Comparison of Maternal Serum Levels of Thrombopoietin among Pregnant Women with and without Preeclampsia

مقارنة بين مستويات الثرومبوبويتين من مصل الدم للأم بين النساء الحوامل المصابات والغير مصابات بمتلازمة ما قبل الأرتجاع

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الخلاصة:
خلفية البحث: متلازمة ما قبل الارتجاع (Preeclampsia) هي واحدة من اهم الاسباب التي يمكن ان تؤدي الى امراض و وفيات الام والجنين. تتميز هذه المتلازمة ببداية جديدة لأرتفاع ضغط الدم والسائل الزلالي بعد الأسبوع العشرون من الحمل، متلازمة ما قبل الارتجاع هي اضطراب يصيب العديد من اعضاء الجسم ويحدث اثناء فترة الحمل وليس له علاج فعال. الثرومبوبويتن هو المنظم الرئيسي لنضوج الخلايا الضخمة وانتاج الصفيحات الدموية. الثرومبوبويتن يبدأ تأثيراته البايولوجية من خلال الأرتباط بمستقبلات خاصة على جدار الخلايا المتخصصة في انتاج بقايا انواع الخلايا الدموية.
الأهداف: تهدف الدراسة الى مقارنة مستويات الصرومبوبويتين في مصل الدم عند النساء الحوامل اللواتي يعانن من متلازمة ما قبل الأرتجاع والنساء ذوات الحمل بدون متلازمة ما قبل الأرتجاع (الحمل الطبيعي).
المنهجية: تم أجراء هذا البحث من خلال استخدام دِراسَةُ الحالاتِ و الشَّواهِد (المقارنة) في مستشفى الزهراء للنسائية والتوليد و الاطفال في النجف، العراق للفترة من ١ / شباط / ٢٠١٩ الى ١ / كانون الاول / ٢٠١٨. حيث تم تقدير الثرومبوبويتين في الدم في ٨٨ امرأة حامل ، مقسمة إلى مجموعتين. المجموعة ١ (مجموعة الدراسة): تتكون من ٤٠ مَرِيضَةٌ يعانن من متلازمة ما قبل الأرتجاع. المجموعة ٢ (المجموعة الضابطة): تتكون من ٤٨ امرأة غير مصابة بمتلازمة ما قبل الأرتجاع في حالة مخاض.
النتائج: مستوى الثرومبوبويتين هو اعلى عند النساء الحوامل اللاتي يعانن من متلازمة ما قبل الأرتجاع من النساء الحوامل الطبيعيات. مستوى الثرومبوبويتين بمجموعة متلازمة ما قبل الارتجاع كان (M = 309.63, SD = 86.32 ±) و في المجموعة الضابطة كان (M= 215.69, SD= 63.52±)؛ لذا فإن قيمة p <0.001 وهي ذات دلالة إحصائية.
الاستنتاج: كان مستوى الثرومبوبويتين في الدم مرتفعًا بشكل ملحوظ في النساء الحوامل المصابات بمتلازمة ما قبل الأرتجاع ، وكان تعداد الصفائح الدموية أقل بشكل ملحوظ في المجموعة متلازمة ما قبل الأرتجاع.
التوصيات: الحاجة لمزيد من الأبحاث لعينات مختلفة وكبيرة لتوثيق دور الثرومبوبويتين في الدم كمؤشر حيوي يتنبأ بشدة متلازمة ما قبل الأتجاع.
الكلمات المفتاحية: الصفائح الدموية. متلازمة ما قبل الأرتجاع، النساء الحوامل، ثيرومبوبويتين.

