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ORIGINAL ARTICLE

SERUM LEVEL OF β-hCG IN NORMOTENSIVE AND PREECLAMPTIC PREGNANT WOMEN IN TIKUR ANBESSA SPECIALIZED HOSPITAL

Abebaw Nigussie, MSc[1], Tewabech Zewde, MSc[1], Yirgu G/Hiwot. MD[2]

ABSTRACT

Introduction: *Preeclampsia remains a major cause of prenatal morbidity and mortality worldwide. It is considered to be a likely trophoblastic disorder and since β-hCG is secreted by trophoblastic cells, its serum level may be essential in diagnosing preeclampsia. Hence the aim of this study was to determine serum levels of β-hCG in normotensive and preeclamptic pregnant women.*

Methods: *A case control study was conducted in TikurAnbessa Specialized Hospital from June, 2013 to March, 2014. Cases were preeclamptic pregnant women and controls were normotensive pregnant women. Serum levels of β-hCG were determined by an electrochemiluminescence immunoassay, , and compared among the patient groups. Binary logistic regression and bivariate correlation analysis were done to determine associations between β-hCGother obstetric factors.*

Results: *A total of 76 pregnant women (38 cases and 38 controls) were included in this study. Mean serum levels of β-hCG in the case group was 34439.2+/- 28223.67mIU/ml and significantly higher that of the control group,20582 +/- 17588.31. Serum levels of β-hCG in the case group was positively correlated with mean arterial blood pressure and negatively correlated with maternal age, gestational age and parity. Witha cutoff point of 12,953mIU/ml, the sensitivity and specificity of maternal serum level of β-hCG as a diagnostic test for preeclampsia was 76.3% and 52.6% respectively.*

Conclusions: *In this study, maternal serum level of β-hCGand family history of preeclampsia were significantly associated preeclampsia.*

Key words: *Preeclampsia, normotensive, human chorionic gonadotropin.*

INTRODUCTION

Over half a million women die each year from pregnancy related causes, 99% from low and middle income countries. Overall, 10 to 15% of direct maternal deaths are associated with preeclampsia and eclampsia (1). The incidence of preeclampsia in developing countries (~ 2.8% of live births) is seven times greater than in developed countries (~ 0.4% of live births) (2).

Preeclampsia is a pregnancy-specific syndrome which usually occurs after 20 weeks of gestation. It is determined by increased blood pressure greater than 140 mmHg systolic or 90mmHg diastolic accompanied by proteinuria (≥300mg/24hr or ≥+1 dipstick). Preeclampsia may be subdivided into mild preeclampsia, BP of 140/90 mm Hg or higher with proteinuria of 0.3 to 3 g/day, and severe preeclampsia, BP greater than 160/110 mm Hg, proteinuria of 3 to 5 g/day and additional adverse features (3). Preeclampsia may be early onset, if it occurs before 34 weeks of gestation, or late onset, if it occurs after 34 weeks of gestation (4).

Though the etiology of preeclampsia remains unknown, there are a number of hypotheses including placental, immunological and genetic origins. Preeclampsia has been observed only in the presence of the placenta and its resolution begins with the removal of the placenta at delivery. Abnormal trophoblast invasion and excessive placental tissue have both been implicated as the underlying pathology in preeclampsia (5). Trophoblast invasion is under the influence of several cytokines, and immune maladaptation, mediated by an increased decidua release of Th1 cytokines, proteolytic enzymes, and free radical species, may cause shallow cytotrophoblast invasion of spiral arteries and endothelial cell dysfunction (6).

Abnormal level of human chorionic gonadotropin (hCG) may be an early sign of placental dysfunction. hCG belongs to a family of glycoprotein hormones and is produced by syncytiotrophoblast cells of the placenta. It consists of two dissimilar subunits, α and β, held together by non-covalent interactions. The α-subunit is similar to the other glycoprotein hormones; follicle stimulating hormone (FSH), luteinizing hormone (LH) and thyroid stimulating hormone (TSH). However, the β-subunit

[1]Addis Ababa University, College of Medicine and Health Sciences, Department of Physiology
[2]Addis Ababa University, College of Medicine and Health Sciences, Department of Obstetrics and Gynecology
Corresponding author email: abnm85@gmail.com

which confers biological specificity is different among all the glycoprotein hormones (7). β-hCG is mainly increased during early pregnancy, whereas free α subunit is increased during late pregnancy (8).

