

Nitric oxide in different types of hypertension during pregnancy

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(Received 20 December 1996/10 July 1997; accepted 25 July 1997)

1. Serum nitric oxide (NO) levels (determined by its products of oxidation) were assessed in non-pregnant women, normal pregnant women and patients suffering from mild pre-eclampsia (MPE), severe pre-eclampsia (SPE), chronic hypertension (CHT) and CHT with pre-eclampsia (CHT+PE). The levels of NO products were significantly reduced during pregnancy in MPE ($P < 0.001$), CHT+PE ($P < 0.01$) and SPE ($P < 0.05$). Significant reductions of NO products were also observed in puerperium ($P < 0.001$) in all groups except CHT+PE ($P < 0.05$).

2. In normal pregnancy, three events were related to NO levels: (1) negative correlations were found between the levels of nitrite ($r = -0.73$, $P = 0.0003$), nitrate ($r = -0.53$, $P = 0.017$) and the number of weeks of gestation; (2) in the caesarean section group, the levels of NO at puerperium were significantly lower ($P < 0.05$) than those during pregnancy; and (3) there was a significant reduction in NO levels in the pregnant women carrying male fetuses as compared with female fetuses ($P < 0.05$).

3. In SPE, the patients with a family history of hypertension had lower levels of NO compared with the patients without such a history ($P < 0.05$).

4. A negative correlation was observed between systolic blood pressure, diastolic blood pressure and NO levels in MPE ($r = -0.62$, $P = 0.013$ and $r = -0.68$, $P = 0.0049$ respectively) and SPE ($r = -0.72$, $P = 0.004$ and $r = -0.53$, $P = 0.037$ respectively).

5. In SPE, positive correlations were observed between platelet count and nitrite ($r = 0.67$, $P = 0.006$) and nitrate levels ($r = 0.56$, $P = 0.028$).

6. In MPE, patients with anti-hypertensive treatment showed significantly ($P < 0.05$) higher levels of NO compared with the non-treated patients.

7. NO may be important in the physiopathology of hypertension during pregnancy, although several factors may affect its levels.

INTRODUCTION

Different factors have been associated with pre-eclampsia (PE)/eclampsia: multifetal gestations, molar pregnancy, certain chromosomal alterations of the fetus, familiar history of hypertension, personal history of PE, chronic hypertension (CHT), diabetes, renal diseases [1–2] and the sex of the fetus [3–4]. However, the aetiology and physiopathology of pregnancy-induced hypertension (PIH) is not fully understood. Damage of endothelial cells and organs in PE have been associated with increased vascular reactivity [1–2, 5–7] due to an imbalance between vasoconstrictive and vasoactive substances, mainly prostacyclin, thromboxane, endothelins and nitric oxide (NO) [8–25].

NO, initially identified as an endothelial factor responsible for the relaxation of smooth muscle cells [26–27], is generated by the enzymic conversion [by nitric oxide synthase (NOS)] of arginine to citrulline using NADPH as a cofactor. The end product is quickly transformed into a radical which is readily active and can be transformed into nitrites and subsequently into nitrates [26–27]. Several reports have shown that pharmacological inhibition of NOS is related to vasoconstriction [15–16, 24–25].

The role of NO in PIH is still controversial. Some authors have reported a relationship between low NO production and PE [13–23]; on the other hand, other researchers have reported either no difference or increased NO production in PIH [24, 25]. Since NO has been involved in the control of vascular tone and inflammation, a tentative hypothesis was envisioned in which the severity of PE and the events associated with PIH (gestational age, personal or familiar history of hypertension or PE, sex of the fetus and other variables) are related to decreased NO production.

The aim of the present report was to determine the relationship between NO oxidative products and the severity of PE, factors that may predispose to PE, types of hypertension during pregnancy, the

Key words: hypertension, nitric oxide, pre-eclampsia, pregnancy.

Abbreviations: ANOVA, analysis of variance; CHT, chronic hypertension; MPE, mild pre-eclampsia; NO, nitric oxide; NOS, nitric oxide synthase; NP, normal pregnancy; PE, pre-eclampsia; PIH, pregnancy-induced hypertension; SPE, severe pre-eclampsia.

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type of interruption used, puerperium, and the effect of anti-hypertensive therapy. These associations were assessed in normal pregnancy (NP) and in different types of hypertension during pregnancy.

MATERIALS AND METHODS

Patients

The patients studied were among those admitted to the Maternity Hospital (Maternidad Concepción Palacios) of Caracas. The study was accepted by the Ethical Committee of the hospital.

We studied 66 pregnant women, 20 normal (NP), 16 with mild PE (MPE), 17 with severe PE (SPE), 6 with CHT+PE and 7 with CHT. Upon obtaining written, informed consent of all the patients included in the study, two samples of blood were taken (one during pregnancy and one 2 or 3 days after delivery). The controls were 20 normal non-pregnant women in which neither hypertension nor other chronic or viral diseases were recorded.

The characteristics of the patients are described in Table 1. Emphasis was placed on the clinical symptoms and abnormalities frequently observed in the physical examination of pre-eclamptic women (nervous system, gastrointestinal system, skin and visual alterations).

