Research Article

Association between preeclampsia and hyperandrogenemia

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Abstract

Background; Insulin resistance and increased serum free testosterone are well known to be associated with preeclampsia
Type of study; case control
Aim of the study; to compare serum levels of free testosterone among hypertensive preeclamptic women versus control
Methods; two groups were constructed from primigravida women taken from labor ward. Study group (N=30) were preeclamptic versus control (n=30). For all women serum testosterone was assayed and compared and correlated to mean arterial blood pressure and other variables in preeclampsia
Result; serum free testosterone was significantly higher among women with preeclampsia compared with control 5.00±0.72 versus 1.28±0.97, P < 0.001. No Significant change was found between serum estriol levels in both groups. Analysis of co-variants has shown that fetal gender (Male) has no effect on maternal serum testosterone levels.
Conclusions; preeclamptic women in the third trimester have significantly higher free testosterone serum levels than control women. It is not clear yet whether this increase in free testosterone is related to insulin resistance in the placenta or other factors. Further studies are required to explore the significance of increased testosterone levels in the blood with prediction of preeclampsia and assessment of its severity.

Keywords; free testosterone, preeclampsia, insulin resistance

INTRODUCTION

Preeclampsia remains one of the most mysterious disorder affecting uniquely human females. Simply, defective placentation overproduces placental rennin and thromboxane [1]. Rennin is responsible for hypertension while thromboxane is responsible for low grade spread disseminated intra vascular coagulation syndrome [2]. Deposition of fibrin on glomerular basement membrane is responsible for unique type of nephritis leading to proteinuria. At histological level defective secondary wave of trophoblastic invasion in the second trimester is the main histopathological picture [2]. Despite the exact cause of preeclampsia still unknown yet immunological factors play a significant role in its genesis. In 1999 Acromite et al have published a paper in the American Journal Of Obstetrics And Gynecology referring that women with preeclampsia have significantly increased free and total serum testosterone than control [3]. The description of a definite and constant correlation between preeclampsia and insulin Resistance have changed the whole picture [4]. It was well established from the extensive studies among women with polycystic ovary syndrome that insulin resistance is almost always associated with increased serum levels of androgenic steroids [5]. So the aim of this study is to evaluate serum levels of total and free testosterone among women with preeclampsia versus control between 36- 40 weeks of gestation. In addition, to correlate between maternal serum testosterone levels with various hematological, biochemical and biophysical variables associated with preeclampsia.

PATIENTS AND METHODS

This study was conducted in AL-Yarmook Teaching Hospital between April 2011 and October 2012. During this period a total of 60 primigravida women have been chosen to participate in this study. The study was approved by the Iraqi commission for medical specialization. Of the 60 women enrolled in this study

30 women were preeclamptic and represented the study group, while 30 control normotensive women matched for age were selected as control. During the conduct of this study we have encountered two major obstacles. First the number of hormone assays allowed per one day was very limited and sometimes restricted to two patients only. The other obstacle is that sometimes the complete set of hormone assays was not available at many times. And this explains the relatively low number of women in this study. For all the patients their verbal consent were taken and agreed to participate in the study.

Virtually all the women who have been enrolled in this study were slected from the labor ward who bypassed 36 weeks of gestation. Initially thorough medical and obstetrical examination was done to assess the general condition of women. Samples of blood and urine were taken and sent for hemoglobin level, blood sugar, uric acid, platelets count in addition liver function test were sent to exclude complicated preeclampsia. In addition a fraction of blood sample was sent immediately to the endocrine department for estimation of total and free testosterone, estriol and dehydroepiandrosterone sulphate. Ultrasound was done to assess amniotic fluid index. Proteinuria was defined as excretion of 300 mg of protein or more per 24 hours and virtually almost all preeclamptic patients admitted have higher than 1 gram per 24 hours. As all the patients enrolled in this study were at labor, assessment of 24 hours urinary excretion was rather difficult. Instead, we take a simple ratio of albumin/ creatinin in random urine sample as reflection of albumin excretion severity among women. On the other hand hypertension was defined as 2 readings 149/90 mmHg 4- 6 hours apart, or single reading of diastolic blood pressure 110 mmHg or more. Mean arterial blood pressure was assessed by the following formula

$$MAP \simeq DP + \frac{1}{3}(SP - DP)$$

Where MAP ; mean arterial blood pressure, SP systolic blood pressure, DP diastolic blood pressure.

