
**Biochemical Parameters & Method**

- 1) serum 25 (OH) vitamin D level = CLIA Method (Adevia Centaur XP)
- 2) serum B-HCG level. = CLIA Method (Adevia Centaur XP)
- 3) serum AFP level. = CLIA Method (Adevia Centaur XP)

**RESULT AND ANALYSIS**

**TABLE 1. Incidence of preeclampsia in the study population:**

|                           | Number | Percentage |
|---------------------------|--------|------------|
| Outcome: pre-eclampsia    | 17     | 8.5        |
| Outcome: no pre-eclampsia | 183    | 91.5       |

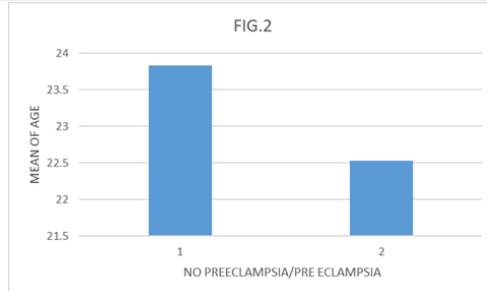


**FIG 1: Pie chart Showing incidence of preeclampsia**

Table and chart showing incidence of pre-eclampsia in the study population which is 8.5%.

**Table 2: Comparison of age with development of pre-eclampsia:**

|              | No pre-eclampsia | Pre-eclampsia | t stat | P value |
|--------------|------------------|---------------|--------|---------|
|              | Mean ± S.D       | Mean ± S.D    |        |         |
| Age in years | 23.83 ± 4.04     | 22.52 ± 3.04  | 1.64   | 0.06    |

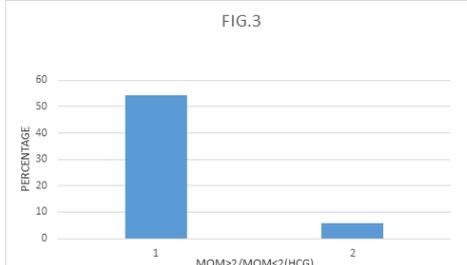


**FIG.2 Bar diagram showing of mean age with preeclampsia and normal cases.**

Table and figure are showing comparison of mean of age with development of pre-eclampsia. It is not significant (p value 0.06).

**Table 3: Showing frequency distribution of preeclampsia according to MOM value of serum HCG (second trimester):**

| Parameter                   | βHCG/MOM>2 (n=11) | βHCG/MOM<2 (n=189) | P value |
|-----------------------------|-------------------|--------------------|---------|
| Outcome: preeclampsia (n/%) | 6(54.5%)          | 11(5.82%)          | <0.001  |

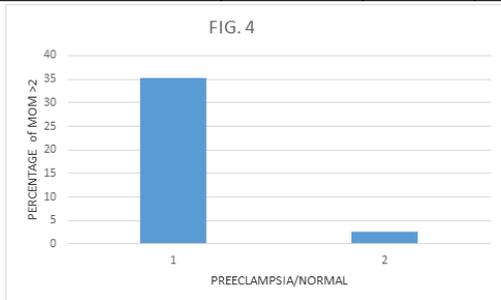


**FIG 3. Bar diagram showing percentage of preeclampsia with MOM values.**

Percentage of preeclampsia (as outcome) is significantly higher in cases with high serum βHCG (MOM>2) than those with low serum βHCG (MOM<2) in second trimester (p value <0.001).

**TABLE 4: Showing comparison of cases with high serum βHCG levels in second trimester (MOM>2) between pregnancies with pre-eclampsia and normal outcome by Chi-squared test:**

| Parameter to be compared | Outcome: pre-eclampsia (n=17) | Outcome: normal (n=183) | P value |
|--------------------------|-------------------------------|-------------------------|---------|
| Cases with MOM>2(n/%)    | 6(35.29%)                     | 5(2.73%)                | <0.001  |



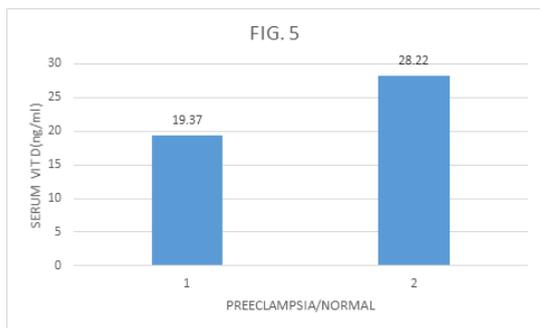
**FIG.4 Bar diagram showing percentage of >2MOM between preeclampsia and normal outcome.**

Percentage of cases with high serum βHCG levels in second trimester (MOM>2) is found to be significantly higher in the pregnancies with preeclampsia than those in pregnancies with normal outcome (p value<0.001).

