The role of Testosterone in Preeclampsia

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Abstract

Background: Preeclampsia is a form of high blood pressure manifested during pregnancy, it is a common major complication causing significant morbidity and mortality; however, its etiology is still unknown.

The systemic vasculature is a target tissue for sex steroid hormone. Estrogen, androgen, and progesterone all influence the function and pathophysiology of the systemic circulation by influencing endothelial derived nitric-oxide pathway.

Objective: *was* to demonstrate the pattern of sex steroid (testosterone) in preeclampsia with respect to normal pregnancy, and the correlation of the above parameter with nitric-oxide pathway.

Subject and methods: The present study is a cross-sectional case-control study includes measurement of nitric oxide, nitric oxide synthase, and sex steroid (testosterone) in 60 patients with preeclampsia. They were classified, according to the gestational age, into two groups:

*Preeclamptics in the second trimester G1: (n=30).

*Preeclamptics in the third trimester G2: (n=30).

The results were compared with 60 apparently healthy pregnants (control group), who were, also, classified according to the gestational age into two groups:

- Pregnants in the second trimester G3: (n=30).
- Pregnants in the third trimester G4: (n=30).

Results: showed a significant reduction in serum NO and NOS in the preeclamptics as compared to the controls which was accompanied by a significant increase in serum testosterone. The inhibitory effect of testosterone on NO production is supported by negative correlation between these parameters.

The disturbance in vasodilation state and testosterone can be attributed to malfunction placenta, and it varies according to the gestational age and advancing disease state; being the best in G4 (normal pregnants in the third trimester), and the worse in G2 (preeclamptics in the third trimester) as indicated by NO measurement.

Conclusion: preeclamptics (in different gestational age groups) experienced vasospasm, hyperandrogenemia when compared with healthy pregnants matched with their age and gestational age.

Key words: preeclampsia, nitric oxide, testosterone,Testosterone in preeclampsia.

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Introduction

Preeclampsia is defined as the onset of hypertension and the presence of proteinuria during pregnancy, usually occurring after the 20th week of gestation in a previously normotensive woman and resolving completely by the sixth week after delivery of fetus [1, 2].

The pathophysiology of preeclampsia is thought to represent a defective response to the physiologic demands of normal pregnancy [2, 3]. Endocrine changes in pregnancy are largely dependent on the concerted production of protein and steroid hormones by the fetoplacental unit [4]. These endocrine changes support the successful establishment, maintenance, and termination of pregnancy [4]. It has

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been established that high androgen level, primarily dependent on placental function, is a factor in the etiopathogenesis of preeclampsia [5, 6]. Nitric oxide (nitrogen monoxide) plays an important role in a wide range of physiologic processes [7]. A major mediator of endothelial function, NO, regulates vasodilatory and antithrombotic actions in the vasculature [7]. Impaired NO bioactivity has been postulated as an important pathogenic factor in preeclampsia [7]. Endothelium-dependent arterial vasodilation has been shown to be reduced and vascular impedance to be increased in preeclampsia compared with normal pregnancy [7]. Postpregnancy, women with a history of preeclampsia (3 months postpartum or later) have significantly reduced endothelium-dependent vasodilation compared with women with a history of normal pregnancy [7]. Also, NO is mainly expressed in Leydig cells where it regulates the concentration of testosterone by acting in an autocrine/paracrine fashion. In fact, NO is involved in testicular testosterone synthesis causing a significant decrease of androgen production [8].

The present study was undertaken to elucidate the role of sex steroid (testosterone) on endothelial dysfunction in preeclampsia.

Subjects & Methods

A-Patients: The study was a cross-sectional, case-control study conducted on sixty patients with preeclampsia (PE) attending the Obstetric Consultant-Clinic, Antenatal Clinic, and Labor Ward at Al-Kadhimiya Teaching Hospital, for re-evaluation of newly diagnosed PE, or for delivery.

The diagnosis of PE was based on clinical criteria that were hypertension (absolute BP of 140/90 mmHg twice over 4 hr without prior comparison) [1, 2] and proteinuria (21.5 mg of urinary protein per mmol creatinine) [9].

The exclusion criteria, which were used for cases and controls, were gestational or chronic hypertension, diabetes mellitus, renal disease, multifetal gestation, intrauterine fetal death, and pregnancy less than 20 weeks of gestation.

