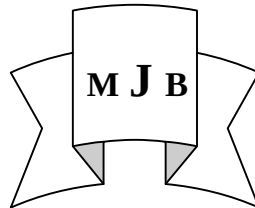


The Association of Serum Androgen Levels with Preeclampsia

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Abstract

Background: Preeclampsia is a major cause of morbidity and mortality during pregnancy. Several independent investigators have demonstrated the association of androgens with hypertension.

Objective: To determine whether maternal serum levels of testosterone and dehydroepiandrosterone sulphate DHEA-S, are higher in patients with preeclampsia than in matched normotensive control subjects.

Method: Case control study included 29 subjects in the 3rd trimester of pregnancy with documented preeclampsia (including 10 cases of mild and 19 cases of severe preeclampsia) and 20 healthy normotensive women in the third trimester of pregnancy used as control group and for comparative purpose another 13 patients with gestational hypertension were also included in our study. All cases had singleton pregnancies. Cases of polycystic ovary syndrome (PCOS), diabetes, chronic hypertension and chronic systemic diseases such as lupus and patients using steroid drugs were excluded.

Serum levels of total testosterone were measured using testosterone enzyme immunoassay test kit, Biocheck (USA), while dehydroepiandrosterone sulfate (DHEA-S) was measured using DHEA-S enzyme immunoassay test kit, DRG (USA, Germany).

Results: Student t-test was used to analyze the difference in androgen levels between the different groups. Serum total testosterone level was significantly higher in patients with severe pre-eclampsia compared to control group (0.41ng/mL versus 0.31ng/mL, P value 0.005), while no significant difference was found between cases of mild and severe pre-eclampsia. Again no significant difference was found when comparing patients with mild pre-eclampsia with control group and cases of gestational hypertension with control group.

Serum level of dehydroepiandrosterone sulfate (DHEA-S) was significantly higher in patients with severe pre-eclampsia compared to patients with mild pre-eclampsia (1.03 µg/mL versus 0.32 µg/mL; P value 0.005) and when compared with control group (1.03 µg/mL versus 0.59 µg/mL; P value 0.02), while the level was not significantly higher when comparing cases of mild pre-eclampsia with control group and cases of gestational hypertension with control group.

Conclusion: Levels of total testosterone and dehydroepiandrosterone sulfate (DHEA-S) were significantly higher in women with severe preeclampsia than in normotensive women. The levels of these androgens were higher in women severe pre-eclampsia when compared to women with mild pre-eclampsia and those with gestational hypertension. This difference may indicate a role for androgens in the pathogenesis of preeclampsia and stimulates research in the potential role of anti-androgens in the management of preeclampsia.

الخلاصة

يعتبر مرض ما قبل الارتعاج احد الاسباب الرئيسية للأمراضية و الوفاة اثناء الحمل وقد وجد بعض الباحثين ان هناك علاقة بين الهرمون الذكري و ارتفاع الضغط.

الهدف من الدراسة هو لمعرفة ما اذا كان مستوى الهرمونات الذكرية (التستوستيرون و الديهايدروابي اندروسترون) اعلى في دم المريضات المصابات بما قبل الارتعاج عند مقارنتهن بالحوامل الطبيعيات.

اشتملت الدراسة تسع وعشرون امرأة حامل تعاني من مرض ما قبل الارتعاج (عشر منهن كانت شدة المرض لديهن خفيفة وتسع عشر كان لديهن المرض شديد) علما ان شدة المرض تقاس وفق مجموعة من المؤشرات. كما اشتملت الدراسة عشرين امرأة حامل طبيعية و ثلاث عشر امرأة حامل يعانين من ارتفاع ضغط الحمل. كانت جميع النساء في الثلث الاخير من الحمل. تم استبعاد النساء اللاتي يعانين من متلازمة تكيس المبايض، ارتفاع ضغط الدم المزمن او أي امراض مزمنة اخرى.