The patients were divided into different groups according to the classification of the hypertensive disorders of pregnancy adopted by the American College of Obstetricians and Gynecologists in 1986 [28, 29].

Blood pressure was measured by the first and fifth Korotkoff sounds with patients in the left lateral decubitus position. The blood pressure recordings were ascertained during hospital admission, before and after starting anti-hypertensive treatment (patients with PE) and immediately before blood collection. Smokers did not smoke for a period up to 4 h before the sample was taken.

All patients were more than 20 weeks pregnant. Patients with recent hypertension, persistently

≥ 140 mmHg systolic or ≥ 90 mmHg diastolic, mild proteinuria or oedema were classified as MPE. Patients with SPE presented one or more of the following symptoms: recent systolic blood pressure persistently ≥ 160 mmHg, diastolic blood pressure persistently ≥ 110 mmHg; proteinuria > 2000 mg/24 h (or $> 3+$ in semiquantitative tests); increased serum creatinine levels ($> 177 \mu\text{mol/l}$ to 2 mg/dl) or oliguria ($< 500 \text{ cm}^3/24 \text{ h}$); platelet count $< 1 \times 10^9/\text{l}$ or evidence of microangiopathic haemolytic anaemia (schistocytes, increase in indirect bilirubin levels or increase in serum free haemoglobin levels); upper abdominal pain, headache, visual disturbances or other cerebral signs. Women with CHT were diagnosed as having essential hypertension before pregnancy. Patients with a previous history of CHT who developed proteinuria, abnormal oedema or any signs of SPE were classified as CHT with superimposed PIH (CHT+PE).

We excluded any patient with fever, other chronic diseases (such as diabetes, renal disorders, cardiopathies, etc.), or patients who were not clearly defined within the aforementioned criteria. In addition, patients who were severely ill (patients with disseminated intravascular coagulation, severe haemolysis, renal, hepatic or cardiac failure), and/or those patients that required intensive care, were excluded as specified by the Ethical Committee of the Hospital. A complete medical record of each patient was kept from hospital admission to discharge.

In the NP group, the caesarean sections were performed due to fetopelvic disproportion or previous caesarean operation.

Hypertensive patients were treated with α -methyldopa, hydralazine or nifedipine, alone or in combination. The doses varied depending on the patient's response.

NO determination

The blood samples were taken during a fasting period of no less than 4 h and no longer than 24 h.

Table 1. Characteristics of the patients. Significant differences were observed in the age of patients (* $P < 0.05$), in the systolic and diastolic blood pressure (** $P < 0.01$, **** $P < 0.001$) and in the percentage of patients that were symptomatic and those that presented alterations in the physical examination (** $P < 0.01$). Using the Chi-squared test with Yates' correction, there is a significantly ($P < 0.005$) higher frequency of previous history of pregnancy PIH in the pre-eclamptic patients (MPE, SPE and CHT+PE) as compared with NP. No significant differences were observed for subjects with a family history of CHT.

	Non-pregnant	NP	MPE	SPE	CHT+PE	CHT
Number of subjects (n)	20	20	16	17	6	7
Age (years)	28 \pm 8	26 \pm 6	22 \pm 7	23 \pm 6	34 \pm 3*	31 \pm 8*
Systolic pressure (mmHg)	116 \pm 9	120 \pm 5	138 \pm 11**	164 \pm 14***	176 \pm 28***	157 \pm 15***
Diastolic pressure (mmHg)	71 \pm 7	72 \pm 7	92 \pm 6***	111 \pm 5***	118 \pm 20***	101 \pm 12***
First pregnancy (%)	—	30	47	18	0	29
Symptomatic (%)	—	15	44**	94**	100**	57**
Abnormal physical examination (%)	—	0	100**	100**	100**	100**
Previous PIH (%)	0	0	20	35	50	57
Family history of CHT (%)	40	55	53	53	100	57
Smokers (%)	20	15	0	0	0	14

Their food intake was similar. All the patients with hypertension (PIH, CHT or CHT+PE) received a diet, given by the hospital, with low contents of nitrate and nitrite, in accordance with the guidelines described elsewhere [30]. The non-pregnant controls (mainly hospital personnel) and the normal pregnant women followed a similar diet.

NO levels were determined indirectly by quantification of their oxidized products of degradation, nitrates and nitrites, using nitrate reductase and the Greiss reagent [31], according to the method of Moshage et al. [32]. Briefly, serum samples were centrifuged at 3000 g, diluted 4-fold with distilled water and incubated with nitrate reductase from *Aspergillus* spp. in order to quantify the total amount of NO products (nitrites+nitrates). In the absence of the enzyme only nitrite concentrations were determined. After 30 min incubation at 37°C in the presence of enzyme and its cofactors NADPH and FAD, and after further incubation for 10 min with sodium pyruvate and lactic dehydrogenase to degrade excess NADPH, the samples were deproteinized with zinc sulphate, and 100 µl of the supernatant was mixed with 100 µl of the Greiss reagent [a solution containing equimolar amounts of solution A (2.5% phosphoric acid, 10% sulphonamide) and solution B (0.1% ethylenediamine)]. A standard curve was obtained using sodium nitrate dissolved in water or in a pool of 20 normal human serums of non-pregnant women. Nitrite concentration was determined at 540 nm using an ELISA plate reader (Labsystems Multiscan MCC/340, Turku, Finland). The inter-assay and intra-assay coefficients of variation for the Greiss reaction were 5.6% and 3.6% respectively.

