Lower circulating angiotensin II levels are related to the severity of preeclampsia and its risk as disclosed by a specific bioassay

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Abstract

Preeclampsia is characterized by an increased sensitivity to angiotensin II (Ang II). We herein assessed whether serum Ang II levels measured by a new developed bioassay are associated with preeclampsia, its severity, and the risk for developing this disease.

Using a cross-sectional design, we studied 90 pregnant women (30 healthy pregnant and 60 with preeclampsia [30 with- and 30 without severe features]). We also used a nested case-control study with 30 women who eventually developed preeclampsia and 31 normotensive controls. Serum samples were collected at diagnosis of preeclampsia or at 4-week intervals (from weeks 12th to 36th). Ang II was measured using a bioassay.

At diagnosis of preeclampsia, serum Ang II concentrations were significantly lower in preeclampsia without and with severe features ($P = .001$ and $P < .001$, respectively) than in healthy pregnancy. In addition, Ang II was different in preeclampsia with severe features than in those without severe features ($P = .048$). Women who subsequently developed preeclampsia had lower Ang II levels than women with normal pregnancies, and these changes became significant at 24 weeks onward. The risk to developing preeclampsia was higher among women with Ang II concentration values in the lowest quartile of the control distribution from 12 weeks onward (odds ratio ranging from 3.8 [95% CI 1.3–11.1] to 6.5 [95% CI 1.6–26.9]).

We concluded that concentrations of Ang II are markedly diminished at diagnosis of preeclampsia and are closely associated with the severity of disease. Changes in circulating levels of Ang II precede the clinical presentation of preeclampsia.

Abbreviations: AGT = angiotensinogen, Ang 1-7 = angiotensin 1-7, Ang I = angiotensin I, Ang II = angiotensin II, AT_1R = angiotensin II type I receptor, AT_1R-AA = autoantibodies against angiotensin II type I receptor, BMI = body mass index, BPPS = Biomarkers and Prediction Preeclampsia Study Program, DMEM = Dulbecco modified Eagle medium, HEK-293 = human embryonic kidney-derived 293, HELLP = hemolysis, elevated liver enzymes, low platelet count, HP = healthy pregnant, IHS = inactivated horse serum, NFAT = nuclear factor of activated T cells, RASS = renin–angiotensin–aldosterone system, SGA = small gestational age.

Keywords: angiotensin II, biomarkers, preeclampsia

1. Introduction

Preeclampsia is a multisystemic pregnancy-specific disease characterized by endothelial dysfunction, leading to the clinical manifestations of this condition, including hypertension and either significant proteinuria or end-organ dysfunction.[1] This disease is presented by 5% to 8% of all pregnant women and remains as a major cause of maternal and perinatal morbidity and mortality worldwide.[2] To date the exact pathogenesis of preeclampsia is not completely understood. Pregnant women experience intense changes in cardiovascular function with a marked vasodilation and increase in blood flow, particularly to the uterus and kidneys in response to demands of the growing fetus and placenta.[3] In addition, during normal pregnancy, the circulating renin–angiotensin–aldosterone system (RAAS) plays a crucial role not only in maintaining circulating blood volume, blood pressure, electrolyte balance, and uteroplacental blood flow, but also in the development of preeclampsia.[4,5]

The circulating RAAS is classically described in the kidney and is defined by the action of renin, an enzyme synthesized and release by juxtaglomerular cells of the afferent renal arterioles in response to low blood pressure or circulating sodium chloride, which cleaves angiotensinogen (AGT) into angiotensin I (Ang I), a physiologically inactive decapeptide. Ang I is converted to the biologically active octapeptide, angiotensin II (Ang II) by angiotensin-converting enzyme (ACE). Ang II acting via the Ang II type 1 receptor (AT_1R, a G protein-coupled receptor) is predominantly vasoconstrictor and it is also a major regulator of aldosterone secretion, as well as angiogenesis and cell proliferation.[4,5]

