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Differences in the synthesis pattern of vasoactive factors in gestational hypertension and preeclampsia

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SUMMARY

Background and objetive: *The gestational hypertension —HG— and preeclampsia —P— are hypertensive diseases whose pathogenic mechanism has not been determined yet. The aim of this work is to define some patterns of vasoactive factors release that allow to explain the origin of the differences between both entities.*

Design: *Prospective case-control study.*

Material and methods: *Two groups of target patients were consecutively selected, GH (n = 21) and P patients (n = 21). Every patient was matched with a pregnant of similar age and week of pregnancy. Two control groups were obtained, one respect to the GH and another one respect to the P group. A biochemistry, blood cell count, coagulation and quantification of vasoactive factors endothelin, nitrites and GMPc were performed in every woman. Results of GH and P groups were compared with their respective control group with the paired Student's* t *Test.*

Results: *Both systolic and diastolic arterial pressures were higher in hypertensive pregnants (GH and P) than in their respective controls. Moreover, blood endothelin and GMPc were higher in GH and P. GH pregnants showed decreased norepinephrine and increased epinephrine urinary excretion, as well as an increased plasma nitrites concentration than control group. P patients did not show statistically significant differences in catecholamines urinary excretion nor in plasma nitrites concentration respect their control group.*

Conclusion: *There are relevant differences in the synthesis patterns of vasoactive factors between gestational hypertension and preeclampsia. These differences could account for a decreased tissue perfusion in preecalmpsia and could also contribute to the genesis of the renal dysfunction of this entity.*

Key words: ***Endothelin. Nitrites. GMPc. Catecholamines. Gestational hypertension. Preeclampsia.***

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DIFERENCIAS EN EL PATRÓN DE SÍNTESIS DE FACTORES VASOACTIVOS EN LA HIPERTENSIÓN GESTACIONAL Y EN LA PREECLAMPSIA

RESUMEN

Introducción y objetivos: *La Hipertensión gestacional —HG— y la preeclampsia —P— son estados hipertensivos del embarazo cuyo mecanismo patogénico no se conoce. Este estudio pretende definir patrones de comportamiento que expliquen el origen de las diferencias entre embarazadas hipertensas y con preeclampsia mediante el análisis de determinados factores vasoactivos.*

Diseño del estudio: *Estudio caso-control basado en casos incidentes.*

Material y métodos: *Se seleccionaron de forma consecutiva dos grupos de pacientes, HG (n = 21) y P (n = 21). Por cada paciente problema se incluyó una gestante normal de similar edad y semana de gestación. Se obtuvieron dos grupos control, uno con respecto al grupo de pacientes HG y otro en relación a las pacientes P. A cada mujer se le realizó estudio de bioquímica, hemograma, coagulación, y cuantificación de los factores vasoactivos endotelina, nitritos y GMPc, así como la excreción urinaria de adrenalina y noradrenalina. Se compararon los resultados de cada grupo de pacientes (HG y P) con su respectivo grupo control.*

Resultados: *La tensión arterial sistólica y diastólica fueron superiores en las pacientes con hipertensión (HG y P) en comparación con sus controles. Igualmente, en las pacientes con HG y en las P se observó un aumento de las concentraciones plasmáticas de endotelina y GMPc. Las pacientes con HG mostraron una eliminación urinaria disminuida de noradrenalina e incrementada de adrenalina, así como una mayor concentración plasmática de nitritos que su grupo control. En las pacientes con P no se observaron diferencias estadísticamente significativas en la eliminación urinaria de catecolaminas ni en la concentración de nitritos en relación con sus controles.*

Conclusiones: *Existen diferencias relevantes en el patrón de síntesis de mediadores vasoactivos en la HG y la P. Estas diferencias condicionarían una perfusión tisular disminuida en la preeclampsia y podrían contribuir a la génesis de las alteraciones renales de este proceso.*

Palabras clave: ***Endotelina. Nitritos. GMPc. Catecolaminas. Hipertensión gestacional. Preeclampsia.***