تم سحب عينات الدم من النساء تحت الدراسة و قياس مستوى التستوستيرون و الديهايدروابي اندروسترون. استنتجت الدراسة ان مستوى التستوستيرون الكلبي و الديهايدروابي اندروسترون اعلى في دم الحوامل اللاتي يعانين من مرض ما قبل الارتعاج الشديد عند مقارنتهن بالحوامل الطبيعيات مما يدل على احتمال وجود دور للهرمونات الذكرية في امراضية ما قبل الارتعاج و اوصت الدراسة باجراء بحوث اوسع لمعرفة اثر مضادات الهرمونات الذكرية في علاج مرض ما قبل الارتعاج.

Introduction

Preeclampsia is a pregnancy-specific, multisystemic disorder that is characterized by the development of hypertension and proteinuria after twenty weeks of gestation in a woman with previously normal blood pressure [1-3]. The disorder complicates approximately 3-5% of pregnancies. Preeclampsia is a major cause of morbidity and mortality during pregnancy [4]. Although the exact cause of preeclampsia remain unclear, systemic vasospasm, vascular hemoconcentration, exaggerated inflammatory response, inappropriate endothelial activation, activation of coagulation cascade and resultant microthrombi formation have all implicated [5].

The general consensus is that preeclampsia is an endothelial cell disorder resulting in mild-to-severe microangiopathy of target organs such as brain, liver, kidney, and placenta [6]. While hypertension may be the most common presenting symptom, it should not be viewed as the initial

pathogenetic process. Evidence of other organ involvement before hypertension becomes fulminant is not uncommon. Several circulating markers of endothelial cell injury have been shown to be elevated in women who develop preeclampsia before they became symptomatic. These include endothelin, cellular fibronectin, plasminogen activator inhibitor-1, and altered prostacyclin/thromboxane profile. Evidence to date suggests that oxidative stress; circulatory maladaptation; inflammation; and humeral, mineral, and metabolic abnormalities may all contribute to endothelial dysfunction and pathogenesis of preeclampsia [7]. Factors that may be responsible for pathophysiological changes include the renin-angiotensin system, eicosanoids and platelets [8].

Several independent investigators have demonstrated, through human and animal studies, the association of androgens, especially testosterone, with hypertension [9-12]. Interestingly accumulating evidence indicates that

androgens have important effects on vascular reactivity, the renin-angiotensin system, eicosanoids, and platelets, in ways that are strikingly similar to those reported for preeclampsia [8]. Some studies have shown that women with polycystic ovary (PCO), a disease associated with hyper-androgenism, are at risk for pregnancy induced hypertension independent of body mass index (BMI) [8]. It has been suggested that overproduction of steroid hormones, especially androgens, is the main factor for appearance of preeclampsia in PCO patients [11]. Recently it has also been found that serum concentrations of inhibin A were higher in patients with preeclampsia than in control subjects with matched pregnancies and this finding was interpreted as further evidence for trophoblastic dysfunction in preeclampsia [13].

Additionally, inhibin was recently shown to increase androgen production by ovarian theca cells, in turn increasing circulating androgen levels in women. It is thus possible that effects of increased serum inhibin in preeclampsia may be manifested through increased circulating androgen levels [8].

Preeclampsia is classified into mild and severe types and, in its extreme, may lead to liver and renal failure, disseminated intravascular coagulopathy, and central nervous system abnormalities, including seizures [14].

Mild preeclampsia is defined as the presence of hypertension (BP >140/90 mm Hg) on 2 occasions, at least 6 hours apart. Proteinuria is defined as the presence of greater than or equal to 1+ protein on random dipstick or at least 300 mg of protein in a 24-hour urine collection. Edema and hyper-

reflexia are no longer considered to be diagnostic criteria.

Severe preeclampsia is defined as the presence of one of the following symptoms or signs in the presence of preeclampsia:

- Systolic BP of 160 mm Hg or higher or diastolic BP of 110 mm Hg or higher on 2 occasions at least 6 hours apart
- Proteinuria of more than 5 g in 24-hour period
- Pulmonary edema
- Oliguria (<400 mL in 24 h)
- Persistent headaches
- Epigastric pain and/or impaired liver function
- Thrombocytopenia
- Intrauterine growth restriction [14]

Aim of Study

To determine the relationship between maternal serum levels of testosterone and dehydroepiandrosterone sulphate (DHEA-S) and the development of pre-eclampsia.

