Evaluation of Serum Human Chorionic Gonadotropin Level in Women with Preeclampsia in Khartoum State

تقييم مستوى هرمون الحمل في مصل الدم لدى النساء اللاتي يعانيين من ارتداد الرحم (تسمم الحمل) في ولاية الخرطوم

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الآية

بسم الله الرحمن الرحيم

قال تعالى:

-O- قُلْ لاَ كَانَ الْبَحْرُ مَدَادًا لِكِلَمَاتِ رَبِّي كَلِمَةً لَن يَنْفَدَةَ الْبَحْرُ قَبْلَ اِنْفَادِ كِلَمَاتِ رَبِّي وَلَوْ جَنَّةً وَلَوْ مَدَادًا

قال تعالى:

وَاللَّهُ خَلَقَ كُلَّ دَابَّةٍ مِنْ مَاءٍ فَمِنْهُمْ مِنْ بُطُنِهِ وَمِنْهُمْ مِنْ يَمِينِهِ وَمِنْهُمْ رِجْلِهِ وَمِنْهُمْ أَرْبَعٌ ﴿۴۵﴾

صِدَقَ اللَّهُ العَظِيم

سورة الكهف الآية (109)

سورة النور الآية (45)
Dedication

To my father and mother

To my sisters and brothers

To my dear family and friends

Hanaa
Acknowledgements

First I thank Allah, who helps me to do this work successfully.
I hope to express my gratitude to my supervisor Dr. Nasereldin Mohamed Ahmed for his efforts and valuable supervision throughout this work.
Also deep thanks to my colleague in Omdurman Military Hospital and Bahry teaching Hospital.
Special thanks to doctor Nuha Algilai Abubkr, the head department of clinical chemistry and Moawia Ali.
And great thanks to doctors Yassir Alsamani and all staffs in Algilai Khalid Musa laboratory.
Finally my thanks for my family and all those encourage me and follow me to achieve this work.
Abstract

**Background:** Pregnancy associated hypertensive disorder and intrauterine growth restriction are common complication responsible for neonatal and maternal morbidity. Most current hypothesis regarding the pathophysiologic mechanisms of pregnancy induced hypertension point to early placenta abnormalities. The production of hCG by the placenta in early pregnancy is critical for implantation and maintainance of the blastocyst. Since it is postulated that preeclampsia is likely a trophoblastic disorder. It may be essential for understanding of this disease, to investigate the pathologic and secretory reaction of the placenta.

**Materials and Methods:** A case control study was conducted at Omdurman military hospital and Khartoum Bahry Teaching Hospital during the period from June to December 2016. Eighty one pregnant women with gestational age 16-36 weeks were selected, they were classified into 3 group: group 1 consist of 47 women with normal pregnancy as control an effort made to match the participant regarding age and gestational age, group 2 consists of 14 patients with mild preeclampsia and group 3 consists of 20 patients with sever preeclampsia. Maternal serum BhCG, blood pressure and proteinuria were measured.

**Results:** The mean concentration of BhCG was significantly increased among preeclamptic pregnant women (17472.59±4470.244 ml IU/ml) in comparison with (12403.67±2647.164ml IU/ml) in normal pregnant women with ( P value 0.012) . The mean concentration of BhCG in mild preeclamptic pregnant women was significantly decrease (14596.50±5037.689mlIU/ml) in comparison (46870.00 ± 21864.876 ml IU/ml) in sever preeclamptic pregnant women ( with Pvalue 0.006) . The mean concentration of BhCG was significantly decreased in second trimester of pregnancy among preeclamptic pregnant women (7540.00±6314.45 ml IU/ml)
in comparison with (24870.65±8006.34ml IU /ml )in third trimester of pregnancy among preeclamptic pregnant women with( P value 0.001) .Maternal serum BhCG is not correlated with systolic blood pressure ( r =0.140 , P- value 0.296 ) . Also no correlation with diastolic blood pressure (r =0.186, P-value 0.161).

Results showed that there is significant increased in BhCG and proteiuria with ( P-value 0.021) in preeclamptic pregnant women.