ABSTRACT:
Background: Preeclampsia is a syndrome and considered one of the most significant causes that can lead to maternal and fetal morbidity and mortality. It is characterized by new onset of hypertension and proteinuria after 20 weeks of gestation. Thrombopoietin is a major regulator of megakaryocyte maturation and platelet production. Thrombopoietin initiates its biologic effects by binding to the myeloproliferative leukemia protein receptor, which is a member of the hematopoietin receptor family.
Aims of the study: The present study is intended to compare the maternal serum levels of thrombopoietin between preeclamptic pregnant women and pregnant women without preeclampsia (normal pregnancy).
Methodology: This research study is conducted by utilizing a case control (comparative) study. This study is carried out in Al-Zahra' a Teaching Hospital of Obstetrics / Gynecology and Pediatric at Al-Najaf, Iraq. The period of this study started from 1st of February to 1st of December 2018. Where serum thrombopoietin was estimated in 88 pregnant women, divided into 2 groups; Group1 (study group): consist of 40 patients with preeclampsia. Group 2(control group): consist of 48 term women non preeclamptic in labor.
Results: Serum thrombopoietin level is higher in preeclamptic than normal pregnant women. The thrombopoietin level of the preeclamptic group was (M = 309.63, SD = 86.32 ±) and in the control group was (M= 215.69, SD= 63.52±); so p value <0.001 which is statistically significant.
Conclusion: Serum thrombopoietin was significantly elevated in preeclamptic pregnant women and platelet counts were significantly lower in preeclamptic group.
Recommendations: More researches are needed for different and large samples to document the role of serum thrombopoietin as biomarker predicting severity of preeclampsia.
Keywords: Platelet; Preeclampsia; Pregnant women; thrombopoietin.

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INTRODUCTION

Few studies have been conducted to measure maternal serum thrombopoietin levels in order to determine the association between the levels of thrombopoietin and preeclamptic pregnant woman. A preeclampsia is considered critical and complicated medical condition due to unknown etiology. The preeclampsia is defined as a systemic syndrome characterized

by new hypertension (blood pressure >140/90 mmHg) occurring after 20 weeks of gestation with significant proteinuria (urinary protein / creatinine ratio of greater than 30 mg/mmol or a validated 24-hour urine collection result showing greater than 300 mg protein) [(1)].

Despite the complicated condition and unknown etiology of preeclampsia that remains not fully understood there is a theory proposed that circulating factors produced by an oxidatively stressed placenta have been suggested as a factor that cause an excessive systemic inflammatory response which leads to disorder in the function of maternal endothelial cells. A shallow placentation, with atypical invasion of cytotrophoblasts and inadequate remodeling process of placenta-supplying maternal uterine spiral arteries, is suggested to be a reason that change circulation and consolidate oxidative stress in the placenta and associated release of endothelial deranging factors to the maternal circulation [(2)].

Preeclampsia or eclampsia, or both, occurring before the 20th week of gestation and has been reported with molar or hydro pic degeneration of the placenta with or without a coexistent fetus .On the other hand, the presence of hypertension, proteinuria and abnormal laboratory tests before (20 weeks) gestation may be due to lupus nephritis, hemolytic uremic syndrome (HUS), antiphospholipid antibody syndrome, or thrombotic thrombocytopenic purpura (TTP). Therefore, pregnant women should be evaluated to rule out the presence of the mentioned disorders [(3)].

Thrombopoietin (TPO) is a glycoprotein hormone manufactured by the liver and kidney which controls the production of platelets. It encourages the production and differentiation of megakaryocytes, the bone marrow cells that bud off large numbers of platelets [(4)]. TPO regulates the differentiation of megakaryocytes and platelets, but studies on the removal of the thrombopoietin receptor show that it effects on hematopoiesis are more versatile. It is a negative feedback that has different from of most hormones in endocrinology; the effector regulates the hormone directly. Thrombopoietin is bound to the surface of platelets by the c-mpl receptor (CD 110) and destroyed, thereby reducing megakaryocyte exposure to the hormone. Hence, the increasing and decreasing platelet concentrations regulate the thrombopoietin levels. Low platelets lead a higher degree of TPO exposure to the undifferentiated bone marrow cells, leading to differentiation into megakaryocytes and further maturation of these cells. On the other hand, high platelet concentrations lead to less availability of TPO to megakaryocytes [(4)].

Most of the circulating TOP is cleared by platelets (and possibly megakaryocytes) by binding to the TPO receptor, followed by internalization and catabolism of the bound ligand. Therefore the circulating TPO level is inversely related to the rate of platelet production. When platelet production is low, less TPO is cleared and its levels rise, whereas when platelet production is elevated, more TPO is cleared and its levels fall [(5)].