**Table 5: Showing comparison of serum vitamin D levels between pregnancies with preeclampsia and normal outcome by independent t-test:**

| parameter    | PREEC (MEAN±S.E) | NORMAL (MEAN±S.E) | t value | P value |
|--------------|------------------|-------------------|---------|---------|
| Vit D(ng/ml) | 19.37±0.42       | 28.22±1.15        | 4.69    | <0.001  |

Serum vitamin D levels (second trimester) in pregnancies with preeclampsia are found to be significantly lower than those in pregnancies with normal outcome (p value<0.001).



**FIG.5 Bar diagram showing Vit D level between preeclampsia and normal outcome.**

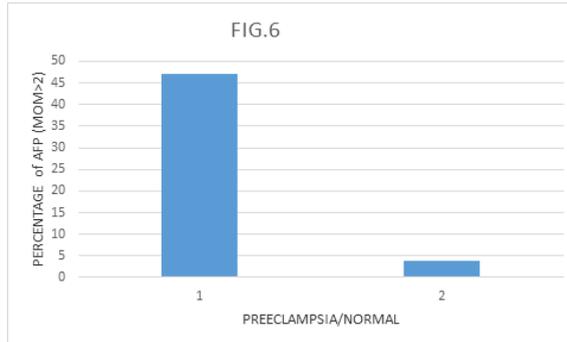
**Table 6: Showing comparison of cases with high serum AFP levels in second trimester (MOM>2) between pregnancies with preeclampsia and normal outcome by chi-squared test:**

| Parameter to be compared | Outcome: pre-eclampsia n=17 | Outcome: normal n=183 | p Value |
|--------------------------|-----------------------------|-----------------------|---------|
| Cases with MOM>2 (n / %) | 8 (47.06%)                  | 7(3.82%)              | <0.001  |

**Table: 8 Sensitivity, Specificity and Predictive values of βHCG (second trimester) in prediction of development of preeclampsia:**

| Parameter β-HCG | True pose | True neg | False positive | False negative | sensitivity | specificity | +vet predictive value | -vet predictive value |
|-----------------|-----------|----------|----------------|----------------|-------------|-------------|-----------------------|-----------------------|
| MOM>2           | 6         | 178      | 5              | 11             | 35.3        | 97.2        | 54.5                  | 94.2                  |

Sensitivity, specificity, positive predictive value, negative predictive value of MOM value of HCG are 35.3, 97.2, 54.5, 94.2 (ref. ROC curve above), when cut off MOM βHCG is 2.

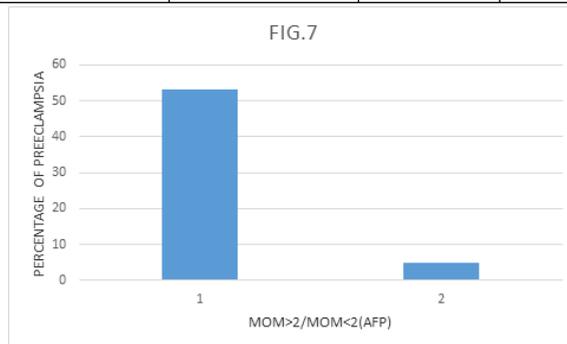


**FIG.6 Bar diagram showing percentage of serum AFP levels between preeclampsia and normal pregnancy.**

Percentage of cases with high serum AFP levels in second trimester (MOM>2) is found to be significantly higher in the pregnancies with preeclampsia than those in pregnancies with normal outcome (p value<0.001)

