The Relationship between Leptin and Some Steroid Hormones in the Sera of Pre-eclamptic Iraqi Women

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Abstract

Background: Pre-eclampasia (PE) is a disease characterized by elevation of blood pressure and proteinuria after 20th week of gestation. It is a common and major complication in pregnancy causing significant morbidity and mortality. The etiology of PE is still poorly understood, and several theories have been put forward.

Aim of the study: To find out the relationship between serum leptin level and some steroids hormone in mild and severe cases of PE.

Patients and Methods: Eighty three pregnant women with PE (All of them in third trimester of pregnancy) were enrolled in this study. They were subdivided into three groups i.e. G1 (comprising 28 women) with mild PE, G2 (comprising 25 women) with severe PE. The third group (G3) (comprising 30 women) was taken as control group. Serum leptin, free testosterone (fT), estradiol (E_2) were determined by using enzyme - linked immunosorbent assay (ELISA).

Results: Serum levels of leptin were significantly higher ($p<0.01$) in mild and severe group compared with control group in the third trimester, Also free testosterone and estradiol were significantly higher ($p<0.01$) in mild and severe groups compared to their control group. A significant positive correlation ($p<0.01$) between leptin and estradiol in preeclamptic patients in mild and severe cases.

Conclusion: Estradiol plays a major part in the induction of leptin synthesis in preeclampsia.

الخلاصة

المقدمة: قبل الشنج مرض يتصف بارتفاع ضغط الدم وبروتين الادرار بعد الاسبوع العشرين من الحمل. يعتبر هذا المرض من اكثر التعقيدات المصاحبة للحمل و المسبب للوفاة. ان اسباب الشنج غير مفهومة بشكل واضح وهناك نظريات عدة وضعت في هذا المجال.

الهدف من البحث: للكشف عن العلاقة بين هرمون اللبتين و بعض الهرمونات الستيرويدية في مرضى مصابين بداء ما قبل الشنج مع الحالات المعتدلة والشديدة .

المرضى وطرق العمل: ثلاثة و ثمانون حامل مصابة بمرض ماقبل الشنج (جميعهم ينتمون الى الثلث الثالث من الفترة الحملية) تم ادخالهم في هذه الدراسة . المصابات بالمرض تم تقسيمهم الى مجموعتيين ، الاولى وتتضمن ثمانية وعشرون مع اصابة معتدلة بالمرض، والثانية خمسة وعشرون مع اصابة شديدة. المجموعة الثالثة والمتالفة من ثلاثون حامل تم اخذهن كمجموعة سيطرة. مصل اللبتين، الاستراديول، التستوستيرون الحر، تم حسابهم باستخدام تقنية الارتباط المناعي للانزيم

النتائج: زيادة هامّة في مستوى اللبتين في مصل المصابات بنوعيه المعتدل والشديد مقارنة بمصل غير مصابات في الثلث الثالث من الفترة الحملية. ايضا زيادة هامّة في مستوى الاستراديول والتستوستيرون في مصل المصابات بنوعيه المعتدل والشديد مقارنة بمصل غير مصاباتهنالك ايضا علاقة ايجابية مهمة بين اللبتين والاستراديول في مصل المصابات بنوعيه المعتدل والشديد.

الاستنتاج: هرمون الاستراديول يلعب دورا رئيسيا في تحفيز زيادة انتاج هرمون اللبتين في مرض ماقبل الشنج.

Introduction

Pre-eclampsia (PE) is a disease characterized by elevation of blood pressure and proteinuria after the 20th week of gestation. It is a common and a major complication in pregnancy causing significant morbidity and mortality. [1] The etiology of PE is still poorly understood, and several theories have

been put forward. PE is considered a two-stage disease. [2] The first stage is characterized by poor placentation resulting from shallow invasion of the trophoblasts into the maternal spiral arteries. Instead of the low-resistance high-flow system seen in the normal placenta, the PE placenta has increased resistance, a decreased blood flow and uneven perfusion leads to hypoxia, ischemia, and reperfusion injuries in the placenta. [3] The second stage of PE is characterized by a general vascular endothelial damage, which eventually affects all maternal organs. Transition from stage one, to stage two of the disease is typically seen before 35 gestational weeks in early onset PE. Placentas from early onset PE more often show histological pathological findings associated with poor placental perfusion, such as infarcts and chronic inflammation.[4] In contrast, placentas from late-onset PE, manifest after 35 gestational weeks, rarely show these pathological findings. The placental factors linking the first and second stage are not fully known. However, some placental factors that may participate in the progression to the second stage have been identified. [5]