Depending on the gestational age, the patients were divided into two groups:

1. Preeclamptics in the second trimester (G1):

Included thirty Preeclamptics in their second trimester of pregnancy. Age range was from 18 to 37 years (mean age $\pm$ SD = 26.1 $\pm$ 6.4 year). The gestational age range was from 20 to 28 weeks (mean gestational age $\pm$ SD = 26.3 $\pm$ 1.5 week).

2. Preeclamptics in the third trimester (G2):

Included thirty preeclamptics in their third trimester of pregnancy. Age range was from 18 to 40 years (mean age $\pm$ SD = 25.1 $\pm$ 6.9 years). Gestational age ranged from 29 to 40 weeks (mean gestational age $\pm$ SD = 35.6 $\pm$ 1.6 week).

Controls: Sixty apparently healthy pregnants attending the Antenatal clinic, and Labor Ward at Al-Kadhimiya Teaching Hospital, for re-evaluation of their pregnancy, or for delivery. The control groups were comparable to the preeclamptic groups regarding the age, gestational age, Depending on the gestational age, the apparently healthy pregnants were divided into two groups:

3. Control pregnants in the second trimester (G3):

They were thirty apparently healthy pregnants in the second trimester of pregnancy. Age range was from 15 to 38 years (mean age + SD = 24.6 + 4.5 year).

Gestational age range was from 20 to 28 weeks (mean gestational age + SD = 25.5 + 1.8 week).

4. Control pregnants during the third trimester (G4):

They were thirty pregnants in the third trimester of pregnancy. Age range was from 18 to 35 years (mean age ± SD = 24.8 ± 4.6 year). Gestational age range was from 29 to 40 weeks (mean gestational age ± SD = 34.6 ± 2.1 week).

B. Blood samples:

Ten milliliters of random venous blood were withdrawn from each patient and control, in supine position, without application of tourniquet. Samples were transferred into clean plane tubes, left at room temperature for 15 minutes for clotting, centrifuged, and the separated sera were then divided into tow parts:

1) An aliquot of serum was transferred into Eppendrof tube, which was used for measuring nitric oxide expressed as nitrite (the end product of NOS), this was done at the same day of collection [10].

2) Another aliquot of the serum was transferred into Eppendrof tube, which was used for measuring sex steroids (estrogen, progesterone, and testosterone) by enzyme linked fluorescent assay (ELFA) method. The tubes were stored at -20^{o} C until analysis, which was done within one month after collection [11].

C-Methods

Nitrite concentration measurement was used as an index of NO synthase activity [10], NO synthase activity is expressed here as the amount of nitrite (in µmoles) formed per minute, whereas the specific enzyme activity is given as the amount of nitrite (in µmoles) formed per minute per mg of protein for plasma[10] (µmol/min/mg protein).

Estimation of serum total testosterone was done in the Al-Kadhimya teaching hospital laboratories by Enzyme Linked Fluorescent Assay (ELFA) methods using the VIDAS instrument [11].

Results

Serum testosterone:

Serum testosterone was significantly higher in preeclamptics (G1 &G2) compared with normal pregnants (G3 & G4) [P < 0.001 for both]. Also serum testosterone was significantly higher in G2 compared with G1 [P < 0.001 for both], but there was no significant difference between G3 & G4 [P< 0.05] as in Table 1.

Serum Nitric oxide (NO) and nitric oxide synthase (NOS):

In preeclamptic pregnants in the third trimester G2, the maternal serum NO and NOS levels were significantly lower than those in the second trimester G1 [P< 0.001 and < 0.05 respectively]. In preeclamptic pregnants G1 & G2, the maternal serum NO and NOS were significantly lower than healthy pregnants G3 & G4 [P < 0.001 for both parameters in both groups], this difference was not found between healthy pregnants in the second trimester G3 and the third trimester G4 [P < 0.05 for both parameters] as in Table 1.

3.3.1. Correlation between serum testosterone and NO in different groups:

There was a significant negative correlation between serum testosterone and serum NO in preeclamptic groups G1 and G2 [r =0.9, P < 0.001 & < - 0.05, figures. 1 & 2 respectively) however, no correlation was seen among the normotensive groups G3 and G4 [r = 0.1, P > 0.05, r = 0.06, P> 0.05; figs. 3 & 4 respectively).