Patients and Methods

The study was conducted in Babylon Teaching Hospital for Gynaecology and Paediatrics from February 2009 to September 2009. The study included sixty two pregnant women in the third trimester with singleton pregnancy. Twenty nine women had pre-eclampsia, nineteen of them had severe pre-eclampsia and ten had mild disease. Twenty women had normal pregnancy with normal blood pressure used as control group. Another 13 women had gestational hypertension included in this study for comparison. Thorough assessment of these women which included history, physical examination and investigations in the

form of complete blood count, liver function test, renal function test, coagulation profile and ultrasound and sometimes Doppler study of umbilical artery. Exclusion criteria included women with previous history of polycystic ovary syndrome, diabetes, chronic hypertension or other chronic systemic diseases and patients using steroid drugs. Blood samples were collected from women under study and sera isolated and stored at -20° C.

Testosterone and DHEA-S ELISA kits are solid phase enzyme-linked immunosorbant assay based on principle of competitive binding. The microtiter wells are coated with a polyclonal antibody directed towards an antigenic site on testosterone or DHEA-S molecule. Endogenous testosterone or DHEA-S of a patient sample competes with a testosterone or DHEA-S horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off. The amount of bound peroxidase conjugate is inversely proportional to the concentration in the sample. After addition of substrate solution, the

intensity of color developed is inversely proportional to the concentration in the patient sample. Serum testosterone was measured in nanogram per milliliter (ng/mL) while serum DHEA-S was measured in microgram per liter ($\mu\text{g/L}$).

Statistical analysis: SPSS (version 10) program was used for statistical analysis. The results were represented through mean, standard deviation and standard error of the mean. Student t-test was used to compare between means and to study the significance of the difference. P value < 0.05 was considered to be statistically significant.

Results

Table no. 1 shows the maternal characteristics regarding patients' age and gestational age for the different groups of women under study. The mean maternal age was lowest in patients with mild PE (24.5 years) while it was highest in patients with gestational hypertension. Mean gestational age was lowest in patients with severe PE while it was highest in control group.

Table 1 Maternal age and gestational age in study groups

groups	No.	Maternal age (yr) Mean $\pm$ SD	Gestational age (wk) Mean $\pm$ SD
Severe PE	19	26.9 $\pm$ 5.5	35.3 $\pm$ 3.9
Mild PE	10	24.5 $\pm$ 4.6	37.7 $\pm$ 2.2
Gestational HT	13	28.9 $\pm$ 4.3	38.2 $\pm$ 1.5
Control	20	26.4 $\pm$ 5.1	38.4 $\pm$ 1.8

Table no.2 shows the maternal serum total testosterone level in different groups. The highest value seen in patients with severe PE (0.41 $\pm$ 0.06

ng/mL) while the lowest value seen in normotensive women (0.31 $\pm$ 0.10 ng/mL).

Table 2 Maternal serum total testosterone level in the study groups

Groups	No.	mean S. total testosterone level ng/mL	Standard deviation
Severe PE	19	0.41	0.067
Mild PE	10	0.38	0.101
Gestational hypertension	13	0.38	0.073
Control	20	0.31	0.105

Table no.3 shows a comparison between different groups regarding maternal serum total testosterone level. The comparison performed using student t test. Significant difference was found between patients with severe PE and normotensive women (P value 0.005) while no significant difference

was found when comparing patients with severe PE with patient with mild PE or gestational hypertension. Again no significant difference was found when comparing the two latter groups with each other or with normotensive women.

Table 3 comparison of maternal serum total testosterone level between different study groups using t test

Group comparisons	df	t value	P value
severe PE and mild PE	18	1.7	0.113
Severe PE and gestational HT	24	0.77	0.47
severe PE and control	36	3.2	0.005
mild PE and gestational HT	18	0.023	0.82
mild PE and control	18	1.64	0.13
gestational HT and control	24	1.88	0.83

Table no.4 shows the mean maternal serum DHEA-S level in the groups under study. The highest level is found in patients with severe PE ($1.14 \pm$

$0.9 \mu\text{g/mL}$) while the lowest level is found in patients with mild PE ($0.22 \pm 0.17 \mu\text{g/mL}$).