All hormone assays conducted in this study were done with ELIZA technique using the DRG instrument made in Germany. Results whether biochemical or hormonal were expressed as mmol/litter Exclusion criteria included any hypertensive women with other risk factor like diabetes, placental previa were excluded immediately from the study to reduce bias in results and women with complications of severe preeclampsia. Eventually we have study group (N=30) consist of primigravida women with preeclampsia versus control group (N=30) who are primigravida with low risk pregnancy. For both groups labor was monitored throughout labor as active management. After delivery birth weight was measured accurately and recordered for statistical analysis.

Statistical analysis

Power analysis was done to estimate the minimum sample size required for each group and found to be 25 patients. Continuous data were expressed as mean and standard deviation while categorical data were expressed as mean and standard deviation. Chi square was used to compare categorical variables while t student test for continuous data. Regression lines with calculation of coefficient of correlation was done among continuous data taking free serum testosterone as primary independent variable against which various variables were correlated. Analysis of co- variants or ANCOVA was used to rule out the fetal sex in causing differences in serum testosterone levels among study groups. P values less than 0.05 were considered as significant.

RESULT

In table 1 the overall epidemiological characteristics are shown. As far as table 1 is concerned the main points to refer here is the significantly higher values of systolic, diastolic and mean arterial blood pressure. Platelets count as well as serum uric acid is also higher among women with preeclampsia. However the main point to notice is the significantly higher total and free serum testosterone levels in women with preeclampsia; 2.04 versus 0.36 for total testosterone and 5 versus 1.28 mmol/l, for free testosterone. Women in the study group have significantly earlier onset of labor in weeks than control. However no statistically significant differences were found among the two groups with regard to age, body mass index and offspring gender. Serum dehydroepiandrosterone sulphate and estriol are both higher in study groupversus control yet not statistically significant.

In order to show a basic correlation between serum free testosterone and systolic and diastolic blood pressure readings, a 3 dimensional surface plot was constructed as in figure 1. Clearly as the systolic and diastolic values in x and y axes increase, serum testosterone increase in y axis. This gives a hint that a proportional relationship exist between them.

Figure 2 simplifies basically the hypothesis upon which this paper is built. A regression line is constructed between free serum testosterone as independent variable and means arterial blood pressure as the dependent variable shows simply a proportional relationship between the two variables.

Table 1 the epidemiological characteristics for both study groups

characteristics	*Study group (N=30)*	*Control Group (N=30)*	*P Value*
Maternal Age (years)	**24.57± 5.66**	**23.95±3.9132**	**P = 0.6878**
Gestational age at delivery (Weeks)	**37.4211±0.6925**	**38.7500±0.9105**	**P < 0.0001**
Body Mass Index	**27.73±3.07**	**29.55±2.5644**	**P = 0.0522**
Systolic Blood Pressure mmHg	**170.00±13.74**	**121.25±6.46**	**P < 0.0001**
Diastolic Blood Pressure mmHg	**109.21±9.46**	**74.00±5.52**	**P < 0.0001**
Mean Arterial Pressure/ mmHg	**129.45±10.19**	**89.63±4.82**	**P < 0.0001**
Fetal Weight (Gram)	**2857.89±332.19**	**3480.00±320.52**	**P < 0.0001**
Amniotic Fluid Index (cm)	**8.89±1.79**	**10.3000±1.62**	**P = 0.0144**
Male Gender (newborn)	**8(42%)**	**12(51.3%)**	**0.4254**
Platelets Count (per cubic mm)	**184.47±41.19**	**243.00±13.41**	**P < 0.0001**
Serum Uric Acid (mmol/L)	**277.94±84.56**	**208.05±16.87**	**P = 0.0009**
Protein/ Creatinin Ratio	**0.84±0.35**	**0.084±0.031**	**P < 0.0001**
Total Testosterone (mmol/L)	**2.04±0.77**	**0.36±0.23**	**P < 0.0001**
Free Testosterone (mmol/L)	**5.00±0.72**	**1.28±0.97**	**P < 0.0001**
Dehydroepiandrosterone Sulphate(mmol/L)	**1.22±0.56**	**1.21±0.42**	**P = 0.9449**
Estriol (mmol/L)	**165.05±36.29**	**160.30±39.08**	**P = 0.6966**