Statistics

Comparative analysis among groups was performed using paired and unpaired Student's *t*-test, linear regression, analysis of variance (ANOVA) and the Chi-squared test with Yates' correction.

RESULTS

The characteristics of the patients are depicted in Tables 1–3. As expected, differences in arterial blood pressure, percentage of symptomatic patients and abnormal physical parameters were observed between groups. Significant differences were also observed in the ages of the different groups; the patients with MPE and SPE being younger than the patients with CHT and CHT+PE ($P < 0.05$). The frequency of women having their first pregnancy was higher in the MPE group as compared with patients in the SPE and CHT+PE groups ($P < 0.05$). In every group there were women with family histories of CHT (grandparents, parents, uncles, brothers or sisters). All CHT+PE patients had family histories of CHT. Patients with pre-eclampsia (MPE and SPE) and hypertension (CHT and CHT+PE) had experienced previous episodes of PIH. None of the patients with MPE, SPE and CHT+PE were smokers. Only 20% of the non-pregnant women (mean 11 cigarettes/day), 15% of the NP (mean 18 cigarettes/day) and 14% of CHT (mean 2 cigarette/day) patients smoked.

Three parameters, frequency of first pregnancy, previous PIH and family history of CHT, were compared using contingency tables among groups. Significant differences between the groups ($P < 0.00001$) were observed. In comparison with NP, MPE and SPE groups, CHT+PE patients had the highest frequencies of previous PIH and family history of CHT and the lowest frequency of first pregnancy.

In Table 2, it can be observed that the CHT+PE group had the lowest number of weeks of gestation when their pregnancy was interrupted ($P < 0.001$ with respect to NP). There were no differences in the number of caesarean sections performed in each group. On the other hand, the complications of the neonate were more frequent in the CHT+PE and SPE groups than in the other groups ($P < 0.01$).

Some of the laboratory tests are presented in Table 3. Interestingly, the leucocyte count was higher in SPE compared with NP ($P < 0.05$). Several

Table 2. Gynaecological and obstetrical characteristics of patients. The only significant parameters found were: the week of interruption (second sample) in the CHT+PE and SPE groups as compared with NP (**** $P < 0.001$ and **** $P < 0.005$), the weight of the neonate, which is significantly less in the SPE and CHT+PE patients (** $P < 0.01$), and the neonatal mortality (** $P < 0.01$) as compared with normal pregnancy.

	NP	MPE	SPE	CHT+PE	CHT
No. of weeks of gestation					
First sample	38 ± 3	37 ± 2	36 ± 3	31 ± 5	33 ± 2
Second sample	39 ± 1	38 ± 1	36 ± 3***	32 ± 4****	38 ± 2
Caesarean section (%)	67	64	53	50	60
Fetal death (%)	0	0	6	17	0
Weight of neonate (g)	3150 ± 250	3110 ± 390	2440 ± 570**	2100 ± 1140**	2860 ± 780
Female neonate (%)	50	43	47	40	40
Neonatal mortality (%)	0	0	0	17**	0
Maternal mortality (%)	0	0	0	0	0

other alterations were observed for haemoglobin concentration, creatinine values, uric acid, urea, alanine aminotransferase and proteinuria. Most of the alterations were observed in patients with SPE or CHT+PE. Minor differences were observed in the patients with CHT or MPE.

Anti-hypertensive treatment was not required in 53% of the patients with MPE and only 19% were treated with MgSO₄. In contrast, most of the patients with SPE (84%) required at least two anti-hypertensive drugs to control their blood pressure, and 69% were treated with MgSO₄. Likewise, 67% of CHT+PE patients required three anti-hypertensive drugs (100% treated with MgSO₄) as compared with 57% of the patients with CHT who required two hypotensors (43% treated with MgSO₄). The amount of drugs required was significantly higher in the CHT+PE group as compared with the MPE group ($P < 0.01$).

The values of NO are depicted in Fig. 1. In Fig. 1(A), the samples obtained during pregnancy are reported along with the values of non-pregnant women (control group, C). Significant differences were observed when the values of non-pregnant women were compared with MPE ($P < 0.001$), SPE ($P < 0.05$), CHT ($P < 0.01$) and CHT+PE ($P < 0.01$). The levels in the MPE group were the lowest. The samples during puerperium are presented in Fig. 1(B). The values for the NP group and for patients in puerperium were lower than for non-pregnant women. Significant differences were observed when the values for non-pregnant women were compared with those for NP ($P < 0.001$), MPE ($P < 0.001$), SPE ($P < 0.001$), CHT ($P < 0.001$) and CHT+PE ($P < 0.05$). When the values of NO during pregnancy were compared with those recorded at puerperium, a significant reduction was observed in the NP group ($P < 0.0001$) and the SPE group ($P < 0.05$) and no differences were observed between the other groups. On the other hand, the levels of NO were similar in pregnancy and puerperium in the MPE, CHT+PE and CHT groups.