The association between elevated maternal serum hCG with adverse pregnancy outcomes such as preterm labor, pregnancy-induced hypertension, and fetal growth retardation has been documented in some clinical reports (9,10). Clinical reports have hypothesized a relation between elevated second trimester hCG levels and hypertensive pregnancy disorders. Hence, the purpose of this study was to determine the association between maternal serum levels of β-hCG with preeclampsia.

PATIENTS AND METHODS

***Study area and period*:** This study was conducted at Tikur Anbessa Specialized Hospital from June, 2013 to April, 2014.

***Study design*:** Case-control study design was applied.
Sample size determination: The desired Sample size was calculated using difference of means formula

$$n = (\frac{r+1}{r})\frac{\sigma^2 (Z_\beta + Z_{\alpha/2})^2}{(\mathrm{x - y})^2}$$

Where:
n = sample size in the case group
r = ratio of cases to controls
Z_β= 0.84 for 80% power
$Z_{\alpha/2}$ = 1.96 for 95% confidence level
x-y= difference of means between cases and controls
σ^2 = difference of variance between cases and controls

Based on the literature (11), the values of:

x-y = 34691 – 18111.7 = 16579.3
σ = 38760 – 15147.9 = 23612.1
Therefore, taking a 1:1 ratio of cases to controls, and inserting all the above values into the equation, and with a non-response rate of 10%, the total sample size was n =70. However, 38 cases and controls each were included in the study.

Sampling procedure: Cases and controls were selected by systematic random sampling. About 3 preeclamptic and 20 normotensive pregnant women attend ANC clinic at Tikur Anbessa Specialized Hospital per day. Taking 3 attendances per day and considering 45 days of data collection, a total of 135 pregnant women were used to calculate the sampling interval. Thus, by dividing the total population by the sample size, the sampling interval was found to be 2. After random selection of the first sample, every 2nd unit were included in the study. Cases and controls were identified by using their blood pressure level from the mother's record card at ANC clinic.

***Exclusion criteria*:** Women with multiple pregnancies were excluded from the study, given the likelihood of a larger placenta in such women and hence higher levels of hCG.

Data collection: About 3ml of blood was collected from pregnant women who gave consent during their ANC visit. The serum was separated by centrifugation and stored at -20°c until determination of β-hCGlevel. A semi-structured and interviewer administered questionnaire was used to collect data on socio-demographic characteristics, disease conditions and obstetric factors.

***Data processing and analysis**:* Serum level of β-hCG was determined by the electrochemiluminescence immunoassay (ECLIA) using the cobas e 411immunoassay analyzer. Every questionnaire was given a unique code and checked for completeness and consistency. The data was analyzed using IBM SPSS version 21.0 statistical software. T-tests were done to compare different characteristics of the study participants between normal and preeclamptic groups. Binary logistic regression and bivariate correlation analysis were done to identify the association between serum levels of β-hCG and preeclampsia, and to determine correlation of obstetric factors with serum levels of β-hCG respectively. A level of $p< 0.05$ was considered statistically significant.

Ethical consideration: The research proposal was approved and ethically cleared by the Ethical Review Committee of the Departments of Physiology and Obstetrics and Gynecology. Participants were provided with clear information and asked if they were willing to participate in the study. Data collection started after written informed consent was obtained from those who were willing to participate. Confidentiality of response was maintained throughout the study.

RESULT

Table 1 summarizes patient characteristics of preeclampsia and control subjects. Maternal age, gestational age, weight, height and BMI were nearly the same in both groups. Higher level of Serum β-hCG was observed in preeclamptic group (34439.18 ± 28223.67mIU/ml, range 2300.00-108735.00mIU/ml) than the normal group (20582.00 ± 17588.31mIU/ml, range 2340.00 - 68961.00 mIU/ml), and this was statistically significant (p=0.013). Serum levels of β-hCG in the case group were also higher than the control group when evaluated over multiple gestational weeks (Fig 1).

Table 1: Demographic and obstetric characteristics of cases and controls, Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia, 2013-2014.