INTRODUCTION

Several conditions are grouped under the term "gestational hypertensive conditions" such as gestational hypertension, preeclampsia, chronic hypertension, and preeclampsia overlapping with chronic hypertension. They represent 10-15 % of all pregnancies.[1] According to the Committee on Terminology of The American College of Obstetricians and Gynecologists", the diagnosis of hypertension during pregnancy may be done with the occurrence of at least one of the following changes: increase in 30 mmHg or more of usual systolic arterial blood pressure, increase in 15 mmHg or more of usual diastolic blood pressure, arterial systolic blood pressure equal or higher than 140 mmHg o arterial diastolic blood pressure equal or higher than 90 mmHg, whenever this value or its increase is sustained and observed at least on two times at least 6 hours apart.[1,2] Gestational hypertension (GH) is the occurrence of AHT in a previously normotensive woman during the pregnancy, at delivery, or the immediate post-natal period, with return to baseline within the first 10 post-natal days. If proteinuria (> 300 mg/24 hours) onsets under these conditions, then preeclampsia (P) is considered.[1]

The mechanisms implicated in the development of these hypertensive estates have been extensively studied. In women with preeclampsia impairments in multiple neurohumoral blood pressure regulating systems have been described, which may explain the elevations of this parameter. Thus, some studies have shown that preeclampsia women have higher catecholamines production than their corresponding controls.[4-6] Similarly, it has been postulated that P may be the consequence of an unbalance in the synthesis of

vasoactive endothelial factors, particularly a decrease in nitric oxide[7,8] and an increase in endothelin.[9-11] Cyclic GMP has also been studied, which is an indirect marker of the activity of nitric oxide itself and of circulating natriuretic peptides in preeclamptic patients, and the decrease in the synthesis of this second messenger has been evidenced.[12-14]

However, in spite of these analysis and similar others focused on the study of other bioactive metabolites, it has not been possible to determine the common pathogenic mechanism that would explain vascular dysfunction in P. This has been due to the lack of homogeneity in the information generated, since not all studies agree assigning the seam role to the different studied mediators. Moreover, it is very likely that a multifactorial mechanism exist with partial, although not definitive, contributions of the above-mentioned vasoactive metabolites.

By contrast with the large amount of information on P, there are no relevant studies exploring the genesis of GH. It has been assumed that pathogenic mechanisms should be similar, but there has not been a explanation why a hypertension developing during pregnancy, as a result of the feto-placental unit, progresses or not to renal dysfunction and, in some cases, to ischemia in certain areas of the body. The present studies were designed trying to answer this question. By analyzing several vasoactive factors, in hypertensive and preeclamptic pregnant women, we have tried to define patterns that would allow explaining the origin of these differences.

MATERIAL AND METHODS

Subjects

Two groups of study patients were consecutively selected, patients with GH (n = 21) and patients with P (n = 21). All were previously normotensive, their gestational age calculated by ultrasound, was older than 32 weeks, and in all of them AHT was detected, defined according to the criteria of the Committee on Terminology of the American College of Obstetricians and Gynecologists.[1] Besides, P patients had proteinuria, according to the definition of the Society for the Study of Hypertension in Pregnancy and the World Health Organization.[15] For each problem patient, the first normal pregnant woman followed-up at the clinic, was included into the study, provided that the following conditions were met: same gestational age (± 1 week, calculated by ultrasound), same age (± 2 years), same number of offspring, similar socio-economic status, and same level of smoking status (± 2 cigarettes). Thus, two control groups with GH were obtained (C-GH, n = 21), and another one for patients with P (C-P, n = 21). None of the women included in the study received drug therapy but iron supplements and usual vitamins during pregnancy, and none had a history of drug addiction. All gave their written informed consent after being informed, and eventual hospital admission was decided upon their clinical situation. From a clinical point of view, patients were managed at any time with usual procedures of the Hospital obstetrics department, although an additional Doppler study was carried out to assess umbilical blood flow.

Determinations

Between 8:00 and 9:00 a.m. of the following morning of entering the study, 3 blood samples and one urine sample were obtained. The first blood sample was used to carry out full blood count and coagulation study. The serum of the second sample was used to get different biochemical determination (liver enzymes, glucose, uric acid, lipids, total proteins, creatinine, urea, sodium, potassium, and calcium). The plasma from the third sample was used to quantify endothelin, nitrites, and cGMP. The urine was used to measure epinephrine and norepinephrine, with additional measurement of creatinine and calcium levels. After this first stage of blood sampling, 24-hours urine was collected to assess creatinine clearance as well as daily protein and sodium losses. The samples not being analyzed right away were stored at– 20ºC until further analysis.