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Although preeclampsia is characterized by an increased vascular resistance and a reduced intravascular volume, remarkably most components of the circulating RAAS are down-regulated.[4,6,7] In addition, compared with healthy pregnancies, patients with preeclampsia have an increased sensitivity to intravenous infusion of Ang II, showing an exaggerated pressor response.[8–10] On the other hand, there is no agreement in the literature about Ang II levels in pregnancies complicated with preeclampsia. Whereas some studies have reported no differences in Ang II levels between women with normal pregnancy and patients with preeclampsia,[11–14] other works have found that patients with preeclampsia exhibit lower,[6,15–17] or higher[18] Ang II levels. These discrepancies may stem from methodological difficulties with the Ang II assay, as well as to differences in the population characteristics studied, or to the limited sample size. To date, only the immunometric assays have been used to determine plasma Ang II levels; however, these methods are cumbersome and technically demanding. An alternative to assess serum Ang II is the measurement of circulating bioactive Ang II. Previously, we have applied a heterologous in vitro bioassay for Ang II for the detection of stimulating autoantibodies against Ang II type I receptor (AT_1R-AA) in women with preeclampsia. This bioassay uses human embryonic kidney-derived 293 (HEK-293) stably transfected with plasmids carrying the cDNA encoding the rat AT_1R and the firefly luciferase reporter construct under the control of the nuclear factor of activated T cells (NFAT) transcription factor.[19] In the present study, we validated and applied this in vitro bioassay model system to the measurement of circulating bioactive Ang II in serum samples from healthy pregnant (HP) women and patients presenting with the diagnosis of preeclampsia, and attempted to correlate bioactive Ang II levels with disease severity, and also to investigate whether differences in circulating bioactive Ang II may identify those women at risk to develop preeclampsia. To this end, we chose 2 samples of women belonging to a large cohort of pregnancies attended in our institute: 1 sample was integrated by women who eventually developed preeclampsia and the other by women who had an uneventful pregnancy and term labor.

2. Patients and methods

The study protocol was approved by our institute's review board. Written informed consent was obtained from all participants. All women were patients attended to the Hospital of Gynecology and Obstetrics of a tertiary care level hospital (which also provides prenatal, labor, and delivery services to healthy pregnant women). For the cross-sectional design, study participants were at gestational age 20 weeks or older and had a diagnosis of preeclampsia and were divided into those who had preeclampsia without severe features and those who had preeclampsia with severe features. For the longitudinal study, women who are participants in the Biomarkers and Prediction Preeclampsia Study Program (BPPS)[20] were used in this study. BPPS involves only singleton pregnancies in healthy nulliparous women or women with a second pregnancy and history of preeclampsia in their first pregnancy. For the purpose of the present study, samples from all 30 women who developed preeclampsia as well as from 31 HP women matched by maternal age, nulliparity, body mass index (BMI), smoking status, and history of preeclampsia in their first pregnancy, who remained normotensive throughout their pregnancies, and who delivered a healthy term infant ($\geq$38 weeks) were included. None of the women studied had

preexisting hypertension or diabetes, renal diseases, connective tissue disorders, and/or other high-risk obstetric condition.

For the longitudinal study, routine visits to the hospital were scheduled at 4-week intervals starting from week 12th of gestational age and ending at week 36th. For both studies, blood was drawn from an antecubital vein after 30 minutes of rest in the supine position and the serum obtained after centrifugation was aliquoted and stored at −80C until assayed.

Hypertension was defined as systolic or diastolic blood pressure $\geq$140 mm Hg or $\geq$90 mm Hg, respectively, measured twice at least 4 hours apart or 1 systolic or diastolic blood pressure $\geq$160 mm Hg or $\geq$110 mm Hg, respectively treated with antihypertensive medication, and that returned to normal values within 3 months after delivery. Preeclampsia was defined according to The American College of Obstetricians and Gynecology criteria.[21] Preeclampsia without severe features was considered hypertension and significant proteinuria ($\geq$300 mg protein in a 24-hours urine specimen or a protein to creatinine ratio $\geq$0.30 mg/mg in a random urine sample[22]). Preeclampsia with severe features was considered when either hemolysis, elevated liver enzymes, low platelet count (HELLP) syndrome, eclampsia, or preeclampsia with severe hypertension (systolic or diastolic blood pressure $\geq$160 mm Hg or $\geq$110 mm Hg, respectively) was present. Other parameters included, even in the absence of significant proteinuria, were new-onset cerebral or visual disturbances, upper right quadrant or epigastric pain, abnormal liver enzymes levels (to twice normal concentration), thrombocytopenia ($<$100,00/μL), serum creatinine $\geq$1.1 mg/dL or pulmonary edema. Small gestational age (SGA) infant was defined as an infant whose birth weight was below the 10th percentile.