Variable	Mean ± SD Cases (n=38)	Controls (n=38)	p
Maternal age[a]	28.3 ± 4.6	27.3 ± 4.8	0.374
Gestational age	35.7 ± 4.1	36.6 ± 3.7	0.361
Maternal weight	63.5 ± 11.8	62.9 ± 8.1	0.813
Maternal height	1.6 ± 0.1	1.6 ± 0.1	0.268
Maternal BMI	23.7 ± 3.7	24.0 ± 2.5	0.669
SBP[b]	156.8 ± 21.2	111.6 ± 12.2	0.001
DBP	101.8 ± 11.1	75.0 ± 8.3	0.001
MAP	120.2 ± 13.3	87.4 ± 8.9	0.001
Maternal Serum β-hCG	34439.2 ± 28223.67	20582.0 ± 17588.31	0.013

[a]Unit of measurement for Maternal age= years, Gestational age = weeks, weight=kg, height = m, BMI = kg/m^2, BP = mmHg, β-hCG = mIU/ml)
[b]Abbreviations: Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP), Mean Arterial Pressure (Map), Body Mass Index (BMI)

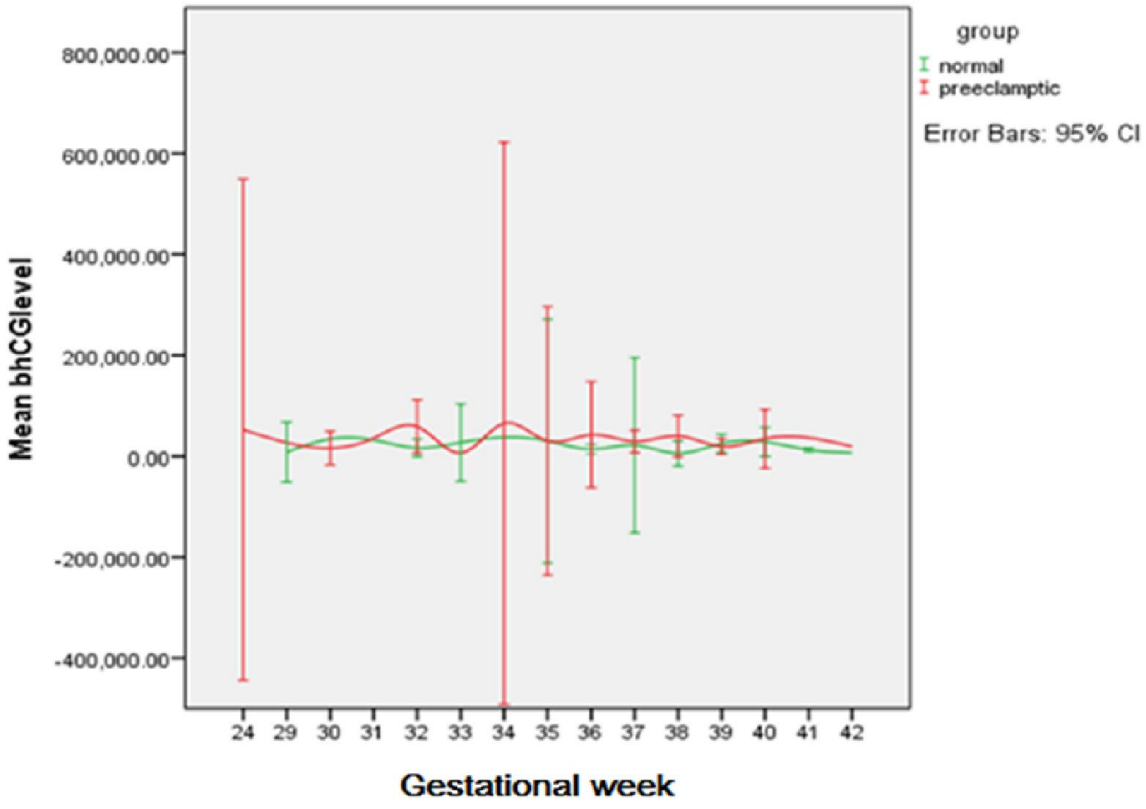

Figure. 1 Serum level of β-hCG in normal and preeclamptic women with respect to gestational weeks, Tikur Anbessa Specialized Hospital, Addis Ababa, /Ethiopia, 2013-2014

Table 2 evaluates β-hCG as a function of number of pregnancies. Normotensive pregnant women in their first pregnancy had significantly higher serum level of β-hCG than normotensive multigravida women (p=0.045). By contrast multigravida preeclamptic women had higher β-hCG level than primigravida preeclamptic women, but this did not reach statistical significance.