**Conclusion:** The study concluded that BhCG is higher in preeclamptic pregnant women than normal pregnant women.
المستخلص
خلفية الدراسة: اضطرابات الضغط أثناء الحمل وقصور النمو داخل الرحم هي من أكثر المضاعفات المسئولة عن موت الأمهات والأجنة. ومعظم البحوث الحديثة تعتبر آليات حدوث المرض التي تؤدي إلى ارتفاع الضغط أثناء الحمل هي مشاكل المشيمه المبكرة. خلال الحمل تصنف المشيمه عدة هرمونات منها هرمون الحمل وله عدة فوائد منها غرس وحفظ البويضة في الرحم. وله طبيعة هذا المرض يجب دراسه تغيير افرازات المشيمه وتفاعالاتها.


النتائج: أوضحت الدراسة أن هناك زيادة ذات دلاله إحصائيه في متوسط تركيز هرمون الحمل عند النساء ذوات ارتداد الرحم ( ml IU/ml 4740 ± 2447.59) (مقارنة بالنساء ذوات الحمل الطبيعي ( ml IU/ml 2647.164 ± 12403.67) (مقارنة بالنساء ذات ارتداد الرحم العالي 5037.689 ± 14596.50) (مقارنة بالنساء ذات ارتداد الرحم المتوسط 21864.876±646870) (0.121) ، وأن هناك نقصان في متوسط تركيز هرمون الحمل عند النساء ذوات ارتداد الرحم العالي ( ml IU/ml 24870.65 ± 8006.34) (مقارنة بالنساء ذات ارتداد الرحم المتوسط 6314.45 ± 7540) (مقارنة بالنساء ذات ارتداد الرحم العالي ، القيمة الإحتمالية (0.006) ، ونسبة نقصان في متوسط تركيز هرمون الحمل في الثلث الثاني من الحمل ( ml IU/ml 8006.34 ± 24870.65) (0.001). كما بنت الدراسة أنه لا يوجد ارتباط بين مستوى هرمون الحمل وضغط الدم الانقباضي (R=0.142, p value 0.296) وضغط الدم الانبساطي (r=0.186 p value 0.161). كما أوضحت أن هناك ارتباط طردي بين مستوى هرمون الحمل وبروتين البول والقيمة الإحتمالية (0.021) لدى النساء ذوات ارتداد الرحم.

الخلاصة: خلصت الدراسة إلى أن هناك زيادة في متوسط تركيز هرمون الحمل لدى النساء ذوات ارتداد الرحم مقارنه بالنساء ذوات الحمل الطبيعي.
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<td>PE</td>
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<td>BhCG</td>
<td>Human chorionic gonadotropin</td>
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<td>HTN</td>
<td>Hypertension</td>
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<td>DM</td>
<td>Diabetes mellitus</td>
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Chapter One

Introduction

Literature review
1. Introduction

1.1 Human chorionic gonadotropin and preeclampsia

Human BhCG is glycoprotein with lipid structure that is expressed in trophoblast and various malignant tumor. Human placenta synthesis steroid, protein, glycoprotein throughout gestation (Petralgia, 1990). The production of hCG by placenta is crucial for implantation and maintenance of blastocyst since it postulate that PE is is trophoblastic disorder, it has become to investigate the pathologic and secretory reaction of placenta (Redman, 1990). Twins pregnancies and molar pregnancy produce higher levels of hCG and they are associated with higher incidence of PE than uncomplicated singleton pregnancies (Curry etal, 1975). An association between PE and elevated third trimester hCG levels considerable evidence suggests an association between serum hCG and PE (Onderoglu etal, 1997; Anneli etal, 1998). Physiological concentration of hCG is significantly increased invitro capillary formation and migration of endothelial cells in dose dependent manner and has novel function in uterine adaptation to early pregnancy (Zygment etal, 2002).