2.1. Bioassay of Ang II

The bioassay was performed as described.[19] Briefly, HEK-293-rAT_1R-NFAT-Luc cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, penicillin (50 U/mL), streptomycin (50 μg/mL), and containing 500 μg/mL of geneticin (Invitrogen, Gaithersburg, MD). Cells were trypsinized, washed twice, switched to DMEM containing 10% heat inactivated horse serum (IHS), and seeded at a density of 1×10^4 cells in 100 μL per well in 96-well plates and cultured at 37C in humidified, 5% CO_2 atmosphere. Twenty-four hours later, 100 μL of serum-free DMEM containing different concentrations of Ang II (Sigma Chemical Co, St. Louis, MO) or 100 μL serum samples at different dilutions in DMEM/10% IHS were added to each culture well. Specific inhibition of the effects induced by Ang II was achieved by adding the nonpeptide AT_1R antagonists (telmisartan, losartan, valsartan, or candensartan [Sigma Chemical Co]). After incubation for 20 hours, the media were aspirated and the cells were lysed for 15 minutes in 25 μL lysis buffer (Promega, Madison, WI). Luciferase activity (measured in relative light units) was determined in cell lysates (20 μL) for 10 seconds using a luminometer (Sirius L, Berthold Detection Systems GmbH, Pforzheim, Germany).

3. Statistical analysis

Data shown are the means $\pm$ SEM or SD from 3 or more independent experiments or from representative experiments in triplicate incubations, unless indicated. Differences between continuous variables were determined by the unpaired Student t test (or the Mann–Whitney U test for non-normally distributed

variables). Differences between categorical variables were determined by the χ^2 test with Yates continuity correction or the Fisher exact test for small samples (or the Mantel–Haenszel $\chi2$ test with linear tendency for variables with >2 categories). Differences among ≥ 3 continuous variables were determined by 1-way analysis of variance followed by post hoc procedures (Scheffe test) or by the Kruskal–Wallis 1-way test followed by the Mann–Whitney U test for non-normally distributed variables.

For each gestational age interval analyzed, the association between circulating bioactive Ang II and the subsequent risk of preeclampsia was calculated. On the basis of the distribution exhibited by the samples from HP women, the serum concentrations of Ang II at each gestational age were divided into quartiles and the odds ratio (OR) was calculated and employed to assess the association between quartiles and the risk of preeclampsia. Due to the sample size, women in the 3 highest quartiles were collapsed and used as reference category. A 2-tailed $P < .05$ was considered statistically significant.

4. Results

4.1. Specificity of the response of rat Ang II receptor type 1 and nuclear factor of activated T cells in human embryonic kidney-293 cells

As shown in Fig. 1A, addition of increasing amounts of Ang II to cells led to a dose-dependent increase in luciferase activity, indicating that the rat AT_1R expressed was functionally active. Maximal and minimal activities were achieved at doses of 1000 ng/mL and 4 ng/mL Ang II, respectively and the within-assay and between-assay coefficients of variation were 4.8% and 6.9%, respectively. The effect induced by increasing concentrations of Ang II, Ang I, or Ang 1–7 is shown in Fig. 1A. Only Ang II activated the Ang II-responsive reporter gene, whereas addition of Ang I and Ang 1–7 had no effect. The antagonistic effects of telmisartan, losartan, valsartan, or candansartan on the induction of luciferase activity by Ang II (at 1 µmol/L [1000 ng/mL] concentration) are shown in Fig. 1B. Ang II-stimulated luciferase activity was inhibited by all the nonpeptide AT_1R antagonists in a dose-dependent manner, with complete abrogation at 1 µmol/L. All these data show that rAT1R-NFAT-Luc cells are specifically stimulated by Ang II.

4.2. Bioassay of Ang II in human serum

Addition of increasing amounts (10–50% v/v) of human serum to cells stimulated luciferase activity in a concentration-dependent manner. Thus, each sample was tested in at least 3 different dilutions to ascertain for parallelism with the Ang II standard curve and to determine more accurately serum Ang II bioactivity in cells. The effects of telmisartan on the induction of luciferase activity by human serum are shown in Fig. 1C. When telmisartan was added to the cultures, the induction of luciferase activity was completely abrogated. These data indicated that Ang II accounted for all of the biological response of cells to Ang II present in sera. As previously reported,[19] none of the patients included in this study were found to have AT_1R-AA.