Also, multigravida preeclamptic women had significantly higher level of β-hCG than normotensive multigravida women (p=0.004). Early onset preeclamptic women had higher β-hCG level than late onset preeclamptic women, though not statistical significant.

As indicated in Table 3, maternal serum level of β-hCG in the case group had a positive correlation with diastolic and systolic blood pressure. However, it had a negative correlation with maternal age, gestational week and parity. Among the risk factors assessed in the study participants, family history of preeclampsia was a significant determinant factor for the development of preeclampsia (AOR = 6.72 (1.13, 40.05).

Table 2: Serum level of β-hCG in primigravida and multigravida women and in early and late preeclamptic women, Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia, 2013-2014.

Group		Serum β-hCG[a] level (Mean ± SD)	P
Controls (n=38)	Primigravida (n=15)	28332.7 ± 20693.42	0.045
	Multigravida (n=23)	15527.2 ± 13419.94	
Cases (n=38)	Primigravida (n=21)	32094.4 ± 30629.95	0.576
	Multigravida (n=17)	37335.7 ± 25557.94	
	Early onset (n=9)	39578.6 ± 30891.12	0.539
	Late onset (n=29)	32844.2 ± 27728.08	

[a]Unit of measurement for β-hCGis mIU/ml

Table 3: Pearson's correlation coefficients between maternal serum level of β-hCG and obstetric factors in preeclamptic women, Tikur Anbessa Specialized Hospital, Addis Ababa, /Ethiopia, 2013-2014.

Variable		DBP[a]	SBP	MAP	Maternal age[b]	Gestational age	Parity
Serum β-hCG[c] level	r	0.23	0.05	0.15	-0.15	-0.21	-0.06
	p	0.17	0.79	0.36	0.36	0.20	0.71

[a] Abbreviations: r=correlation coefficient, Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP), Mean Arterial Pressure (MAP)
[b] Unit of measurement for Maternal age = years, Gestational age = weeks, BP = mmHg
[C] β-hCG units asmIU/ml

A receiver operating characteristic curve was determined and identified a cut off point at 12,953mIU/ml which yield a sensitivity and specificity of maternal serum level of β-hCG for preeclampsia diagnosis at 76.3% and 52.6% respectively (Fig 2).

Converting continuous to binary data with this cut-off value, binary logistic regression analysis indicated that pregnant women with serum β-hCG level greater than 12,953mIU/ml were 3.58 times more likely to develop preeclampsia (Table 4).