In 1998 Frolich et al. reported for the first time that TPO levels were significantly greater in pregnancies complicated by the hemolysis, elevated liver enzymes and low platelets syndrome [(5)]. However, many studies have been conducted to evaluate platelet count and hemostatic activity during normal pregnancy, and pregnancies complicated by preeclampsia. Pregnancy is accompanied by significant alterations in hemostatic mechanisms and parameters. Studies have indicated an overall increase in plasma procoagulant factors, suppression of fibrinolysis, increase in soluble fibrin, and an increase in fibrin degradation products [(6)]. A decrease in platelet number has also been reported to occur, and it has been suggested that pregnancy is a state of chronic intravascular coagulation. Preeclampsia and possibly the syndrome of hemolysis, elevated liver enzymes, and low platelets (HELLP) appear to be exaggerated states of this phenomenon. The role of platelets in this altered state of hemostasis remains disputed and unclear. Thrombocytopenia, frequently observed in pregnancy induced hypertension or the HELLP syndrome, appears to be the result of platelet consumption at the micro vascular level [(7)].

However, the regulation megakaryopoiesis and platelet production has not been well studied in human pregnancy. The major regulator of circulating platelet levels is believed to be a cytokine termed thrombopoietin. Thrombopoietin, also known as c-mpl ligand, has been isolated and cloned by several groups. It promotes proliferation of committed megakaryocytic precursors and differentiation of immature megakaryoblasts in vitro and in vivo. Recent studies of experimental thrombocytopenia induced in animals by chemotherapy, radiation, or antiplatelet antibodies indicate an inverse relationship between circulating platelet mass and thrombopoietin levels. However, other observations, particularly in humans, indicate that megakaryocyte mass may be the principal regulator of TPO levels.

AIM OF THE STUDY

The purpose of the present study is to compare the maternal serum levels of thrombopoietin between preeclamptic and normal pregnant women.

METHODOLOGY

- **Study Design and Setting:** This study was conducted by utilizing a case control study comparative design. The study carried out in Obstetrics/ Gynecology and Pediatric of Al-Zahra' a Teaching Hospital at Al-Najaf City, Iraq. The period of this study started from 1st of February to 1st of December 2018. The protocol of the study is approved by the Scientific Council of the Iraqi Board for Medical Specialization and ethical approval was also obtained from institutional review board.
- **Sample of the study:** The current study includes 88 pregnant women who have attended labor room either for labor or for controlling blood pressure. Oral consent was taken from all participants. Eligibility criteria included gestational age ranged from 28 to 40 weeks and their age between 16- 39 years old, who visited the above-mentioned hospitals. The studied women were further divided into two groups: Group one (study group): consist of 40 patients with preeclampsia, subdivided to; 32 patients with mild preeclampsia and 8 patients with severe preeclampsia. Group two (control group): consist of 48 term women non-preeclamptic in labor. The data of each pregnant woman in this study were taken and recorded on the data sheet which included maternal age, parity, abortion, occupation, residency and gestational age (determined by LMP and confirmed by early ultrasound).

A detailed clinical history regarding personal history, family history, past medical, obstetrical and gynecological history are taken, examination (general and obstetric examination) was done. The blood pressure had been measured in semi recumbent posture with a left lateral tilt, in the right arm by Sphygmomanometer. Laboratory investigations included; blood group and Rh, complete blood picture, random blood sugar and general urine examination (sample for albumin in urine by dipstick method), liver function test(LFT), renal function test (RFT) and blood coagulation profile.

The preeclamptic patients were subdivided into:

1. Mild Preeclampsia (PE): This includes cases of sustained rise of blood pressure of more than 140/90 mm Hg, but less than 160 mm Hg systolic or 110 mm Hg diastolic without significant proteinuria [(7)].
2. Severe PE: (1) A persistent systolic blood pressure of >160 mm Hg or diastolic pressure of >110 mm Hg. (2) Protein excretion of >5 gm/24 hr. (3) Oliguria (<400 ml/24 hr). (4) Platelet count < 100,000/mm3. (5) HELLP syndrome. (6) Cerebral or visual disturbances. (7) Persistent severe epigastric pain. (8) Retinal hemorrhages exudates or papilledema. (9) Intrauterine growth restriction of the fetus. (10) Pulmonary edema [(8)].

The patients who found to be elevated blood pressure were treated according to the hospital protocol and evaluated for IUGR and other complications of preeclampsia.

Pregnant women are recruited if they met the inclusion and exclusion criteria.

a. Inclusion criteria:
   1. Singleton pregnancy.
   2. Gestational age: pregnant lady with gestational age ranging from (20-40) weeks of gestation.
b. Exclusion criteria:
   1. Chronic hypertension.
   2. Cardiac, renal or liver disorders.
   3. Autoimmune disorders.
   4. Stroke, D.M. and sepsis.
   5. Multiple pregnancies.
   6. Family or personal history of thromboembolic disease and bleeding disorders.
   7. Morbid obesity.
   8. Fetal congenital malformation.
   9. Immune thrombocytopenic Purpura.