1.2 Human placenta hormones

Human placenta synthesis steroid, protein, glycoprotein hormone throughout gestation (Petralgia, 1990)

1.2.1 Steroid hormone

Steroids hormones produced by placenta as estrogen, progestrone, and relaxin

1.2.2 Protein hormone

Protein hormone produced by placenta as human chorionic somatomammotropin (hCS) or human placental lactogen (hPL)
1.2.3 Glycoprotein hormone
Glycoprotein produced by placenta as human chorionic gonadotropin (hCG) or choriogonadotropin (Carl et al, 2008)

1.3 Human chorionic gonadotropin
Human chorionic gonadotropin is hormone produced during pregnancy that is made by the developing embryo after conception and later by the syncytiotrophoblast (part of placenta). BhCG is glycoprotein with lipid structure that is expressed in trophoplast and various malignant tumor (Carl et al, 2008). BhCG can be detected by blood test around eight to eleven days past ovulation. The production of hCG by placenta in early pregnancy is crucial for implantation and maintenance of blastocyst since it is postulate that preeclampsia is trophoblastic disorder (Redman, 1990), twin pregnancies (Long and Oat, 1987) and molar pregnancies (Curry, 1994) produce higher levels of hCG and they are associated with a higher incidence of preeclampsia than uncomplicated singleton pregnancies. An association was reported between preeclampsia and elevated third trimester hCG levels (Hsu, 1994), whereas early experience with second trimester levels suggests a link between increased hCG and other adverse pregnancy outcomes (Wenstrom, 1994, Onderoglu, 1997).

1.3.1 Function of Human chorionic gonadotropin
hCG maintains the corpus luteum, which is responsible for progesterone production in early pregnancy that is help to keep the lumen of uterus thick for healthy pregnancy, to maintain endometrium for the first trimester, to stimulate fetal gonad development and androgen synthesis by the fetal testes and to stimulate secretion of estrogen and development of placenta (Carl et al, 2008)
1.3.2 Human chorionic gonadotropin during second and third trimester of pregnancy

In most cases hCG levels will double every 13-72 hours in early pregnancy the level will reach its peak at around 8-11 weeks of pregnancy and then will decline and level off for the remainder of the pregnancy

1.4 Type of hypertention during pregnancy

Type of hypertention during pregnancy are gestational hypertention, preeclampsia and eclampsia

1.4.1 Gestational Hypertention

Gestational hypertention is the development of new hypertention in pregnant women after 20 weeks gestation with out the presence of protein in urine or the signs of preeclampsia. Gestational hypertention is defined as having blood pressure higher than 140/90 mmHg measured on two occasion, more than 6 hours apart, with out the presence of protein in the urine and diagnosed after 20 weeks of gestation (Mission and Cougher, 2013)

1.4.2 Preeclampsia (PE)

Preeclampsia is gestational hypertention plus protein in a 24 hour urine sample. Sever preeclampsia involves blood pressure greater than 160/110 mmHg with additional medical signs and symptoms, mild preeclampsia when blood pressure greater than 140/90 and less than 160/110.

Preeclampsia is a multi systemic disorder involving the placenta, liver, kidneys, blood and neurological and cardiovascular system (wagner, 2004). It is a relatively common syndrome, dangerous for mother and infant, unpredictable in its onset and
progression, unpredictable except through termination of pregnancy (Redman, 1991).

The symptoms of this multisystemic disorder which appear during the second and third trimester of pregnancy are caused by increased vasoconstriction, which result in maternal hypertension, decreased uteroplacental blood flow, edema, proteinuria, abnormal clotting liver and renal dysfunction (James 2006, Cromble 2008). Generalized dysfunction of maternal cells may underlie most of the clinical symptoms such as hypertension, fluid retention and clotting abnormalities. Hormonal changes contribute to the physiological maternal adaptation during human gestation, these hormonal changes are different in pathological pregnancy and may be monitored for diagnosis or risk prediction of gestational disease taking into account both hormonal levels and preexisting maternal risk factor (Reis et al., 2002). Excessive or deficient release of some placental hormone in association with gestational diseases may be of an adaptive response of the placental and fetal membranes to adverse environmental conditions such as hypertension, hypoxia and infection or to malformation of the fetus and placenta (Larry, 2006).

The high concentrations of these placental hormones in maternal peripheral blood, in fetal (cord) blood, and in the amniotic fluid are clinically accessible signs of increased placental hormone synthesis (Reis, 2002). The secretion of several placental hormones is augmented in preeclamptic patients such as hCG, estrogen and inhibin A.

1.4.3 Eclampsia

Eclampsia is occur when tonic–clonic seizure appear in pregnant women with high blood pressure and proteinuria.
1.5 Risk factor

1.5.1 Maternal causes
Maternal causes such as obesity, age 43 or more, past history of DM, HTN, renal disease, adolescent pregnancy, new paternity, thrombophilia and having donated a kidney.