**Table 7: Frequency distribution of preeclampsia according to MOM value of serum AFP (second trimester):**

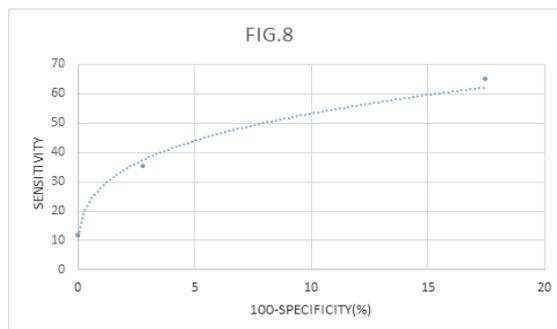
| parameter                   | AFP/MOM>2 (n=15) | AFP/MOM<2 (n=185) | P value |
|-----------------------------|------------------|-------------------|---------|
| Outcome: pre-eclampsia(n/%) | 8(53.3%)         | 9(4.86%)          | <0.001  |



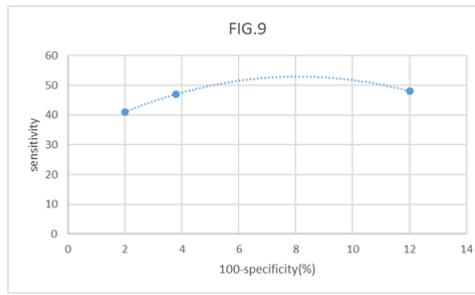
**FIG.7 Bar showing percentage of preeclampsia depending upon serum AFP MOM values.**

Percentage of preeclampsia (as outcome) is significantly higher in cases with high serum AFP(MOM>2) than those with low serum AFP (second trimester) and p value is <0.001.

**FIG.8: ROC Curve of MOM value of maternal βHCG**



**FIG.9: ROC Curve of MOM value of maternal AFP:**



**Table 9: Sensitivity, specificity and predictive values of serum AFP (second trimester) in prediction of development of preeclampsia:**

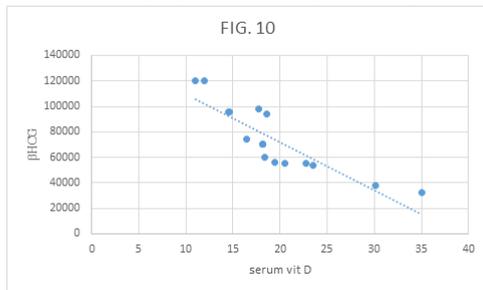
| Parameter of serum AFP | True +ve | True - +ve | False +ve | False - +ve | sensitivity | specificity | +ve predictive value | -ve predictive value |
|------------------------|----------|------------|-----------|-------------|-------------|-------------|----------------------|----------------------|
| MOM>2                  | 8        | 176        | 7         | 9           | 47.05       | 96.2        | 53.3                 | 95.13                |

Sensitivity, specificity, positive predictive value, negative predictive value of MOM value of AFP is 47.05, 96.2, 53.3, 95.3 (ROC curve above), when cut off of MOM AFP is 2.

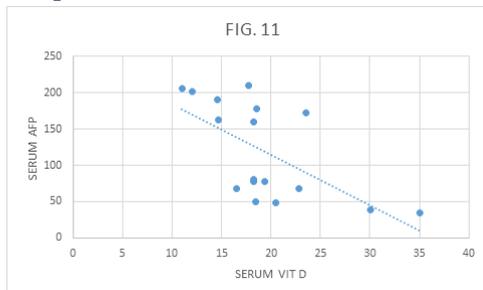
**Table 10: Pearson's correlation of serum vitamin D levels with serum βHCG and serum AFP levels in pregnancies with preeclampsia:**

| Parameter                  | Serum βHCG | Serum AFP |
|----------------------------|------------|-----------|
| Correlation coefficient(r) | -0.80      | -0.62     |
| P value                    | <0.001     | <0.05     |

The above table showed there is significant negative correlation of serum vitamin D levels with serum βHCG and serum AFP levels in pregnancies with preeclampsia



**Figure 10: Scatter diagram showing distribution of serum vitamin D and serum βHCG levels in pregnancies with preeclampsia.**

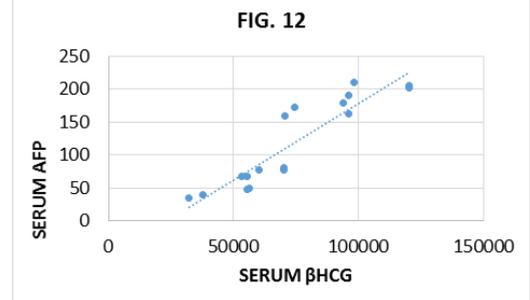


**Figure 11: Scatter diagram showing distribution of serum vitamin D and serum AFP level (second trimester) in pregnancies with preeclampsia.**

**Table 11: Pearson's correlation of serum βHCG levels with serum AFP levels in pregnancies with preeclampsia:**

|                                      | Serum AFP |
|--------------------------------------|-----------|
| Pearson's correlation coefficient(r) | 0.91      |
| P value                              | <0.05     |

The above table showed that there is significant positive correlation of serum βHCG with serum AFP levels (second trimester) in pregnancies with preeclampsia.