No differences in NO levels were recorded among patients of different races or ages (results not shown). Furthermore, no differences in NO levels

were observed when patients were treated with fenitoin or MgSO₄ (results not shown).

In order to assess the effects of different obstetric parameters in NO determination, NP samples were analysed for number of weeks of gestation and the effect of parturition (normal vaginal delivery) or caesarean section. These results are presented in Fig. 2. Fig. 2(A) represents the negative correlation between the number of weeks of gestation and nitrite ($r = -0.73$) and nitrate ($r = -0.53$) levels. A similar correlation (not shown) was observed with total NO products ($r = -0.67$, $P < 0.005$). This correlation was not observed in the patients with different types of hypertension. Figure 2(B) presents the effect of parturition or caesarean section on NO levels. In the NP group, in which a caesarean operation was performed, the values were significantly reduced in puerperium compared with the values observed during pregnancy ($P < 0.05$, paired t -test). However, in patients with hypertension, no differences were observed between groups with caesarean section compared with parturition (results not shown).

In Fig. 3(A), normal pregnant women carrying male fetuses had significantly lower nitrite, nitrate and total NO products than the female group ($P < 0.05$). This difference was not observed in the other groups and at puerperium. In Fig. 3(B), in the SPE group, patients with a family history of CHT had significantly lower ($P < 0.05$) levels of NO compared with patients without such a history. These differences were not observed in NP, nor in patients with MPE and CHT (results not shown). All patients with CHT+PE had a family history of CHT (see Table 1).

In Fig. 4, the correlation between blood pressure and the levels of total NO products is shown for MPE (Fig. 4A) and for SPE (Fig. 4B). A negative correlation was observed between systolic blood pressure, diastolic blood pressure and NO levels in MPE ($r = -0.62$, $P = 0.01$ and $r = -0.68$, $P = 0.005$ respectively) and SPE ($r = -0.72$, $P = 0.004$ and $r = -0.53$, $P = 0.037$ respectively). These correlations were not observed in the groups of patients with chronic hypertension (CHT and CHT+PE).

Table 3. Laboratory parameters. Significant differences were observed in the leucocyte count, haemoglobin concentration, creatinine, uric acid, urea, ALT and proteinuria (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$, **** $P < 0.0001$).

	NP	MPE	SPE	CHT+PE	CHT
Total leucocytes/mm ³	10800 ± 5800	11500 ± 4000	15500 ± 5300*	12100 ± 2900	11000 ± 3600
Platelets/mm ³	275000 ± 125000	248800 ± 79000	232000 ± 92000	195000 ± 61000	264000 ± 54000
Haemoglobin (g/dl)	11 ± 1.5	9.9 ± 1.95*	9.9 ± 1.3*	10.6 ± 0.7	11.3 ± 1.1
Haematocrit	34.8 ± 4.9	32.3 ± 5.07	32.2 ± 3.8	33.5 ± 2.2	35.1 ± 3.8
Creatinine (mg/dl)	0.6 ± 0.2	0.66 ± 0.13	0.81 ± 0.19***	0.83 ± 0.12**	0.73 ± 0.1
Total bilirubin (mg/dl)	0.4 ± 0.2	0.42 ± 0.13	0.48 ± 0.4	0.5 ± 0.25	0.63 ± 0.23
Uric acid (mg/dl)	3.2 ± 0.6	5.0 ± 1.6**	5.9 ± 2.7****	6.0 ± 2.4***	5.2 ± 1.2*
Urea (mg/dl)	18.5 ± 3.5	14.7 ± 3.1	24.3 ± 8.1*	27.3 ± 14.2**	17.3 ± 5.0
ALT (units/l)	22 ± 12	20.3 ± 4.2	37.7 ± 28.3	86.8 ± 130.2**	27.3 ± 14.3
AST (units/l)	20 ± 10	11.4 ± 3.6	21.8 ± 15.6	26.2 ± 25.2	21.2 ± 13
Semiquantitative proteinuria	0	0.5 ± 0.5	2.3 ± 1.4****	2.5 ± 1.3****	0.5 ± 0.5

In patients with MPE and SPE, other parameters were correlated with NO levels. As shown in Fig. 5, there was a positive correlation in SPE between the platelet number and the level of nitrites ($r = 0.67$, $P = 0.0061$) and nitrates ($r = 0.56$, $P = 0.0276$). A similar correlation (not shown) was observed with total NO products ($r = 0.65$, $P = 0.009$).

Furthermore, in MPE, anti-hypertensive treatment appeared to affect NO levels. As can be seen in Fig. 6, patients treated with anti-hypertensive drugs had significantly higher levels of nitrites, nitrates and total NO products ($P < 0.05$) compared with the non-treated patients. The levels of NO

during pregnancy were significantly lower ($P < 0.0001$) in non-treated MPE patients (nitrates 13.3 ± 2.7 and nitrites $3.8 \pm 1.8 \mu\text{mol/l}$, $n = 8$) when compared with non-pregnant women (nitrates 19.3 ± 3.3 and nitrates $7.5 \pm 2.0 \mu\text{mol/l}$, $n = 20$) and with NP (nitrates 18.7 ± 4.1 and nitrites $6.4 \pm 2.0 \mu\text{mol/l}$, $n = 20$); values given \pm SD.