Table 4 Maternal serum DHEA-S level in groups under study

Groups	No.	mean S.DHEA-S level $\mu\text{g/mL}$	Standard deviation
Severe PE	19	1.14	0.90
Mild PE	10	0.22	0.17
Gestational hypertension	13	0.82	0.078
Control	20	0.52	0.057

Table no.5 shows a comparison of mean maternal serum DHEA-S level between the different groups. The level was significantly higher in patients with severe PE when compared to patients with mild PE and normotensive women ($1.14 \pm 0.9 \mu\text{g/mL}$

versus $0.22 \pm 0.17 \mu\text{g/mL}$ and $0.52 \pm 0.05 \mu\text{g/mL}$), P value was 0.005 and 0.02 respectively. No significant difference was found when comparing patients with mild PE and gestational hypertension with each other or with the control group.

Table 5 comparison of maternal serum DHEA-S level between different groups using t test

Groups comparisons	df	t value	P value
severe PE and mild PE	18	3.7	0.005
Severe PE and gestational HT	24	0.81	0.43
severe PE and control	36	2.4	0.02
mild PE and gestational HT	18	0.02	0.06
mild PE and control	18	0.011	0.27
gestational HT and control	24	1.26	0.23

Discussion

The pathophysiology of pre-eclampsia is still obscure. Steroid levels have been reported to be abnormal in women with pre-eclampsia.

In our study, maternal serum total testosterone level was found to be significantly higher only when

comparing patients with severe PE with normotensive women. However, a study done by Acromite et.al, who compared serum total testosterone in women with PE (without classifying them into mild and severe) with normotensive women found that the level was significantly higher in women with PE.[8] Similar result was found in a study done by Emanouel

et.al.[15] Another study done by Ziotopoulou M et.al who compared serum leptin, androgens and estrogen in women with PE, with normotensive women found similar results.[16] Youssef H et al, found in their study that total and free testosterone levels were significantly higher in primigravid women with preeclampsia and antepartum eclampsia than in normotensive women with similar gestational and maternal ages.[17]

This may be explained by the effects of testosterone on an enzyme, epoxide hydrolase, associated with preeclampsia. Steegers E.A., reported a connection between genotype variability of the gene for "epoxide hydrolase" and the incidence of preeclampsia. "Microsomal epoxide hydrolase is an important enzyme involved in the metabolism of endogenous and exogenous toxicants." The "high activity genotype" occurs more often (29%) in preeclampsia than in controls (16%). He concluded that: "Women with the high activity genotype in exon 3, which could reflect differences in metabolic activation of endogenous or exogenous toxic compounds, may have enhanced susceptibility to pre-eclampsia." [18]

On the other hand a study done by S.Taghavi showed that there is no significant difference in total serum testosterone level when comparing women with PE with normotensive women.[19] Similar results were found by Valadan M. et.al who compared women with severe and mild PE with normotensive women.[20] Miller NR et.al. also found no significant difference in total serum testosterone level when compared preeclamptic patients with normotensive women.[21]

Regarding serum DHEA-S, we found in our study significantly higher

level in patients with severe PE when compared with cases of mild PE and the control group. While studies done by Acromite et.al,[8] Emanouel et.al, [15] Ziotopoulou M et.al [16] and others found no significant difference in serum DHEAS level when comparing women with preeclampsia with normotensive women.

Conclusions

1. Levels of the serum total testosterone were significantly higher in women with severe preeclampsia than in normotensive women
2. the levels of serum DHEA-S were significantly higher in women with severe PE compared to women with mild PE and normotensive women.
3. This difference may indicate a role for androgens in the pathogenesis of preeclampsia.

Recommendations

1. Further studies about the association of other androgens with preeclampsia are needed.
2. studies involving the role of androgens in the pathogenesis of preeclampsia are needed
3. Researches in the potential role of anti-androgens in the management of preeclampsia are encouraged.

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