Discussion

In this study the level of the potent androgen testosterone was found to be significantly higher in women with preeclampsia than in healthy controls with similar gestational age, and chronologic age as in Table 1 & Figure 1.

Several independent studies showed that androgens could cause physiologic changes strikingly similar to those seen in preeclampsia [12]. High circulating androgen concentrations (in the male range) and exogenously administered androgens have both been linked to hypertension in vivo and in vitro [6].

Maternal serum androgen levels have been shown to be elevated in healthy pregnant women compared with levels in those who were not pregnant; this can be attributed to the increase in sex hormone binding globulin concentration induced by estrogen, or to the effect of hCG hormone which results in increasing maternal and lowering fetal testosterone [13]. Other suggestions may involve the increase in inhibin –A found in preeclamptic women which leads to increase androgen synthesis by the ovarian theca cells , with a reduction in the placental aromatization enzymes for androgens in preeclamptic women[6] .

Our findings suggest a possible effect of the enzyme deficiency, as well as a possible mechanism for its association with preeclampsia [6].

Alternatively, it could be argued that the testosterone increase observed in the patients with preeclampsia could have been caused by decreased intravascular volume found in preeclampsia [6, 14].

Nitric oxide mediates many functions of endothelium, including vasodilatation and inhibition of platelet aggregation [15]. Preeclampsia may be associated with nitric oxide deficiency [15], and the results of this study provide an evidence to support this hypothesis. As shown in Table 1, NO level in blood was similar in both healthy pregnants groups; it was unchanged during physiological pregnancy. During preeclampsia, the NO was decreased compared to the control level. This suggests that during preeclampsia the low activity of endothelial NO-synthases and redox-dependent transformation of NO in peroxynitrite provoke a decrease in the blood nitric oxide level [16]; these results are comparable to those of Meher & Duly [15], Khetsuriani et al. [17], Choi et al. [16], and Nishikawa & Miyamoto [18].

The reduction of NO in preeclampsia and other cardiovascular disease can be attributed to either the association of a subset of endothelial nitric oxide synthase gene (NOS3) polymorphisms (Glu298Asp, intron 4, -786>C and -786CC) with cardiovascular disease, preeclampsia and recurrence of pregnancy negative events[19,20] , or to testosterone increment in preeclampsia[15].

Arginase is often colocalized with NOS and they maintain a complex relationship, regulating each other and competing with one another for their common substrate [21]. There is evidence that when either arginase or NOS is activated, it competitively inhibits the action of the other [21].

During late pregnancy, arginase activity increases significantly in animals [21]. Kidney arginase was also increased in these animals [21]. This suggests that the placenta is required for maximal increase in arginase activity [21].

Rats and sheep have also been shown to have an increase in arginase that peaks in late pregnancy [21].

Arginase is also found in the human placenta [21]. One study that evaluated the levels of serum hydrolases in human pregnancy found no increase in serum arginase activity in the first, second, or third trimester of pregnancy [21]. It is quite possible that arginase activity in pregnancy is increased significantly in the involved tissues, while does not increase in the serum [21]. One study on the arginase activities of various tissues in rats also found that while there was an increase in arginase activity during late pregnancy, it was not reflected in circulating urea levels [21]. Why arginase activity is increased during pregnancy is unknown. In rats, inhibiting uterine arginase activity had arrested the embryonic development [21]. This could be secondary to its effects on polyamine synthesis [21]. The timing of the increase in arginase activity at the end of pregnancy and the decrease in NO production at this time may reflect normal enzyme interaction. It is quite feasible that the increase in arginase activity is part of the trigger that normally decreases the myometrial NOS activity just prior to, and in preparation for parturition [21].

In studies on rats and mice, testosterone has been shown to stimulate arginase activity [21]. It was found that testosterone elicited a 50% decrease in the enzyme ornithine carbaomoyl transferase (OCT). Inhibiting OCT may cause a significant decrease in endogenous L-arginine production [21].