5. Cross-sectional design

5.1. General description of the population studied

A total of 90 consecutive pregnant women were included in the study. There were 30 women who did not meet the criteria for
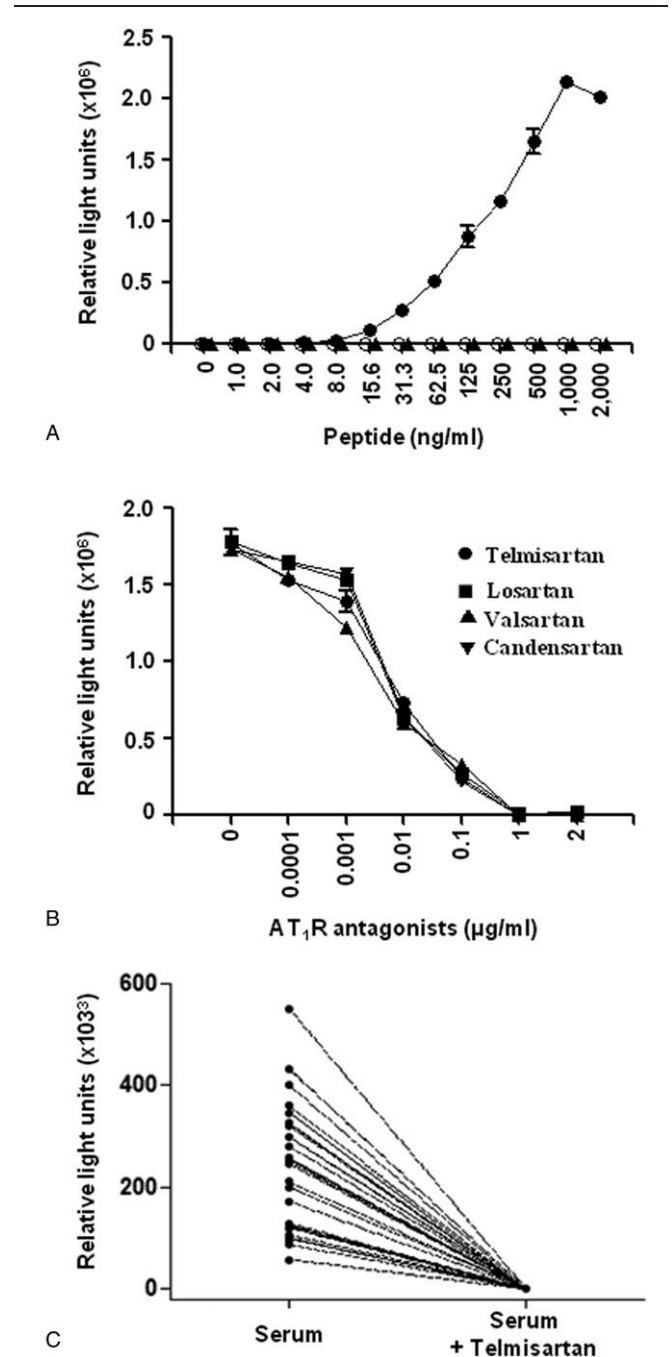

Figure 1. Functional characterization of the transcriptional bioassay in rat angiotensina II receptor type 1 (AT_1R)–nuclear factor of activated T cells–Luc human embryonic kidney-293 cells. A, Luciferase response (expressed as relative light units) in cells exposed to increasing concentrations of Ang II (●), Ang I (▲), or Ang 1–7 (○). B, The antagonistic effects of telmisartan, losartan, valsartan, or candansartan on Ang II-stimulated luciferase activity. Cells were incubated with Ang II (1 µmmol/l [1 µg/mL]) and increasing doses of AT_1R antagonists. C, The antagonist effect of telmisartan on cells luciferase response by serum samples from pregnant women. Values are the mean ± SEM from a representative experiment in triplicate incubations.

hypertensive disorders of pregnancy in further evaluations and were considered HP; the remaining 60 women were diagnosed as having preeclampsia: 30 without severe features and 30 had severe features (including 2 patients with HELLP syndrome). There were no significant differences in maternal age or BMI as well as nulliparity, history of preeclampsia, or smoking status

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Table 1

Demographic and clinical characteristics of women with normotensive pregnancies and women with preeclampsia.