**Figure 12: Scatter diagram showing distribution of serum βHCG levels with serum AFP levels (second trimester) in pregnancies with preeclampsia.**

**DISCUSSION**

Amongst 200 pregnant mothers included in our study as per inclusion and exclusion criteria for study parameters estimation in their second trimester, 17 mothers developed preeclampsia (8.5%) (Table 1, Fig1).

Sensitivity, specificity, positive predictive value, negative predictive value of MOM value of HCG are 35.3, 97.2, 54.5, 94.2 (ref. ROC curve above), when cut off MOM HCG is 2 and this result is more or less consistent with the result of SMS Medical College, Jaipur (2008-2009), where positive predictive value of the test is 83.99%

in our study, sensitivity, specificity, positive predictive value, negative predictive value of MOM value of AFP is 47.05, 96.2, 53.3, 95.3 (ROC curve above) respectively, when cut off of MOM AFP is 2. Ratty et al in their study stated that high mid-trimester serum AFP values may help in the prediction of severe pre-eclampsia.

Another possible explanation may be inadequate trophoblastic remodelling of the maternal uterine vasculature, with an absence of normal physiologic changes in the spiral arteries leading to placental hypoxia and HCG overproduction.

Unexplained MS AFP elevations in such cases are most likely the result of transplacental leakage of AFP from the foetal to the maternal circulation. (15) This may be due to functional or structural abnormalities of the placenta providing an increased area of transport or due to a defective endothelial barrier.

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

Therefore, vitamin D is likely to play an important role in the immune and cardiovascular changes necessary for a healthy pregnancy, highlighting the need to ensure that the existing public-health recommendations for vitamin D intake by pregnant mothers are endorsed. The table (10) showed there is significant negative correlation of serum vitamin D levels with serum  $\beta$ HCG ( $<0.001$ ) and serum AFP ( $<0.05$ ) levels in pregnancies with preeclampsia. In order to establish causation, randomized controlled intervention trials need to be undertaken.

Our study is to discuss about detecting serum  $\beta$ -HCG and AFP levels in second trimester of pregnancy as a predictor of preeclampsia. The table (11) showed that there is significant positive correlation of serum  $\beta$ HCG with serum AFP levels (second trimester) in pregnancies with preeclampsia ( $<0.05$ ). Unexplained MSAFP elevations in such cases are most likely the result of transplacental leakage of AFP from the foetal to the maternal circulation. (19) This may be due to functional or structural abnormalities of the placenta providing an increased area of transport or due to a defective endothelial barrier. Indeed, placental and cord anomalies have been found in association with increased MSAFP level in several studies. This may suggest that early placental pathology permits a more rapid diffusion of AFP from the fetoplacental compartment to the maternal compartment. (12,13) Given that the underlying pathophysiologic reason of many obstetric complications involves placental dysfunction at some level, it would be logical to claim that pregnancies complicated by elevated MSAFP and/or  $\beta$ HCG levels could have higher rates of adverse events (10) like preeclampsia and also, about detecting second trimester serum vitamin D status and its correlation with serum HCG and AFP levels in preeclampsia cases for the purpose of investigating the extent of justification of using it as an independent marker of developing preeclampsia.

#### Limitation

The main limitation of this study was that the study population was small. Therefore, the results of this study did not stimulate many other epidemiological studies conducted elsewhere.

- Besides in this study primipara and multipara are not separately dealt with.
- The study would be able to establish serum Vitamin D as an independent marker of preeclampsia, if baseline /pre conceptional Vit-D level is measured.
- Since this is a hospital-based study, the results were not a true reflection of the preeclampsia incidence and prevalence in the community.

#### CONCLUSION

Our study shows that serum  $\beta$ -HCG and AFP levels in second trimester of pregnancy has predictive value for developing Preeclampsia. Also, vitamin D may have a decisive role in predicting pre-eclampsia and has a potential to emerge as an independent marker of developing pre-eclampsia.

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