Patients with preeclampsia have been shown to have higher levels of testosterone than the level of testosterone typical of nonpreeclamptic pregnants. If testosterone stimulates the arginase in humans, then this could potentially decrease the L-arginine available to NOS and thus increase production of O_2^-; this was supported by the negative correlation between NO and testosterone serum levels found in preeclamptics, which was lost in normal gestation as seen in Figures:1.2,3 & 4

Biochemical changes in preeclampsia appear to be driven by over-production of testosterone (probably induced by placental dysfunction) which may lead to a reduction in nitric oxide synthesis (as evident by low serum nitrite). While measuring NO and testosterone before 20^{th} week gestation can be used as predictor of the disease.

Table 1: The mean serum testosterone, nitric oxide and nitric oxide synthase (NOS) in different preeclamptic and control groups (presented as mean ± SD).

Variable	G1	G2	G3	G4
testosterone (ng/ml)	1.89 ± 0.6**	2.9± 2.4**	0.85 ± 0.7	0.72 ± 0.3
Nitric oxide (µmol)	6 ± 0.9**	4.1± 2.4**	8.1 ± 3	8.8 ± 3.3
NOS (µmol/g/min)	0.08±0.01*	0.06±0.03*	0.1 ± 0.04	0.11 ± 0.04

NOS activity is expressed as nitrite / g protein / min.
** p< 0.05 , ** p < 0,01*

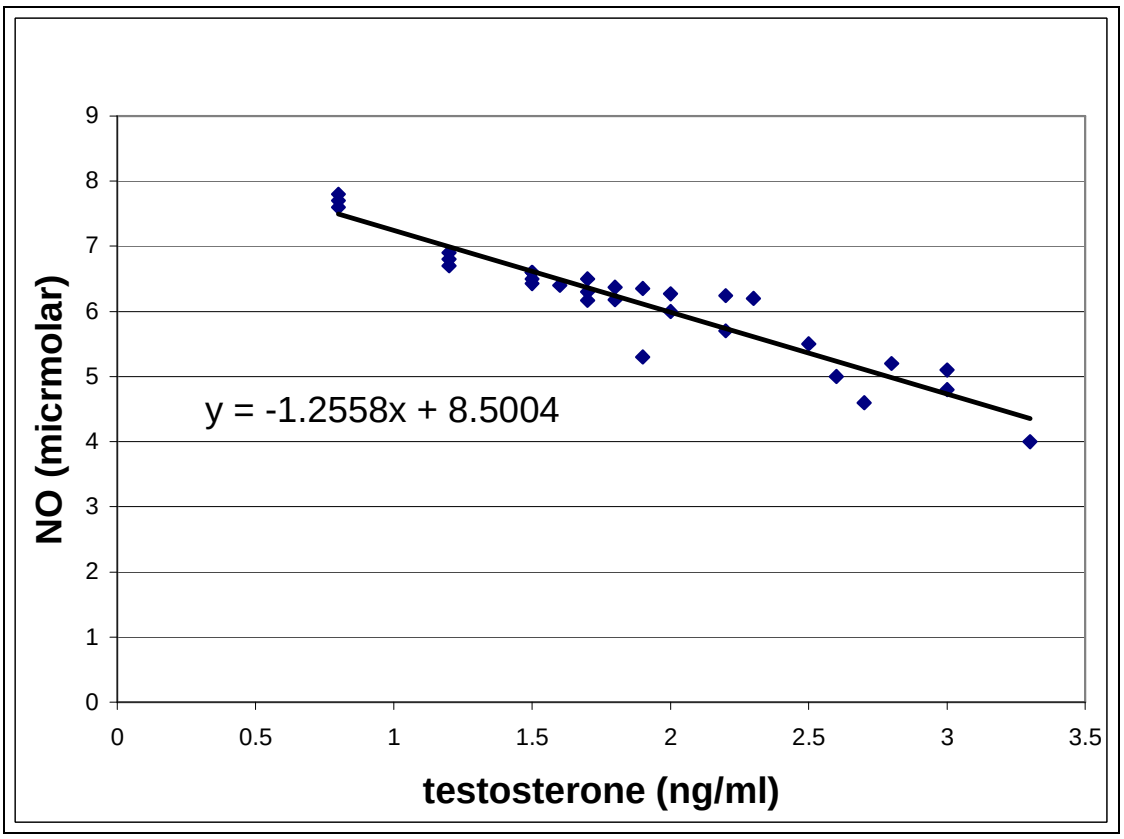

Figure 1: Correlation between testosterone & NO in sera of preeclamptics in the second trimester G1 (n = 30; r= -0.9; p< 0.001).