Variables	Healthy pregnancies (n=30)	Preeclampsia without severe features (n=30)	P value	Preeclampsia with severe features (n=30)	P value
Maternal age, y, mean±SD	31.7±4.6	29.4±6.0		29.5±6.4	
Body mass index, mean±SD	28.1±6.2	26.7±5.6		26.2±4.3	
Nulliparous, n, %	10 (33.3)	12 (40.0)		14 (46.7)	
Previous preeclampsia, n, %	7 (23.3)	7 (23.3)		8 (26.7)	
Smoked during pregnancy, n, %	6 (20.0)	4 (13.3)		4 (13.3)	
Gestational age at enrollment, wk, mean±SD	36.0±0.0	36.1±2.3		33.5±3.3	<.001 [*,†]
Gestational age at delivery, wk, mean±SD	38.4±0.2	36.2±2.1	<.001 [*]	33.6±3.2	<.001 [*,†]
Infant birth weight, g, mean±SD	3,053±219	2,537±587	<.001 [*]	1,726±621	<.001 [*,†]
Small-for-gestational-age infants, n, %	0 (0)	7 (23.3)	.01 [*]	16 (53.3)	<.001 [*] .03 [†]
Serum Ang II, ng/mL, median (IQR)	70.4 (14.4–213.8)	15.2 (4.5–43.4)	.001 [*]	7.6 (2.5–19.9)	<.001 [*] .048 [†]
Patients sampled at gestational age ≥36 wk Serum Ang II, ng/mL, median (IQR).	70.4 (14.4–213.8) (n=30)	17.6 (7.8–53.3) (n=19)	.006 [*]	3.0 (1.7–18.0) (n=10)	<.001 [*] .008 [†]

P value is given only for significant differences.
SD = standard deviation, IQR = interquartile range.
[*] Versus healthy pregnancies.
[†] Versus patients with preeclampsia without severe features.

among the 3 groups studied (Table 1). Compared with HP women, patients with preeclampsia had lower gestational age at delivery ($P<.001$), delivered infants with lower birth weights ($P<.001$), and had a greater proportion of SGA infants ($P\le.01$) regardless of the severity of preeclampsia. These outcomes were more pronounced in patients with severe features than in those without severe features.

5.2. Serum Ang II concentrations

Table 1 shows serum Ang II levels. Serum Ang II levels were significantly higher in HP women than in patients with preeclampsia ($P\le.001$) and in patients with preeclampsia without severe features than in preeclampsia with severe features. Further considering that gestational age may distinctly affect serum Ang II levels and thereby explain the lower Ang II concentrations in preeclampsia, we made a subanalysis. For this, we only included the data from all women who were sampled at gestational age ≥36 weeks. This new analysis confirmed the previous results showing that serum Ang II levels were

significantly lower in patients with preeclampsia without severe features and even more in those with severe features than in those with HP (Table 1).

6. Longitudinal study

6.1. General description of the population studied

The demographic and clinical characteristics of the participants are shown in Table 2. As expected, there were no significant differences in maternal age or BMI as well as nulliparity, history of preeclampsia, or smoking status between women who developed preeclampsia and those who did not. Among 30 women who developed preeclampsia, 9 had early onset (<34 weeks) preeclampsia and 21 had late-onset preeclampsia (≥34 weeks), including 3 patients who developed HELLP syndrome and 1 eclampsia. Compared with HP, patients with preeclampsia had lower gestational age at delivery, delivered infants with lower birth weights, and had a greater proportion of SGA infants and preterm delivery ($P<.001$).

Table 2

Demographic and clinical characteristics of women with normotensive pregnancies and women who eventually developed preeclampsia.

Variables	Healthy pregnancies (n=31)	Women with preeclampsia (n=30)	P value
Maternal age, y, mean±SD	31.4±4.0	32.5±5.0	
Body mass index, mean±SD	26.6±5.5	28.05±6.1	
Nulliparous, n, %	9 (29.0)	8 (26.7)	
Previous preeclampsia, n (%)	10 (32.3)	16 (53.3)	
Smoked during pregnancy, n, %	8 (25.8)	6 (20.0)	
Gestational age at delivery, wk, mean±SD	38 (38–39)	35 (32–37)	<.001
Infant birth weight, g, mean±SD	3003 (2920–3200)	1920 (1175–2480)	<.001
Small-for-gestational-age infants, n, %	0 (0)	13 (43.3)	<.001
Delivery at <37 wk, n, %	0 (0)	11	<.001
Delivery at <34 wk, n, %	0 (0)	9	<.001
Neonatal deaths, n, %	0 (0)	2	

P value is given only for significant differences.
SD = standard deviation.