DATA COLLECTION PROCEDURE

The blood sample of 3 ml was taken through a standard venipuncture of the antecubital vein from each candidate in the labor room. The gel tubes were transferred into the laboratory and centrifuged for 20 minutes at approximately 1,000^{x}g. to obtain serum. Serum thrombopoietin is estimated by ELISA (Enzyme-Linked Immunosorbent Assay), organism species: Homo sapiens (Human). The kit name was (cloud-clone corporation), serial No.:25D251CD44. A midstream clean-catch urine sample was also collected in disposable caps and sent to a laboratory where urine albumin was measured by using the dipstick method.

RESULTS:

Table (1): Sociodemographic characteristics of all participants (N = 88)

Variable		Mean	Range
Age (Years)		24.27±5.3	16-39
Parity		2±1	1-4
Gestational age/weeks		37.8±1.8	28-40
Albumin in urine			2-3 +
		Frequency	**Percentage**
Residence	**Rural**	12	13.6
	Urban	76	86.4
Occupation	**Employer**	10	11.4
	Housewife	78	88.6

A total of 88 pregnant women included in this study, 40 of them were complaining from PE while 48 without PE (control group). The sociodemographic characteristics of all women are shown in table 1.

Table (2): Comparison between PE and Control Group

Variable	PE group (*n*=40)	Control group (*n*=48)	*P*-value
	Mean ±SD	Mean ±SD	
Age/years	23.48±5.62	24.94±4.98	0.199

BMI Kg/m^2	27.14±1.46	24.89±2.07	<0.001
Gestational age/weeks	36.78±2.08	38.73±0.86	<0.001
Systolic BP(mmHg)	150.88±5.97	112.5±5.25	<0.001
Diastolic BP(mmHg)	100.25±5.98	67.92±6.17	<0.001

Table (2) shows the systolic, diastolic BP and BMI significantly higher in PE group, but the gestational age significantly higher in the control group. There is no significant difference in age between the two groups.

Table (3): Thrombopoietin level and platelet count in case and control groups

Variable	**PE group (n=40)**	**Control group (n=48)**	**P value**
	Mean ±SD	**Mean ±SD**	
Thrombopoietin level pg/ml	309.63±86.32	215.69±63.52	<0.001
Platelets count × 10^3/mL	189±50.03	229.50±109.05	0.033

Table (3) shows significant difference in the thrombopoietin level between the two groups where it is higher in the PE group while platelet level significantly lower in PE group.

Table (4): Comparison between mild and sever PE

Variables	**Sever PE (n=8)**	**Mild PE (n=32)**	***P*-value**
	Mean±SD	**Mean±SD**	
Thrombopoietin level pg/ml	264.75±80.85	320.84±85.14	0.101
Platelets count × 10^3/mL	188±60.91	189.2±48.07	0.951

Table 4 above shows there is no significant difference between mild and sever PE in thrombopoietin level and platelet counts.

Table (5): Correlation between thrombopoietin levels with different variables among cases

Variable	**R**	**P value**
Age/years	-0.137	0.399
BMI Kg/m^2	-0.103	0.526
Gestational age/weeks	-0.053	0.745

The above table indicates there is no significant correlation between thrombopoietin level and age, BMI and gestational age among cases.

Table (6): Correlation between thrombopoietin levels with different variables among control

Variable	**R**	**P value**
Age/years	0.023	0.879
BMI Kg/m^2	-0.255	0.080
Gestational age/weeks	-0.052	0.723

The above table demonstrates there is no significant correlation between thrombopoietin level and age, BMI and gestational age among controls.

DISCUSSION:

The findings of this study will be discussed here in relation to previous research studies. As noted earlier, the objective of this research study was comparing serum thrombopoietin level between pre-eclamptic and normal pregnant women. Pre-eclampsia is a critical condition which needs to be managed immediately in order to prevent life threatening complications.