1.5.2 Pregnancy
Pregnant women with multiple gestation, placental abnormalities as hyperplacentosis and placental ischemia.
1.2 literature review

Study was done in Egypt from June 2009 to June 2010 on 90 pregnant women classified into 3 group: group 1 consist of 30 normotensive non proteinuric pregnant women ; group 2 consist of 30 women with mild preeclampsia ;and group 3 consist of 30 women with sever preeclampsia .it found the maternal serum B-hCG was significantly higher in group 3 than in group 2 and group 1

The maternal age; maternal serum levels B-hCG showed a significant positive correlation with SBP/DBP and albuminuria in group 2 . The maternal serum B-hCG and age showed asignificant positive correlation with SBP/DBP and albuminuria in group 3(Elhadi, 2011).

Study was done in Dhaka medical college hospital ,Dhaka between January and July 2013 on 74 pregnant women with preeclampsia and 76 normotensive pregnant women .It found significant difference between the B-hCG level in the preeclamptic women compared to the normotensive pregnant women and severity of preeclampsia increase with further rise of B-hCG level(Begumz etal,2014) .

Study was done in china on 142 normotensive and 43 preeclamptic women .The study conclude that that B-hCG level might reflect the degree of disorder activity of placenta induced hypertention and could be utilized as marker for in determining PIH (Feng etal ,2000)

Study was done in Iraq on 120 pregnant women divided into 30 healthy pregnant women and90 pregnant women with preeclampsia whose divided into 37 pregnant women with mild preeclampsia and 53 pregnant women with severe preeclampsia .this study conclude that preeclamptic women with severe cases but not mild cases had significant increased levels of serum hCG as compared with healthy pregnant (Suaad et al ,2012 )
Study was done in Turkey on thirteen women with severe preeclampsia and twenty-one normotensive, healthy pregnant women with singleton pregnancies in the third trimester were matched. It conclude there is a relationship between severe preeclampsia and elevated serum B-hCG levels (Remzi et al., 2000).

Study was done in Iran on 66 preeclampsia pregnant women matched by age and gestational age to 66 normotensive pregnant women, the study concluded that the mean of maternal serum B-hCG level in patients with preeclampsia was significantly higher than control (Yousif and Moslemizadeh, 2013).

Study was done in Nigeria on 70 pregnant women with preeclampsia and 80 healthy normotensive pregnant women, the study concluded that there is low serum beta human chorionic gonadotropin in preeclamptic pregnant women (Adeosun, 2016).
1.3 objectives:

1.3.1 general objective:
To study the beta human chorionic gonadotropin on preeclamptic pregnant women.

1.3.2 specific objective:
1. To measure and compare between BhCG in preeclamptic pregnant women and normal pregnancy.

2. To measure and compare between BhCG level in mild and severe preeclampsia.

3. To measure and compare between second and third trimester BhCG in preeclamptic pregnant women.

4. To correlate between BhCG and systolic and diastolic blood pressure in preeclamptic pregnant women.

5. To find association between BhCG and proteinuria in preeclamptic pregnant women.
1.4 Rationale

Preeclampsia is a common syndrome dangerous for mother and infant, unpredictable in its onset and progression and untreatable except through termination of pregnancy (Redman, 1990). It affects up to 7% of pregnant women and is considered a leading cause of fetal growth restriction and perinatal morbidity and mortality. Number of theories have been put forward where different biochemical markers have been implicated in the causal association of preeclampsia. Several studies have reported an association between unexplained increases in maternal serum hCG levels in the second trimester of pregnancy and subsequent development of preeclampsia (Hsu, 1991).

To our knowledge up to now no research has been diverted to solve or to illustrate the correlation between hormone BhCG and preeclampsia.
Chapter two

Material

&

Methods
2-Material and Methods

2.1 Study design
This is a case control study.

2.2 Study area
This study was conducted on Omdurman Military Hospital and Bahry Teaching Hospital.

2.3 Study populations
Eighty pregnant women were selected for this study of thirty three pregnant women with preeclampsia (as case) depending on clinical sign and forty seven normal pregnant women (as control)

2.3.1 Inclusion criteria
Specimens were collected from Pregnant women with preeclampsia and apparently healthy pregnant women with gestational age 20-36 weeks

2.3.2 Exclusion criteria
Pregnant women with chronic hypertension, multiple pregnancy, diabetes mellitus and women suspected to have premature delivery were excluded from study

2.4 Ethical approval
The research was granted ethical approval by Sudan University research committee and informed consent was given by each participants (appendix).