No correlation was found between NO and total leucocyte count and NO and other laboratory parameters that were altered in SPE. Moreover, no correlations were found between NO and other parameters in CHT and CHT+PE patients.

DISCUSSION

Little information is available currently about the physiological implications of NO in pregnancy, the

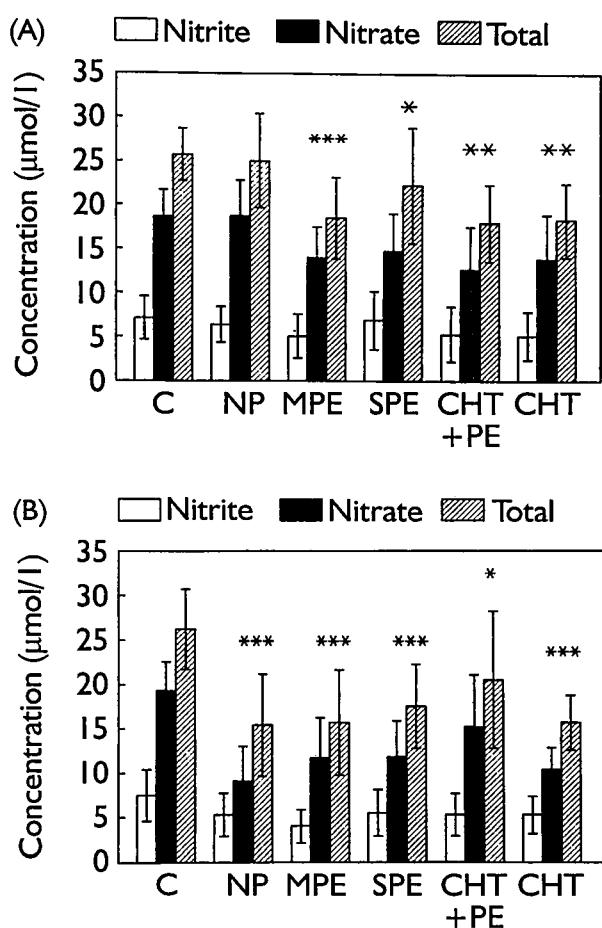


Fig. 1. Levels of nitrites, nitrates and total NO products during pregnancy and at puerperium in the different groups in comparison with non-pregnant women. The levels correspond to the means \pm SD of the nitrite, nitrate and total NO products in each group. Non-pregnant women are designated controls (C). Nitrites and nitrates were determined as described under Materials and methods. (A) Levels of NO during pregnancy. The values obtained were compared with non-pregnant women (C). The ANOVA analysis was highly significant ($P < 0.0001$). Significant differences were observed when the total values in C were compared with the patients with MPE ($***P < 0.001$), CHT and CHT+PE ($**P < 0.01$) and SPE ($*P < 0.05$). (B) Values observed at puerperium. The ANOVA analysis was highly significant ($P < 0.0001$). Significant differences were observed when the values of the total NO products in C were compared with NP, MPE, SPE and CHT ($***P < 0.001$) and CHT+PE ($*P < 0.05$).

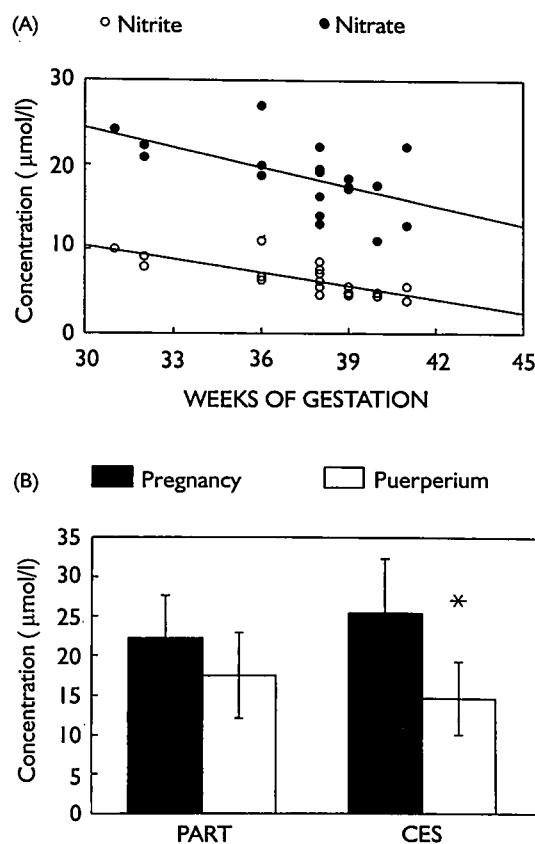


Fig. 2. Effect of gestational age and type of delivery on NO levels in NP. (A) Correlation observed between gestational age (weeks) and the levels of nitrites and nitrates determined as described under Materials and methods. A negative correlation was observed with nitrites ($r = -0.73$, $P = 0.0003$, $y = -1.01x + 43.86$) and nitrates ($r = -0.53$, $P = 0.017$, $y = -0.75x + 46.6$). A similar correlation was found with the total NO products ($r = -0.67$, $P = 0.0013$, $y = -0.38x + 47.1$) (not shown). (B) The levels of total NO products recorded during pregnancy and at puerperium of normal pregnant women whose interruption was parturition (normal vaginal delivery; PART), or caesarean section (CES). A significant difference was observed when total NO products recorded during pregnancy were compared with NO levels recorded at puerperium in the caesarean group ($P < 0.05$).

importance of the different events that have been associated with PIH and their relationship with NO levels. The aim of the present study was to assess the influence of the different factors studied, in NP and PIH, on the levels of NO products.