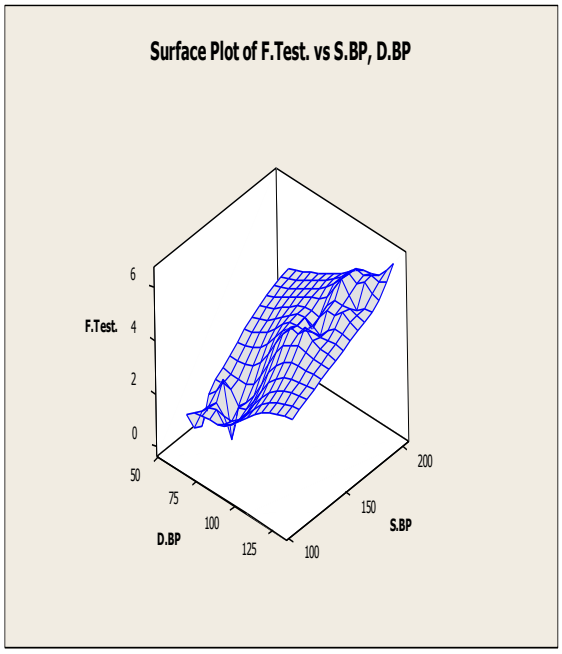

Figure 1 a three dimensional surface plot for free testosterone as z axis versus systolic and diastolic blood pressure values as x and y axes.

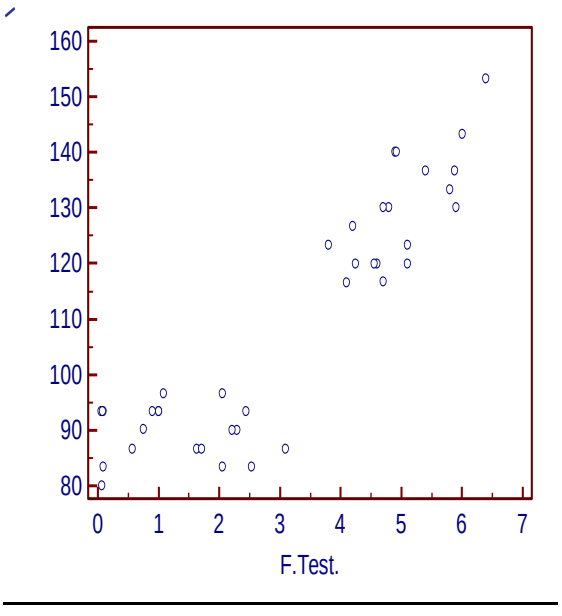

Figure 2 a regression line between mean arterial pressure and free serum testosterone

Regression Equation

y = 79.7555 + 9.4462 x					
Parameter	Coefficient	Std. Error	95% CI	t	P
Intercept	79.7555	2.7470	74.1895 to 85.3214	29.0338	<0.0001
Slope	9.4462	0.7404	7.9460 to 10.9464	12.7578	<0.0001
Coefficient of Correlation		0.8148			

In the same context a series of regression lines with calculation of the coefficients of correlations has been done. In table two above simply the coefficient value with its associated P significance are shown. From the first glance all the correlations are significant. Those variables chosen here in this table represents the variable which were found to be significant in table 1 as well as such correlated to the severity of preeclampsia. Both systolic and diastolic blood pressures are shown to be directly associated with free serum testosterone in the last 4 weeks of pregnancy. While on the other hand fetal weight, gestational age at delivery, amount of liquor is inversely related to maternal serum free testosterone. Platelets count is also inversely correlated signifying the degree of low level disseminated intravascular coagulation syndrome in the blood. Lastly protein over creatinin ration or in other words total 24 hours urinary protein excretion is significantly and inversely related to serum free testosterone level among preeclamptic women.

Table 2 showing coefficients of correlation between free testosterone and some variable taken in this study

Parameter	**Correlation coefficient r**	**P values**
Systolic Blood pressure versus testosterone	0.8874	P<0.0001
Diastolic Blood Pressure versus testosterone	0.8921	P<0.0001
Serum Uric Acid versus testosterone	0.4774	P<0.0001
Platelet Count versus testosterone	-0.7502	P<0.0001
Amniotic Fluid Index versus testosterone	-0.4590	P=0.0033
Protein Over Creatinin ratio versus testosterone	0.8630	P<0.0001
Fetal Weight versus testosterone	-0.7633	P<0.0001
Gestational Age at Delivery versus testosterone	-0.6914	P<0.0001

Analysis of fetal sex as covariates (ANCOVA)

In table 3 in which analysis of co variants taken in this study is shown in clear cut way. Neither maternal age, gestational age at delivery, were serum estriol and dehydroepiandrosterone serum levels associated significantly with maternal serum free testosterone. More importantly fetal sex is clearly shown in this study that fetal gender has nothing to do with maternal serum testosterone level; P Value= 0.523. Even when interaction with the only variable responsible for variability in serum testosterone the P value was not significant, P= 0.418.