2.5 Data collection
The data were collected by using a direct interviewing questionnaire (appendix)
2.6 Sample size
About 34 patients visited the hospital during the period June to December 2016 and matched group by 47 healthy pregnant as control group from the Hospital.

2.7 Sample collection
Sample collected using vacotainer system, tourniquet was used to make veins more prominent, 3 ml blood sample was collected in plane container and was collected under septic condition. All blood sample in plane container were allowed to clot at 25 c, then they were centrifuged at 4000 Rpm to obtain serum and stored at -20 until analysis. Also urine samples were collected and examined directly for protein which was measured by dipstick test.

2.8 Sample analysis

2.8.1 Methods
TOSOH AIA System Analyzer was used for measurement of BhCG.

2.8.2 Principle of the assay
Brief according to manufacture, the STAIA-PACK BHCG is tow–site immunoenzymomorphic assay for the intact hCG molecule and beta subunit which is performed entirely in the STAIA–pack BhCG test sups. Intact hCG molecule and beta subunits present in the test sample are bound with monoclonal antibody immobilized on magnetic solid phase, and then a distinctly different antigenic site on the beta subunits is bound with enzyme–labeled monoclonal antibody in the test cups. The magnetic beads are washed to remove unbound enzyme labeled monoclonal antibody and are then incubated with a fluorogenic substrate 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme–labeled monoclonal antibody that binds to the beads is directly proportional to the beads is directly proportional to the BhCG concentration in the test sample. Standard
curve is constructed, and unknown sample concentration are calculated using this curve.

2.9 Quality control

For internal quality control, normal control sera and pathological control sera were included in every batch of chemical analysis.

2.10 Statistical analysis

The data was analyzed using statistical package of social science (SPSS computer program), independent t test; one way ANOVA; and correlation were used to correlate and compare between study parameter and test variables.
Chapter three

Results
3 Results

This study included 34 preeclamptic pregnant women and 47 normal pregnant women. The mean concentration of BhCG was significantly increased among preeclamptic pregnant women (17472.59±4470.244) in comparison with (12403.67±2647.164) in normal pregnant women with P value 0.012 which is presented in figure 3:1.

The mean concentration of BhCG in mild preeclamptic pregnant women was significantly decrease(14596.50±5037.689) in comparison(46870.00±21864.876) in sever preeclamptic pregnant women with P 0.006 which is presented in figure 3:2.

The mean concentration of BhCG was significantly decreased in second trimester of pregnancy among preeclamptic pregnant women (7540.00±6314.45) in comparison with (24870.65±8006.34) in third trimester of pregnancy among preeclamptic pregnant women with P value 0.001 which is presented in figure 3:3.

Pearson’s correlation showed maternal serum BhCG is not correlated with systolic blood pressure (r =0.140, P-value 0.296) which is presented in table 3:1. Also no correlation with diastolic blood pressure (r =0.186, P-value 0.161) which is presented in table 3:1.

One way ANOVA used to compare between BhCG and proteinuria which showed that there is significant increased in BhCG and proteinuria (with P-value 0.012) in preeclamptic pregnant women which is presented in Figure 3:4.
Table 3.1: Correlation between BhCG and systolic and diastolic blood pressure

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<th>P-value</th>
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<td>Systolic blood pressure</td>
<td>0.140</td>
<td>0.296</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>0.186</td>
<td>0.161</td>
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Figure 3: B-hCG level in preeclampsia pregnant women and normal pregnancy women

Results express as mean ± SD, significant considered as P-value < 0.05
Figure 3:2 B- hCG level in mild and severe preeclampsia pregnant women

Results expressed as mean ±SD, significant the P-value <0.05
Figure 3:3 BhCG level in second and third trimester of pregnancy in preeclamptic pregnant women

Results express as mean ±SD; significant the P-value <0.05
Figure 3:4 relationship between the BhCG and proteinuria