Hematological parameters were processed by an automated H1 system (Technicon). Biochemical blood and urine quantifications were done by a Hitachi 717 auto-analyzer (Roche Diagnostics). Catecholamines were determined in 24-hour urine by HPLC with previous extraction by a cationic exchange extraction column. Endothelin and cGMP were determined by radioimmunoassay (Amersham) and plasma nitrites were quantified by Griess' reaction.[16]

Statistical analysis

Data are shown as mean ± standard error of the mean (SEM), median, range or percentage, according to the type of variable. For numerical variables, it was checked that data followed a normal distribution, and the results for each group of patients (GH or P) were compared with their corresponding control group by using the paired-samples Student's t test. Statistically significant differences were considered when the p value was less than 0.05

RESULTS

The general characteristics of the patients and their controls are summarized in Table I; no significant differences were observed for any of the parameters analyzed. About BP values at the time of study inclusion, and according to a priori set up criteria, both SBP and DBP were higher in hypertensive patients (groups GH and P) as compared to their corresponding controls, the values getting back to normal after delivery (Table II). Although the study design did not allow a direct comparison between patients only having hypertension and those having preeclampsia, the percentage increase of mean BP was comparable in both experimental conditions (GH vs. C-GH: 141%. P vs. C-P: 146%). The most relevant perinatal aspects are also summarized in Table II. In preeclampsia patients, delivery occurred earlier, with 2 weeks of advance on average as compared to their corresponding controls.

The main results relating to biochemical determinations done on these women are shown in Table III. In both preeclamptic and hypertensive patients an increase in serum levels of liver enzymes (GGT, GPT and alkaline phosphatase) and triglycerides, together with a decrease in calcium urinary extraction were observed, being statistically significant as compared to their controls. By definition, preeclamptic patients had proteinuria that was accompanied by a significant decrease in protein plasma levels. Higher levels of hemoglobin, creatinine, urea, and uric acid were found in these patients' sera, with decreased natriuresis. Hypertensive women without proteinuria did not present these changes.

About analysis of vasoactive mediators or metabolites reflecting their synthetic or activity pattern, the data are shown in Table IV. An increase in endothelin and cGMP plasma levels was observed in both GH and P patients, which was statistically significant in all cases as compared to their corresponding controls. About urine catecholamines output and plasma nitrite levels, the findings were diverse. In patients with preeclampsia, no significant differences were observed in these parameters as compared to their controls. Hypertensive patients without proteinuria showed decreased and increased urinary output of norepinephrine and epinephrine, respectively, as well as higher plasma concentration of nitrites then the control group.

Table I. General characteristics of the studied population

	C-GH	GH	C-P	P
Age (years)	28.7 ± 1.0	27.3 ± 0.9	28.7 ± 1.0	28.6 ± 1.2
Num. of offsprings	0 (0-4)	0 (0-4)	0 (0-5)	0 (0-5)
Familial history of AHT	19	15	14	19
Cigarette smoking (Num. of cig.)	1.3 ± 0.5	0.3 ± 0.2	4.8 ± 1.8	3.2 ± 1.0
Study week	36.3 ± 0.6	36.9 ± 0.6	34.9 ± 0.6	35.8 ± 0.7

C-GH: Control group of patients with gestational hypertension. **GH:** Group of patients with gestational hypertension. **C-P:** Control groups of patients with gestational preeclampsia. **P:** Group of patients with preeclampsia. Values are expressed as mean ± SEM (age, number of cigarettes, and study week), percentage (history) and median and range (number of offsprings).

Table II. Mother-fetus parameters

	C-GH	GH	C-P	P
SBP (pregnancy) (mmHg)	105 ± 2	141 ± 3*	103 ± 2	144 ± 2*
DBP (pregnancy) (mmHg)	61 ± 2	90 ± 1*	61 ± 2	93 ± 2*
SBP (postpartum) (mmHg)	112 ± 2	124 ± 2	117 ± 2	123 ± 3
DBP (postpartum) (mmHg)	66 ± 1	73 ± 2	69 ± 2	73 ± 2
Pathological placenta (%)	7	10	5	33*
Umbilical Doppler (s/d)	2.8 ± 0.2	2.5 ± 0.2	2.9 ± 0.2	3.0 ± 0.1
Delivery week	39.4 ± 0.6	38.0 ± 0.4*	39.1 ± 0.3	37.1 ± 0.5*
Umbilical artery pH	7.27 ± 0.01	7.29 ± 0.01	7.29 ± 0.01	7.25 ± 0.02*
Umbilical vein pH	7.34 ± 0.02	7.33 ± 0.01	7.36 ± 0.02	7.30 ± 0.02*
Newborn weight (g)	3173 ± 83	3152 ± 135	3126 ± 78	2564 ± 143*
Placental weight (g)	609 ± 46	575 ± 36	694 ± 54	507 ± 52*