PE is considered mild when the blood pressure is ≥ (140/90) mmHg and proteinuria is 1+ in two occasions collected 4 hour apart in urine samples. [6] The disease is classified as mild in 75% of cases, and 10% of cases occur before 34 weeks' gestation. [7] Severe PE was defined according to the American College of Obstetricians and Gynecologists criteria as new onset proteinuric hypertension and at least one of the following:

1- severe blood pressure elevation systolic blood pressure (SBP) ≥160 mm Hg or diastolic blood pressure (DBP) ≥110 mm Hg on two occasions at least six hour apart). 2- Proteinuria ≥ 2+ on dipstick analysis in urine samples collected 4 hour apart. 3- oliguria less than 500 mL in 24 hours; severe fetal growth restriction; pulmonary edema or cyanosis; or cerebrovascular accident. 4- Symptoms of central nervous system dysfunction (blurred vision, scotomata, altered mental status, severe headache, or headache that persists and progresses despite analgesic therapy). [8] 5- Symptoms of liver capsule distention (right upper quadrant or epigastric pain, nausea, vomiting); hepatocellular injury (serum transaminase concentration at least twice normal). 6- Thrombocytopenia (<100,000 platelets $/mm^3$). [9]

Previous studies indicated the changes in steroid hormone levels in preeclamptic patient. [10, 11] Other study reported the role of leptin in placental functions. [12] In this study, the relationship between leptin and some steroid hormones was investigated in mild and severe cases of the disease.

Patients and Methods

This study was conducted in Babylon Maternity and Pediatric Teaching Hospital and in the laboratory of Biochemistry Department, College of Medicine , University of Babylon in the period starting from 1st February 2012 to the end of November 2012. Fifty three pregnant women with pre-eclampsia (all of them were in the third trimester of pregnancy). These selected pre-eclamptic pregnants were divided into two groups according to gestational age and severity of disease (using the criteria of American college of Obstetrician and Gynecologist):-

The first group (G1) included 28 patients with mild pre-eclampsia , their age ranged between (20 - 42) years, with Mean ± SD (28 ± 6.3) and gestational age range was (28-38) weeks, with Mean ± SD (33.3 ± 3.24). While the second group (G2) included

25 patients with severe pre-eclampsia, their age ranged between (20 - 39) years, with Mean ± SD (27.8 ± 4.28) and gestational age range was (28-38) weeks, with Mean ± SD (34.7±3.5). All the patients were at the third trimester of pregnancy.

Thirty pregnant women apparently healthy were taken as a control group. The values Mean ± SD of systolis and diastolic blood pressure for each group are outlined in table (1). Other clinical symptoms concerning the severity of the disease were investigated by the consultant gynecologist.

After an overnight fasting (14 hours) Five milliliters of blood were obtained from patients and healthy pregnant women, then collected in tubes without anticoagulants and were left for 15 minutes at room temperature to clot. After that, the blood samples were centrifuged at 1500 xg for approximately 10 minutes. The serum was isolated and divided into three eppendrof tubes (1ml) and stored at (-20 °C) until time of use.

Serum leptin, estradiol, free testosterone were determined using ELISA kit provided by DRG, USA. The determination of Serum Estradiol and free testosterone is based on the principle of competitive binding. While Determination of leptin level was carried out using ELISA technique which is based on sandwich principle.

All statistical analysis was performed by using SPSS version 18 for windows. Data were expressed as Mean ± SD. The normality of the distribution of all variables was assessed by the Student′s F-test and Pearson correlation analysis that have been used to determine the significant difference between the two groups. P values less than 0.05 is considered significant.