Results expressed as mean ± SD, significant the P-value < 0.05
Chapter four

Discussion

&

Conclusion

&

Recommendation
4.1 Discussions

The normal placenta differentiates during pregnancy with dominance of cytotrophoblast in early gestation and syncytiotrophoblast in late gestation. It is well known that cytotrophoblasts are undifferentiated stem cells and that syncytiotrophoblasts are differentiated from the cytotrophoblast (Kliman et al, 1987). Although the mechanism of regulation of gestational hCG remains largely unknown, it is generally accepted that hCG is secreted only by the syncytiotrophoblast (Fox, 1970). Remzi et al showed that early placental vascular damage leading to decreased oxygen supply might result in increased hCG production by hyperplasic cytotrophoblastic cells (Remzi, et al., 2000). Also, hCG production has been shown to increase when normal placental villi in organ culture were maintained under hypoxic condition. Typically the placenta is the affected tissue in PE (Correa et al, 2007). In PE, placental pathologic examination reveals focal cellular necrosis in the syncytiotrophoblast and increased mitotic activity with cellular proliferation in the cytotrophoblast. In addition, the proliferating cytotrophoblast in severe PE is rapidly transformed into syncytiotrophoblast within 72 hour (Hoshina et al, 1982).

In recent years, many studies have been conducted to determine the relation between maternal serum hCG levels and subsequence development of PE.

This study showed that, the mean concentration of BhCG was significantly higher in preeclamptic pregnant women than that in their control counterpart with P-value 0.012, this finding was in agreement with previous report who stated that there is association between BhCG level and PE( Elhadi et al, 2011; Begumz et al, 2014; Feng et al, 2000; Suaad et al, 2012; Remzi et al, 2000; Yousif and moslemizaden, 2013). In contrast with other study reported contradict our finding.
that, serum BhCG decrease in pregnant women with preeclampsia (Adeosun et al, 2016)

The independent t test showed that there is significant decrease in BhCG in mild preeclampsia in compare to sever preeclampsia with P-value 0.006, this finding was agreement with previous report who stated that there is increase in BhCG in sever preeclampsia than mild preeclampsia ( Begumz et al, 2014 ; Suaad et al, 2012 ; Remzi et al, 2000 ;)

The independent t test showed that there is significant decrease in BhCG in second trimester in compare to third trimester with (P-value 0.001) this finding was agreement with other study who reported that serum BhCG is higher in third trimester in women with preeclampsia ( Remzi etal,2000 )

Pearson correlation showed that there is no correlation between BhCG and systolic and diastolic blood pressure with P-value(0.296, 0.161) respectively, this finding was not agreement with previous report who stated that there is correlation between BhCG and systolic and diastolic blood pressure ( Elhadi et al 2011 , Begzum, 2014).

One way ANOVA showed that there is significant increased in BhCG and proteinuria with P-value (0.021), this finding agreement with other study report who stated that there is significant different when compare between BhCG and proteinuria (Elhadi etal, 2011).
4.2 Conclusion:
The Study concluded that BhCG is higher in preeclamptic pregnant women than normal pregnant women.

4.3 Recommendation:
1. Measure BhCG concentration at early of pregnancy
2. Do cohort study to know consequence of elevation of BhCG in preeclamptic women.
3. Do other study to know the mechanism of elevation of BhCG in preeclampsia
References


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Reis, F.M; Antona, D.D; Petraglia, F. (2002) Predictive value of hormone measurement in maternal and fetal complication of pregnancy. Endocrine review; 23 (2) 230-257.


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Appendix
Questionnaire

For Preeclampsia:

Name ............................................................NO (……)

Age .............................................................years

Gestational age ..............................................

Chronic Hypertension                   Yes (…..)  No (……….)

Multiple Pregnancy                   Yes (…..)  No (……….)

Diabetes Mellitus                   Yes (……) No (……….)

Premature Delivery                   Yes (……) No (……….)

Biochemical finding :

Blood Pressure :

Diastolic ..............................................Systolic Pressure ..............

Beta HCG.............................................mlu/ml

Proteinuria .............................................
For control:

Name ............................................................................................................ No (……)

Age .............................................................................................................. Years

Gestational age .........................................................................................

Biochemical finding:

Blood pressure:

Systolic pressure .......................... Diastolic pressure ....................

Beta HCG.......................................................... mlu/ml

Proteinuria ..........................................................
Consent Form

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