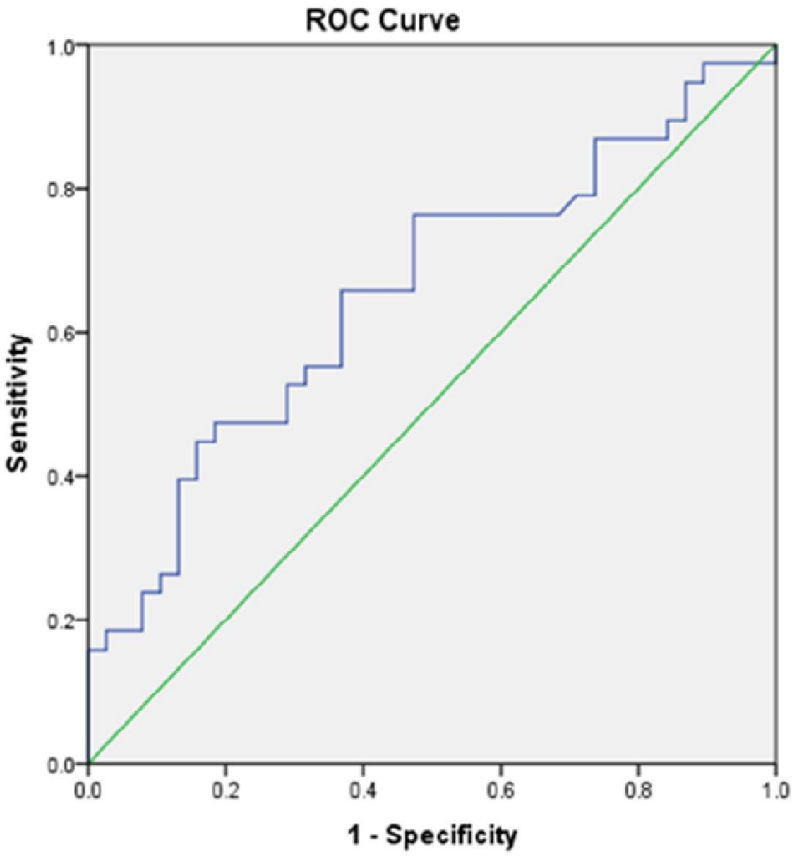

Figure.2 Sensitivity versus specificity of maternal serum level of β-hCG in the diagnosis of preeclampsia, Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia, 2013-2014.

Table 4: Association between preeclampsia and maternal serum level of β-hCG at the optimal cutoff point, Tikur Anbessa Specialized Hospital, Ethiopia, 2013-2014.

	Controls	Cases		
Serum β-hCG level	n (%)	n (%)	OR (95% CI)	P
< 12,953mIU/ml	20	9	1.00	
≥ 12,953mIU/ml	18	29	3.58 (1.34-9.56)	0.01

DISCUSSION

The results of the present study showed a statistically significant association between maternal serum level of β -hCG and preeclampsia. Our results are consistent with previous studies. Significantly higher serum β-hCG concentration was reported in preeclamptic patients compared with normotensive women matched for age and gestation, and β-hCG level were found to rise before the clinical signs of preeclampsia (12). Furthermore, a significant correlation between high serum β-hCG level at early gestation and development of pregnancy induced hypertension later on during pregnancy has been indicated (13,14). One study demonstrated significant increases in serum β-hCG level only in association with severe preeclampsia (15). In contrast, other studies reported no association between elevated β-hCG and preeclampsia (16,17), or a significant association between β-hCG and preeclampsia only among multiparous women (18).

Evaluation of the levels of serum hormones in in pre eclamptic Iraqi pregnancies indicated that there was a significant positive correlation between serum β-hCG and diastolic blood pressure in preeclamptic women (r=0.213) (19). Similarly, in the present study a positive correlation between serum β-hCG and both systolic (r=0.05) and diastolic blood pressure (r=0.23) was observed in preeclamptic women, though not statistically significant. This may be due to the small number of pre eclamptic women in this study.

Theβ-hCG level in normal primigravida women was observed to be significantly higher than normal multigravida women. This is in agreement with the study conducted on the influence of gravidity on Down's syndrome screening with freeβ-hCGwhich suggested that during the first encounter of pregnancy, higher level of hCG is necessary to promote vascular invasion (20).

Studies suggested that early and late onset preeclampsia have different etiologies and clinical expressions. In late onset preeclampsia the behavior of the uterine arteries are normal or only slightly altered but in the early onset disease vascular damages are more prominent (21). Another study reported that early placental vascular damage leading to decreased oxygen supply might result in increased β-hCG production by hyperplasic cytotrophoblast cells (22). This was supported by an *in-vitro* study which showed an increased β-hCG production when normal placental villi in organ culture were maintained under hypoxic condition (23). It seems likely that in this condition the placenta is unable to exert any compensatory effect that may lead to decline of β-hCG levels.

In the present study, cases with early onset preeclampsia (<34 weeks of gestation) had higher mean levels of β-hCG than those with late onset. Though this did not reach statistical significance, our results supports a previous study which showed a significantly higher mean β-hCG level in the early onset group than in the late onset

The higher β-hCG level in early onset preeclampsia may be related to the severity of endothelial cell dysfunction in early onset disease. In contrast to our finding there are studies which reported a higher mean β-hCG level in the late onset group than early onset group (25,26). In general, our result showed association between serum level of β-hCGand preeclampsia and a higher risk of preeclampsia in the first pregnancy.

Conclusion: In this study, maternal serum level of β-hCG was significantly associated with preeclampsia and family history of preeclampsia was found to be a significant predictor of preeclampsia.

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