Several clinical and laboratory parameters have been related to the severity of PE [1–2, 5–13]. A deficit of vasoactive substances and damage of endothelial cells and many organs have been associated with PE [1–2, 5–25]; however, the aetiology and physiopathology of PIH is not fully understood.

In NP, an increment in the circulating levels of NO was observed during the first trimester of pregnancy [14]. In concordance, Delacrétaiz et al. [22] reported an increment in platelet inducible NOS in normal pregnant women. In the present report, a negative correlation was observed (Fig. 2A) between the number of weeks of gestation and the levels of nitrites and nitrates in serum. This observation has not, to our knowledge, been reported previously.

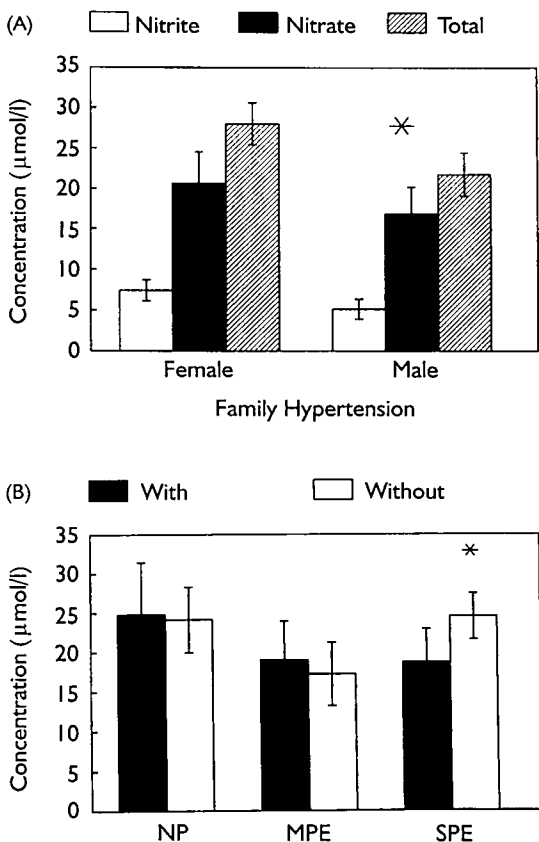


Fig. 3. Effect of the sex of the fetus in NP, and the influence of family predisposition to hypertension on NO levels. (A) Levels of nitrite, nitrate and total NO products, during normal pregnancies that gave birth to a female or male child. A significant decrease ($*P < 0.05$) was observed for each parameter compared. (B) Influence of family hypertension on levels of NO. The black bars represent the patients with a family history of CHT and the white bars the contrary. Only in the group of patients with SPE was there a significant difference in the levels of NO when patients with family hypertension were compared with those without ($*P < 0.05$).

Morris et al. [23] did not find a significant correlation between NO levels, recorded in the exhaled air of pregnant women, and gestational age. This discrepancy may be due to the different type of sample processed.

Recently, increased interest has been shown in NO as a possible important mediator of PE [13–25]. A PE-like phenomenon was observed in rats treated with N^G -monomethyl-L-arginine (an inhibitor of NOS) [13–15], suggesting that an important relationship may exist between NO levels and the vasoconstriction produced in PE. Wang et al. [24] did not find differences in placental NO between NP and PE. However, Davidge et al. [25] reported an increased level of serum nitrates in patients with PE. The issue of NO in pregnancy and PE remains controversial.

Different reports [14–25] have suggested that several factors may affect NO production, which may or may not be directly related to the pathology of PE or any type of hypertension. As described in the present report, in NP, the number of weeks of