Results and Discussion

Table (1) reveals the anthropometric and clinical characteristics of different groups.

The results showed nonsignificant differences in the age and gestational age among different groups. This is due to the selection of subjects who are nearly within the same age and comparable gestational age. In fact, this is an important aspect for the comparison of other parameters especially those which vary with age.

The significant changes in systolic blood pressure (SBP) and diastolic blood pressure (DBP) among different group is quite necessary for the diagnosis of PE and determining its severity where the cases were classified into mild and severe depending on those two parameters besides the presence of priteinuria.

The differences in Body mass index (BMI) were significant between preeclamptic groups (both mild and severe) and control group [table (1)]. The same significance was observed in the comparison between mild and severe groups. This anthropometric parameter is an important measure of obesity and was observed to be positively correlated with leptin in different groups. In our opinion, BMI doesn't reflect fat content accurately during pregnancy in spite of its significant correlation with leptin level because many factors affect BMI in pregnant women. Some of those are the fetus, placenta, amniotic fluid and increased plasma volume during pregnancy. For these reasons, BMI was not considered an important parameter for comparison in this study in spite of its relationship with lipid profile components. The results of this study concerning the relationship between BMI and leptin are in accordance with those obtained by Iftikhar *etal*. [13] The results in [table (2)] revealed a significant increase in free testosterone

in preeclamptic group in comparison with normal pregnancy. Previous studies that are done by Jasim [14], Ameen [15] and Radhi [16] revealed a significant increase in total testosterone levels in preeclampsia. In fact, the increase in serum free testosterone levels in this study is more important because free testosterone reflects the physiological actions of this hormone when the bound hormone is physiologically inactive, in addition to the effect of serum total protein status (especially those concerned with albumin and sex hormone binding globulin (SHBG).

The significant increase in free testosterone levels can be attributed to the following reasons:

1- Decreased testosterone clearance due to changes in kidney function in response to inflammatory factors during PE. Golmahammad *et al* [17] studied such changes in the third trimesters of pregnancy in preeclamptic women.

2- Serum human chorionic gonadotrpin (HCG) increase in pregnancy leads to increase in free testosterone through its stimulatory effect on the ovarian thecal cell where the synthesis of androstenedion and testosterone is induced. [18]

The increase in (E_2) level in the sera of preeclamptic women can be attributed to increased effect of free testosterone since the latter tends to elevate the activity of placental aromatase activity (cytochrome P 450 protein) which catalyzes the conversion of androgens to E_2. [19] In this study, this effect of free testosterone can be seen indirectly from the significant positive correlation between free testosterone and E_2 level in preeclamptic women. Also, the increase in E_2 level was observed to cope with the severity of the disease. The increase of E_2 levels in this study is consistent with those obtained by Baksu *etal*. [20]Valadan *etal*. [21] who estimated the androgen levels in preeclamptic pregnant women and also showed same results. While the results of this study disagreed with the results obtained by Larn *etal*.[22]and Bainbridge and Roberts *etal*.[23]who found no significant differences between preeclamptic women and normal pregnancy. This disagreement can be attributed to ethnic, social and environmental variations in hormone level. Also, there are differences in patient selection criteria and study protocol.

In the current study, the significant positive correlation between E_2 and leptin confirms the role of E_2 in the induction leptin synthesis while the insignificant correlation between free testosterone and leptin indicates the secondary role of free testosterone in increasing the serum leptin levels. Our results concerning changes in leptin levels among different groups are in good agreement with those obtained by Kocygit *etal*. [24]; and Mihu *etal*. [25] All those investigators found a significant increase in leptin levels that correlate well with the severity of disease. There are conflicting results that are obtained by Martinez – Abundis *etal*. [26] and Celik *etal*. [27] Who observed no statistical differences in leptin levels between preeclamptic and normal pregnancy. Such indiscrepancy may be attributed to the changes of dietary habits in different population.

In the present study, Non significant positive correlation between leptin and free testosterone was observed, while a highly significant correlation between estradiol and leptin was noticed. This indicates the role of this hormone in the pathogenesis of preeclampsia. Further future studies are needed to elucidate such a relationship and to confirm the role of inflammatory

factors as a mediator in the pathology of preeclampsia and its progression.