C-GH: Control group of patients with gestational hypertension. **GH:** Group of patients with gestational hypertension. **C-P:** Control group of patients with preeclampsia. **P:** Group of patients with preeclampsia. **SBP:** Systolic blood pressure; **DBP:** Diastolic blood pressure; **S/D:** Systoles/diastole ratio in Doppler velocimetry of the umbilical artery. BP during pregnancy and data on umbilical Doppler refer to values at the time of inclusion into the study. Values are expressed as mean ± SEM (age, number of cigarettes and study week), except for placental pathology that is expressed as percentage. *$p < 0.05$ *vs.* their corresponding control.

DISCUSSION

The importance of certain vasoactive factors in the genesis of GH and P has been studied from several years ago. In fact, as new vascular function-regulating molecules were described, their likely role in the genesis of hypertensive conditions during the pregnancy has been studied. In spite of this, it has not been possible to describe a unique pathophysiologic

Table III. Results of biochemical determinations

	C-GH	GH	C-P	P
Proteinuria (mg/24h)	113 ± 15	107 ± 23	127 ± 27	2.740 ± 809*
Total proteins (g/dL)	6.2 ± 0.1	6.5 ± 0.2	6.2 ± 0.07	5.6 ± 0.1*
Hemoglobin (g/dL)	12.2 ± 0.1	12.5 ± 0.2	12.0 ± 0.2	12.8 ± 0.1*
Fibrinogen (mg/dL)	538 ± 22	586 ± 34	607 ± 20	486 ± 29*
GPT (IU)	14.2 ± 0.9	28.2 ± 7.4*	11.4 ± 0.6	33.5 ± 9.2*
GGT (IU)	7.9 ± 0.6	14.7 ± 1.2*	7.5 ± 0.7	11.2 ± 1.3*
Alkaline phosphatase (U/l)	252 ± 11	362 ± 21*	195 ± 10	297 ± 18*
Triglycerides (mg/dL)	192 ± 16	278 ± 25*	176 ± 9	256 ± 22*
Creatinine (mg/dL)	0.80 ± 0.02	0.77 ± 0.02	0.79 ± 0.01	0.87 ± 0.02*
Urea (mg/dL)	24.5 ± 1.6	26.5 ± 1.4	18.8 ± 1.5	26.9 ± 1.8*
Uric acid (mg/dL)	4.0 ± 0.2	4.8 ± 0.3	3.6 ± 0.2	6.1 ± 0.2*
CrCl (mL/min)	84.9 ± 3.2	74.6 ± 8.2	86.9 ± 2.9	88 ± 4.6
Natriuresis (mmol/day)	122.2 ± 11.7	94.8 ± 9.9	140.6 ± 10.5	102.7 ± 11.9*
Ca/Cr	0.22 ± 0.02	0.11 ± 0.02*	0.20 ± 0.02	0.09 ± 0.02*

C-GH: Control group of patients with gestational hypertension. **GH:** Group of patients with gestational hypertension. **C-P:** Control group of patients with preeclampsia. **P:** Group of patients with preeclampsia. **GPT:** Glutamate-pyruvate transaminase. **GGT:** Gamma-glutamate transaminase. **CrCl:** Creatinine clearance. **Ca/Cr:** calcium/creatinine urine levels ratio. Values are expressed as mean ± SEM. *p < 0.05 *vs.* their corresponding control.