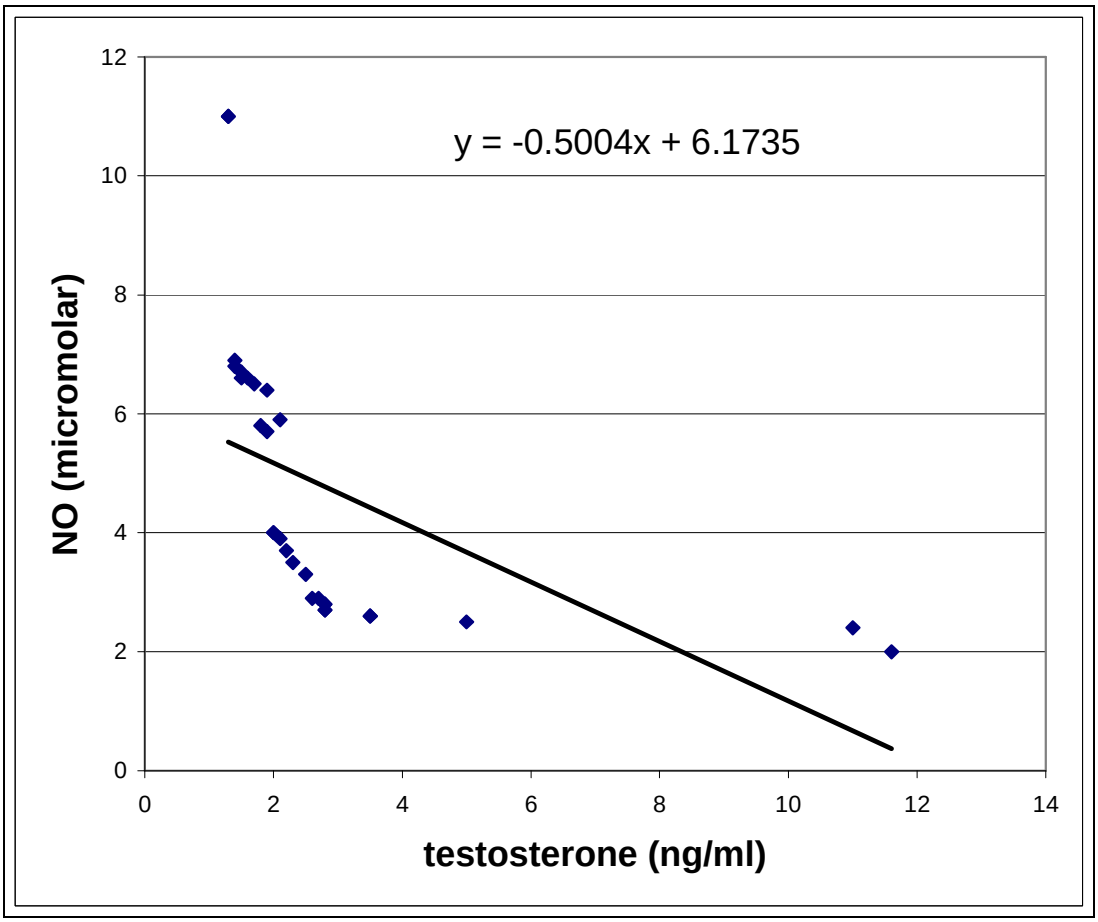

Figure 2: Correlation between testosterone & NO in sera of preeclamptics in the third trimester G2 (n = 30; r = -0.5; p < 0.01).