Table 1 The anthropometric and clinical characteristics of different groups.

Parameters	G1 Mild PE	G2 Severe PE	G3 Controls	P values
Number of subjects	28	25	30	
Age/Years Mean ± SD	28 ± 6.3	27.8 ± 4.28	29 ± 4.9	P1 > 0.05● P2 > 0.05● P3 > 0.05●
BMI Kg/m^2 Mean ± SD	30.5 ± 4.3	34.3 ± 5.9	27.2 ± 7.5	P1 < 0.01** P2 < 0.01** P3 < 0.01**
Gestational age /weeks Mean ± SD	33.3 ± 3.24	34.7 ± 3.5	35.0 ± 3.3	P1 > 0.05● P2 > 0.05● P3 > 0.05●
SBP/mmHg Mean ± SD	146 ± 2.6	168.2 ± 7.2	114.6 ± 5.72	P1 < 0.01** P2 < 0.01** P3 < 0.05*
DBP/ mmHg Mean ± SD	94 ± 2.9	114.6 ± 3.08	73 ± 4.81	P1 < 0.05* P2 < 0.01** P3 < 0.05*

*** Significant = P< 0.05 ** high significant = P< 0.01 ●= non significant (P1= Mild vs control)(P2= Severe vs control) (P3= Mild vs Severe).**

Table 2 Biochemical parameters of pre-eclamptic and control groups.

Parameters	G1 Mild PE	G2 Severe PE	G3 Controls.	P values
Number of subjects	28	25	30	
Leptin ng/ml Mean ± SD	41.55 ± 7.45	67.3 ± 10.7	23.4 ± 4	P1 < 0. 01 ** P2 < 0. 01 ** P3 < 0. 01 **
E2 pg/ml Mean ± SD	1149.96 ± 64.8	1229.5 ± 63.3	1092.7 ± 75.4	P1 < 0. 01 ** P2 < 0. 01 ** P3 < 0. 01 **
fT pg/ml Mean ± SD	2.7 ± 0.13	3.3 ± 0.142	1.52 ± 0.153	P1 < 0.01 ** P2 < 0.01 ** P3 < 0.01 **

***Significant = P< 0.05 **high significant = P< 0.01 ●= non significant (P1= Mild vs control)(P2= Severe vs control) (P3= Mild vs Severe)**

Table 3 Pearson's correlation between the levels of leptin and each of BMI , E2, fT in mild group (G1) (n=28).

Parameters	Leptin	
	r	P values
BMI	0.523	P < 0.01 **
Free testosterone (fT)	0.04	P > 0.05 •
Estradiol ($E_{2)}$	0.61	P < 0.01**

*Significant = P< 0.05 ** high significant = P< 0.01 ●= non significant.

Table 4 Pearson's correlation between the levels of leptin and each of BMI , E2, fT in severe group (G2) (n=25).

parameters	leptin	
	r	P values
BMI	0.603	P < 0.01 **
Free testosterone (fT)	- 0.103	P > 0.05 •
Estradiol ($E_{2)}$	0.78	P < 0.01 **

*Significant = P< 0.05 ** high significant = P< 0.01 ●= non significant

Table 5 Pearson's correlation between the levels of leptin and each of BMI , E2, fT in control group (G3) (n=30).

parameters	leptin	
	r	P values
BMI	0.361	P < 0.05 *
Free testosterone (fT)	0.096	P > 0.05 •
Estradiol ($E_{2)}$	0.55	P < 0.01 **

*Significant = P< 0.05 ** high significant = P< 0.01 ●= non significant.

Table 6 Pearson's correlation between the levels of free teststerone and in different groups (n= 83)

parameters	Groups	Free testosterone (fT)	
		r	P values
Estradiol (E_2)	G1	0.42	P < 0.05 *
Estradiol (E_2)	G2	0.61	P < 0.01 **
Estradiol (E_2)	G3	0.09	P > 0.05 •

*Significant = P< 0.05 ** high significant = P< 0.01 ●= non significant.

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