Table IV. Catecholamines urinary clearance and endothelin, nitrites, and cGMP plasma level in the different experimental groups

	C-GH	GH	C-P	P
Norepinephrine (µg/g Cr)	37.2 ± 2.9	23.6 ± 3.2*	51 ± 4.2	43.6 ± 6.1
Epinephrine (µg/g Cr)	7.9 ± 1.2	17.9 ± 5.4*	12.3 ± 1.8	11.3 ± 1.9
Endotelin (pg/mL)	11 ± 1.0	25 ± 1.4*	9 ± 0.9	18 ± 2.4*
Nitrites (µM)	16 ± 2.5	35 ± 5.8*	14 ± 2.7	20 ± 4.8
cGMP (µM)	4.3 ± 0.4	6.1 ± 0.8*	3.7 ± 0.5	5.8 ± 0.6*

C-GH: Control group of patients with gestational hypertension. **GH:** Group of patients with gestational hypertension. **C-P:** Control group of patients with preeclampsia. **P:** Group of patients with preeclampsia. Value are expressed as mean ± SEM. *p < 0.05 *vs.* their corresponding control.

mechanism, so that this issue still is under investigation. There are not too many studies designed trying to differentiate, from the pathogenic point of view, the differences between GH and P.[2, 5, 8, 11, 12] Given this reason, the present investigational study has been carried out with a case-control design and trying to elucidate the differences existing between GH and P with regards to vasoactive factors.

A large amount of the information obtained confirms two basic facts: patients selection has been appropriate, and the behavior of patients with GH or P is in agreement with previous descriptions. Thus, both study groups had BP levels higher than controls and, evidently, preeclamptic patients had proteinuria, lower gestational age at the time of delivery, lower birth weight of the offspring and of the placenta, and a slightly decrease of umbilical vessels pH. For the remaining variables, the study groups completely compared to their corresponding control groups.

Many of the biochemical changes of preeclamptic patients are explained by a relative decrease in their circulating volume, as a result of either a decrease in plasma oncotic pressure or increased permeability of the vascular wall.[17, 18] Thus, the increase in hemoglobin, urea, and uric acid plasma levels and the decrease in natriuresis are largely the consequence of the decrease in plasma volume with the subsequent renal hypoperfusion.[19-21] Other biochemical changes detected in group P, such as the increase in transaminasas or the decrease in fibrinogen plasma level, have been unexpected. It is well known that fibrinogen consumption and hypertransaminasemia occur in severe forms of preeclampsia,[22, 23] although our results show that, even at not complicated stages of the disease, slight changes in these parameters can be observed, within the normal range, that may precede the occurrence of severe complications.

All hypertensive patients, with or without proteinuria, had hypertriglyceridemia and decreased calcium urine excretion. A higher preeclampsia risk has been observed in pregnant women with hypertriglyceridemia.[24] There controversial opinions about hypocalciuria,[25, 26] although an association between hypocalciuria and preeclampsia is currently accepted.[27] Our own results indicate, however, that hypocalciuria is also present among patients with GH, which would suggest a relationship between hypertension and reduction of urinary calcium, independently of the presence or absence of proteinuria.

The possible importance of certain vasoactive factors in preeclampsia, by contrast to what might occur in gestacional hypertension, has been approached from three perspectives. On the first place, urinary catecholamines clearance has been assessed,[4-6, 28] which is a more accurate reflection of daily synthesis of these metabolites than analyzing their plasma levels. Secondly, plasma levels of vasoactive endothelial factors have been measured, particularly endothelin and nitric oxide,[2, 7, 8, 29] by direct quantification of the immune reactive protein for the former and analyzing

nitrites for the latter. Finally, plasma cGMP level has been used as an indirect indicator of the effect of vasodilating factors activators of guanilate cyclases, such as nitric oxide itself or natriurético peptides.[12-14] These mediators have been selected assuming that all of them have been implicated in the genesis of preeclampsia.

In this study, important differences between the GH and P groups and their corresponding control groups have been detected. In particular, in GH patients, a significant decrease in urinary norepinephrine clearance, an increase in urinary epinephrine clearance, and high nitric oxide levels in peripheral blood were observed; these changes were not observed in preeclamptic patients. These findings may justify important differences in tissue perfusion of all vascular territories, including the kidney, which may condition the presence of structural and functional changes at that level with proteinuria onset and preeclampsia occurrence. The changes observed in endothelin and cGMP plasma levels were comparable between both study groups.

In summary, our results suggest that relevant differences exist in the synthetic pattern of vasoactive mediators between GH and P. These differences, which may condition a decreased tissular perfusion in preeclampsia, might contribute to the genesis of renal impairments in this condition.

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