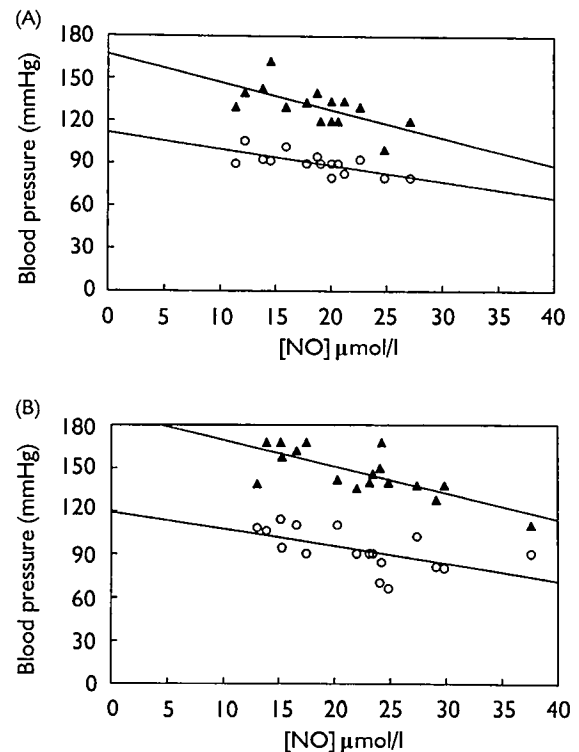


Fig. 4. Correlation between systolic (SBP, ▲) and diastolic (DBP, ○) blood pressure and total NO products. (A) Correlation between the systolic and diastolic blood pressure taken immediately before blood sample collection and NO levels in the group with MPE during pregnancy. Negative correlations were observed for SBP and DBP ($r = -0.62$, $P = 0.012$, $y = -1.92x + 167.6$ and $r = -0.69$, $P = 0.005$, $y = -1.15x + 111.6$ respectively). (B) Correlation observed between the systolic and diastolic blood pressure and the NO levels in the group with SPE during pregnancy. Negative correlations were observed for SBP and DBP ($r = -0.72$, $P = 0.004$, $y = -1.77x + 186.03$ and $r = -0.53$, $P = 0.037$, $y = -1.15x + 119$ respectively).

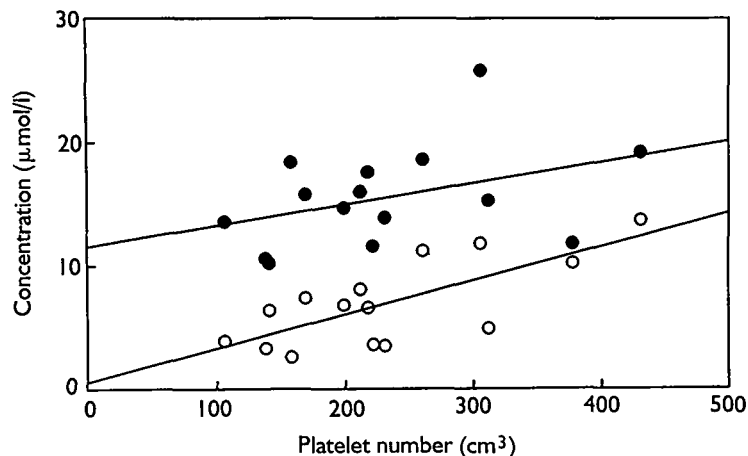


Fig. 5. Relationship between platelet count and nitrite (○) and nitrate (●) levels recorded in the SPE group. Correlation observed between platelet count (number of platelets per cm^3 ; $1 \text{ cm}^3 = 1000 \text{ mm}^3$) and the values of nitrite and nitrate in the group with SPE during pregnancy is shown. A significant correlation was observed with nitrites ($r = 0.67$, $P = 0.0061$, $y = 2.58 \times 10^{-5}x + 0.85$) and nitrates ($r = 0.56$, $P = 0.028$, $y = 2.87 \times 10^{-5}x + 7.79$). A similar correlation (not shown) was observed with total NO products ($r = 0.65$, $P = 0.009$).

gestation, the type of delivery [normal vaginal delivery (parturition) or caesarean section] and the presence of a male or female fetus seem to modify circulating levels of NO. These variables have not been accounted for in some of the reports performed in normal and pre-eclamptic women [18–25]. In addition, only one report compares MPE with SPE [18], and none of them compared patients with CHT and CHT+PE with patients with PE. Another important piece of evidence is the decrease in the levels of NO in the puerperium, reflecting a physiological consequence of parturition and caesarean section.

Most of the reports concentrate on the alterations in the laboratory parameters and do not consider the importance of predisposing factors. In this report, the influence of previous PIH and family histories of CHT were assessed. All patients with CHT+PE had previous pregnancies, had a previous history of PIH, and most of them had a family history of CHT. Furthermore, most of the patients with CHT had a family history of CHT and previous episodes of PIH, and some of the patients with MPE and SPE reported both factors. These results are in agreement with the study by Campbell et al. [33] in which the frequency of PE was found to be

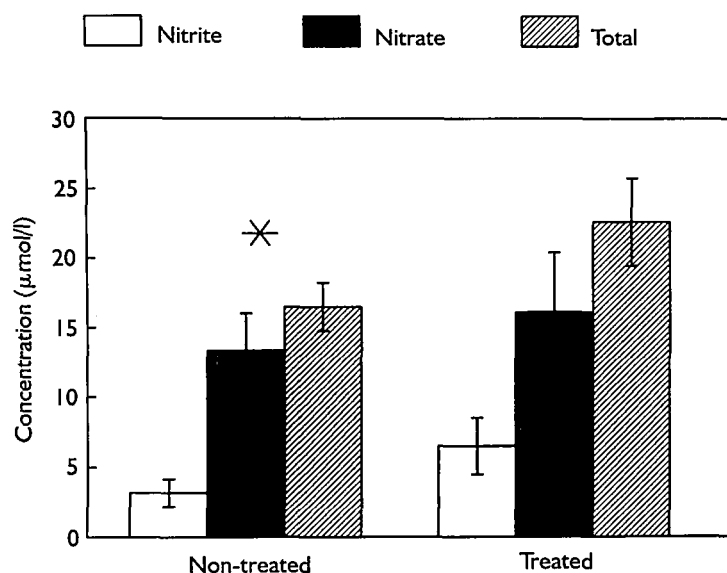


Fig. 6. Effect of treatment on the levels of NO in MPE patients. The levels of NO in MPE patients treated with anti-hypertensive drugs are shown. A significant difference ($*P < 0.05$) was observed between the groups (non-treated $n = 9$; treated $n = 8$) for each parameter studied. Furthermore, a significant difference ($P < 0.0001$) was observed between the total NO products recorded for the non-treated group as compared with the values recorded in NP.

higher in women with an antecedent PIH, as compared with women without PE in the first pregnancy. These observations suggest the importance of genetic factors in PE.