Table 3 showing the significance of co variants taken in this study especially with regard to fetal sex

Variable	**F**	**P**
Maternal Age	**0.403**	**0.530**
Gestational Age At Delivery	**1.932**	**0.174**
Estriol Serum Level	**0.167**	**0.686**
Dehydroepiandrosterone Serum Levels	**2.272**	**0.142**
Fetal Sex (Gender)	**0.418**	**0.523**
Preeclampsia	**84.253**	**<0.001**
F.sex*Preeclampsia	**0.674**	**0.418**

DISCUSSION

As far as the results of this paper are concerned, simply women with preeclampsia have both total and free testosterone levels higher among women with preeclampsia than controls. To explain this finding we shall review the various articles published previously about this subject.

Acromite[6] et all in his study found that free serum testosterone increased in women with preeclampsia versus control. Our study design was based on his paper. Yet all the discussion in his paper was speculative rather than conclusive. Some researchers suspected that women with preeclampsia have increased serum testosterone levels. Valdan et al from the university of Tehran in a prospective study including 120 women half of them are preeclamptic have reached a conclusion that androgen serum levels are not affected by preeclampsia [7]. In his study the preeclampsia group included a wide varieties of women including obese, lean and women with polycystic ovary syndrome. In 2011 a state of the art article was published in American journal of obstetrics and gynecology by John et all [8]. In this paper based on retrospective data found that insulin resistance is significantly associated with preeclampsia incidence and increased testosterone production. Unlike

other situations of insulin resistance like polycystic ovarian women, the placenta itself is resistant to insulin which leads to a complicated chain of events leading to testosterone over production. Inhibin A is secreted from the placenta which has a direct effect on the ovarian theca cells ultimately leading to increased ovarian testosterone release [9]. Te-Yao Hsu [9] from Taiwan University has provided the first clue. Te-Yao has demonstrated that the placentas of preeclamptic women have increased gene mediated androgen receptor in syncytiotrphoblast. In addition serum testosterone levels were significantly increased over control in his study. So the primary pathology in preeclampsia is still lying within the abnormal placenta itself. In addition Te-Yao has noticed that placentas of preeclamptic women have increased production of Inhibin-A. The last mediator is well known factor to increased free testosterone from theca cells in the ovary. However that lead to another question which include why a placenta with insulin resistance mediate increased production of testosterone? The answer came from the work of James et al [10]. Increased testosterone levels modulate smooth muscle fibers in blood vessels in such a way making them more responsive to catecholamines circulating in the blood so to increased blood perfusion to many vital organs, yet not the placenta among them. Johan and his colleagues [11] found that preeclamptic women with male fetus have significantly higher free testosterone and human chorionic gonadotropin. Actually he didn't give actual explanation worth to mention here. We don't know exactly why his results contradict those of this study. Sample size may be a factor in this difference. Other possibility is that the factors taken in his study may be quite different than this study. Nancy et al [12] have found increased levels of activated complement components suggesting that inflammatory process play a significant role in the pathogenesis of preeclampsia. While Stefanović et al[13] have described increased maternal C Reactive protein among women with preeclampsia versus control. The reasons for this are probably two factors. First inhibin- A which is the factor promote increased production of testosterone from the ovary is associated with mild inflammatory process. Or the effect of testosterone itself on small blood vessels is associated with mild inflammatory reaction which is associated with increased inflammation marker. Wolf et al [14] have found that first trimester free testosterone and sex hormones binding globulin levels as predictor of preeclampsia surpass greatly even the state of art Doppler study. However this subject is left for other researcher in the future. Finally Thadhani [15] et al have evaluated the angiotensin versus insulin resistance among preeclamptic women, he found altered angiogenesis and insulin resistance are additive insults that lead to preeclampsia. In other words angiotensin receptor or even yet unknown receptors may play a role on preeclampsia onset and severity. Last word to say that this brief study has shown increased free testosterone among women with preeclampsia which has strong correlation with various variables associated with preeclampsia. Further studies are required to evaluate the role of increased androgen levels and insulin resistance in the pathogenesis, management and prediction of preeclampsia.

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