Nevertheless, pre-eclampsia is a leading cause of maternal and perinatal morbidity and mortality worldwide. In addition, pre-eclampsia is known to affect the function of multiple organs involving metabolism. The most essential feature in preeclampsia is hypertension, which is supposed to be due to a vasospastic phenomenon in kidney, uterus, and placenta and brain [(9)]. However, thrombopoietin (TPO) is the growth factor which responsible for megakaryocyte maturation and platelet production [(10)]. There are few theories regarding the association between TPO and preeclampsia, so we need more studies to make a clear statement about this phenomenon. The results of this study support previous research findings in terms of the relationship between serum thrombopoietin level in preeclamptic and normal pregnant women.

The finding of this study revealed that the serum thrombopoietin level was higher in preeclamptic than normal pregnant women, as shown in table (3). The thrombopoietin level of the preeclamptic group was higher (M = 309.63, SD = 86.32 ±) versus control group (M = 215.69, SD = 63.52±); p value <0.001 statistically significant.

Another finding appeared that the platelet count is decreased in preeclamptic group as compared to non-preeclamptic group (M = 189, SD = 50.03± versus control group M = 229.50, SD =109.05±; p value 0.033).This study supports Johnson et al (2001) who conducted a research study consisted of nineteen patients that they met diagnostic criteria for pre-eclampsia, four of them had HELLP Syndrome. Median platelet count in pre-eclamptic gravid as was 171× 103/mL (range 22-308). Sixteen control patients had a median platelet count of 254× 103/mL (range 158-457) (P = 0 .001). In the pre-eclamptic group, the mean TPO level was 83 pg/ml (range 68-98) and for the control group was 35 pg/ml (range 31-38) (P = 0.001). For the HELLP Syndrome subgroup, the median platelet count was 69× 103/mL, and mean TPO level was (95pg/ml). In addition, a linear correlation was seen between platelet count and TPO level in the preeclamptic group (R = 0.74), no linearity between platelet count and TPO level was demonstrated in the control group [(9)].

The finding of this research study corresponds well to Frolich et al (1998) who assured that the Thrombopoietin levels in normal pregnant women and pregnancies complicated by the hemolysis, elevated liver enzymes, low platelets syndrome were statistically significantly higher than thrombopoietin levels in no pregnant controls. Thrombopoietin levels in healthy pregnant patients (mean = 31.07) and patients with the HELLP syndrome (mean = 44) were significantly higher than thrombopoietin levels in age-matched no pregnant controls (mean = 16.53). The significance of higher thrombopoietin values in the pregnant group becomes obvious when patients are ranked according to thrombopoietin value. None of the patients with severe preeclampsia had platelet counts in the thrombocytopenic range (<150 × 103/mL), whereas platelet counts in pregnancies complicated by HELLP ranged from 38 to 106 × 103/mL. All patients in the latter group had thrombopoietin levels >250 pg/dL [(10)].

The finding also supports a research study conducted by R Caroll (2005). R Caroll conducted a research study that contain 43 patients, 7 were diagnosed as having mild preeclampsia and 4 with severe preeclampsia. The 4 patients diagnosed as severely preeclamptic had average B2R/GAPDH ratios at third trimester and delivery somewhat lower than non-preeclamptic patients (severe PE =1.45±0.45 S.E.M versus normal pregnant = 1.76±0.57, mean ratio±S.E.M) [(11)].

The statistical significance of the present study suggested that serum thrombopoietin level is increased in pre-eclamptic women, which is disagree a study conducted by Caroll (2004). The Caroll's study contained 187 patients, 38 were diagnosed as having mild preeclampsia, 6 with severe preeclampsia and 1 HELLP syndrome, Significant TPO potentiation of collagen activation was observed in non-preeclamptic patients at the first and third trimesters ($P<0.05$, paired t-test). In contrast, preeclamptic patients platelets show no significant TPO potentiation at any time ($P>0.8$, paired t-test), while the mechanism for this difference in thrombopoietin potentiation of platelet activation by collagen as early as the first trimester is unknown **(12)**.

CONCLUSIONS

Serum thrombopoietin significantly elevated in preeclamptic pregnant women. Platelet count significantly decreased in preeclamptic group. There was no correlation between thrombopoietin level and platelet counts.

RECOMMENDATIONS:

1. More researches are needed for different and large samples to document the role of serum thrombopoietin as biomarker predicting severity of preeclampsia.
2. Doing a prospective study, that starts from the first trimester to evaluate if thrombopoietin can be used as predictor for development of preeclampsia.
3. This study can be duplicated by using some modifications that include larger sample and adding HELLP Syndrome patients.

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