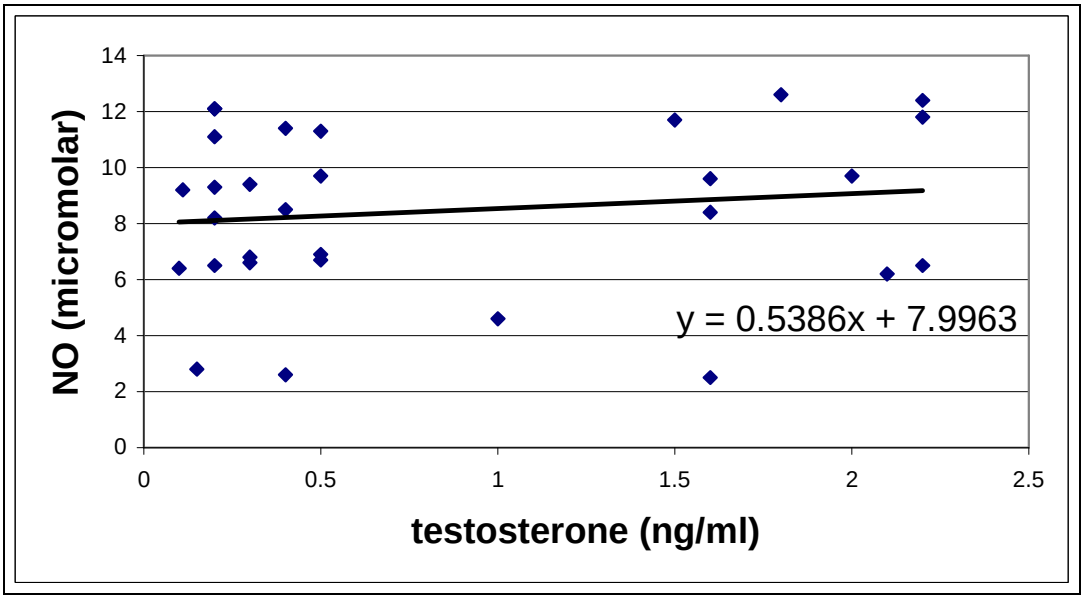

Figure 3: Correlation between testosterone & NO in sera of normotensive pregnants in the second trimester G3(n = 30; r= 0.1; p = 0.05).

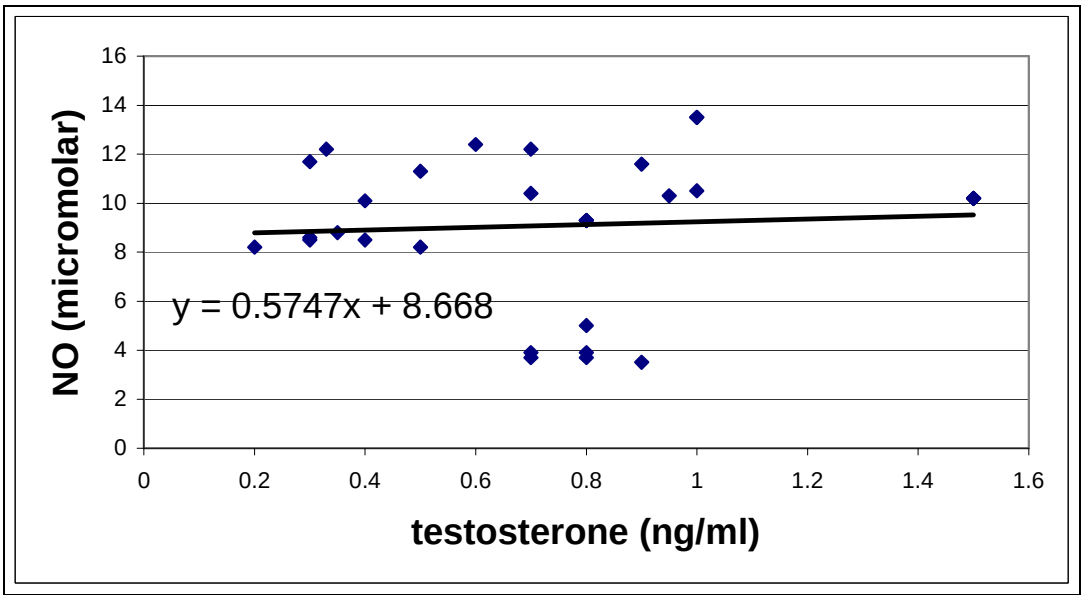

Figure 4: Correlation between testosterone & NO in sera of normotensive pregnants in the third trimester G4 (n = 30; r= 0.06; P = 0.05).

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