Interestingly, a decrease in the levels of NO products was observed in the normal pregnant women carrying male as opposed to female fetuses. This observation was not recorded at puerperium nor in the other groups. Previous reports relating fetal sex to pre-eclampsia/eclampsia have been contradictory. Naeye and Demers [3] observed early gestational blood pressure and weight gain in pre-eclamptic women carrying male fetuses, but a lower proteinuria in these women at the third trimester compared with those carrying female fetuses. In contrast, Sánchez et al. [4] reported that the probability of eclampsia is 1.6-fold higher if the fetus is male. Recently, Bianchi et al. [34] have shown that male fetal cells (CD34+CD38+) may circulate in the maternal blood during pregnancy and for up to 27 years postpartum. It may be proposed that fetal cells may, directly or indirectly, influence maternal NO production; however, this event has to be studied further.

Another important parameter that has been related to NO levels is blood pressure. In the present report, a negative correlation was encountered between blood pressure and NO products in SPE and MPE. Seligman et al. [18] reported a similar correlation of NO levels with systolic blood pressure in pre-eclamptic PE women. However, the authors did not clarify the severity of the pre-eclampsia in the women included in the study. As shown in the present report, the severity of PE clearly modified the slopes of the curves in systolic blood pressure.

Despite the fact that the decrease in platelet number was mild in the SPE group, the modifications in the platelet count parallel the nitrite and nitrate production. This correlation was not observed in the other groups. The positive correlation found suggests a link between NO production and platelet number, which is one of the criteria used to determine the severity of PE. However, the blood pressure (systolic or diastolic) did not correlate with platelet number. Recently, Delacrétaç et al. [22] studied platelet function and constitutive NOS activity and its relationship with NP and PE. The authors reported decreased NOS activity in pre-eclamptic women and a correlation between gestational age (not achieving significance) and NOS activity in NP. However, the authors did not observe a correlation between blood pressure and NOS. This difference may be due to the fact that the assays were performed in a small number of patients.

It is important to emphasize that patients with MPE who received anti-hypertensive drugs had levels of NO higher than those who did not. These differences were observed only during pregnancy and not at puerperium. The increase observed in patients with MPE and anti-hypertensive drugs may

explain the levels of NO encountered in patients with SPE (all of them with hypotensors). It could be suggested that anti-hypertensive treatment may affect NO levels. In concordance with this hypothesis, several reports have shown that hydralazine may increase NO levels [35–38]. Hydralazine induced the transcription, *in vitro*, of NOS in the rat adrenal medulla [37] and it increased cyclic GMP production in the plasma and urine of severe pre-eclamptic patients [38]. However, further studies are required in order to assess the direct effect of anti-hypertensive drugs on NO production and metabolism.

It is not clear from previous reports [18–25] whether the patients tested were treated at the time of collection of the blood sample. In fact, in the report of Davidge et al. [25], the authors did not mention this variable, which may condition the levels of NO in these patients. It is likely that the conclusion reported by the authors is dependent on the treatment used.

Wang et al. [24] have shown that the placental production of NO and endothelin in normal and pre-eclamptic patients was similar. These results are comparable with those of endothelium-dependent relaxation of human resistance arteries in pregnancy [19], but are not comparable with *in vivo* experiments, since *in vitro* incubations were performed for long periods of time. Therefore, the factor or factors that may up- or down-regulate NO production are not present in these *in vitro* incubations.

The absence of severely compromised SPE patients in this report may be responsible for the low number of primips, the absence of maternal deaths, mild alterations in platelet number and a higher gestational age compared with those reported previously. Nevertheless, the gestational age at delivery was lower in the SPE group compared with the NP and MPE groups. The inclusion of severely ill patients may modify several of the parameters and correlations observed in this report.

Overall, it has been shown that serum NO levels in pregnancy can be modified by several parameters, such as the number of weeks of gestation, sex of the fetus, type of interruption and anti-hypertensive treatment, suggesting that several physiological and pathological responses are related to NO production. Even though it cannot be concluded that the decrease in NO is a consequence or the cause of hypertension in pregnancy, the differences encountered, although weak in some cases, contribute to the understanding of the physiological alterations observed in PIH and provide new insights into the possible role of NO in PIH and NP

ACKNOWLEDGMENTS

The authors are indebted to all the patients and control subjects who collaborated with the present study, as well as to the paramedical and medical personnel of the hospital, especially to Drs. Freddy

Bello, Abelardo Lopez, Nellys Avila, María E. Morantes and Isabel Iturria. In addition, we thank Dr. Stephen Tillett for useful suggestions. This research was supported by the GENIC Program and Immunobiología AC (IBAC).

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