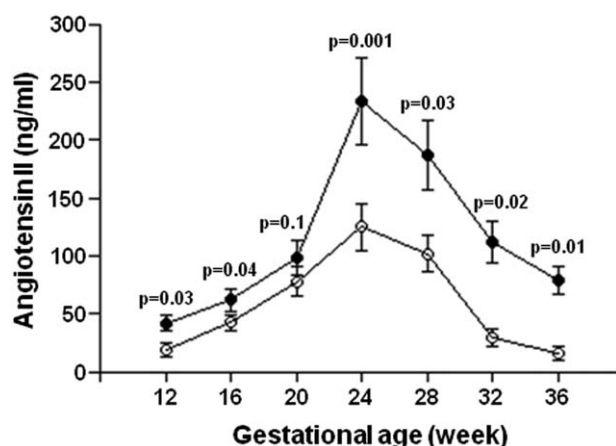Leaños-Miranda et al. Medicine (2018) 97:39

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Figure 2. Comparison of the dynamics of changes of Ang II in maternal serum from women who had normotensive pregnancies (●) or who later developed preeclampsia (○). Values are the mean ± SD. Differences at various gestational age time points between healthy pregnant women and patients who eventually developed preeclampsia at different times are shown.

6.2. Gestational changes in circulating levels of Ang II

Figure 2 shows the serum Ang II levels throughout gestation in HP and in those destined to developed preeclampsia. To assess the changes in Ang II levels throughout gestation, a cross-sectional analysis at each 4-week interval recorded was performed. In HP women, serum Ang II concentrations progressively rose from week 12 to week 24, decreasing thereafter until the end of sampling (at week 36). At each gestational age studied, patients destined to develop preeclampsia had lower serum Ang II concentrations throughout gestation than HP women; these differences became markedly significant from week 24 onward ($P \leq .03$ vs HP women).

6.3. Circulating bioactive Ang II and the risk of preeclampsia

The effect of changes in serum concentrations of Ang II on the association with the development of preeclampsia was investigated by grouping the levels into quartiles based on the distribution of Ang II among HP women. As measure of risk, ORs and 95% confidence intervals were calculated at each gestational age studied. No further adjustments for maternal age, nulliparity, previous history of preeclampsia, BMI, or smoking status were made because these variables were not significantly different between the groups by univariate analysis. ORs for serum Ang II concentrations in the lowest quartile were compared with those in the higher 3 quartiles, which were considered the reference category. Table 3 shows that at each gestational age studied, there was a clear association between Ang II concentrations in the lowest quartile and an increased risk to develop preeclampsia.

7. Discussion

In the present study, employing a new in vitro bioassay for measuring Ang II, the effector of hormonal RAAS in pregnant women, we found that serum Ang II levels at diagnosis of preeclampsia are significantly lower compared with HP women, as previously demonstrated in other studies.[6,15–17] Furthermore, we were able to find that differences in Ang II levels were more

Table 3

The association (odds ratios) between quartiles of angiotensin II concentrations and subsequent preeclampsia.

	Normal pregnancies controls	Preeclampsia	
		Cases	OR (95% CI)
12 wks of gestation			
Q1: ≤21.91	8	17	3.8 (1.3–11.1)
Q2–4: >21.91	23	13	1.0
16 wks of gestation			
Q1: ≤48.83	8	17	3.8 (1.3–11.1)
Q2–4: >48.83	23	13	1.0
20 wks of gestation			
Q1: ≤58.29	8	17	3.8 (1.3–11.1)
Q2–4: >58.29	23	13	1.0
24 wks of gestation			
Q1: ≤75.64	8	18	4.3 (1.5–12.8)
Q2–4: >75.64	23	12	1.0
28 wks of gestation			
Q1: ≤55.26	8	16	4.2 (1.4–12.7)
Q2–4: >55.26	23	11	1.0
32 wks of gestation			
Q1: ≤54.97	8	15	4.8 (1.5–15.2)
Q2–4: >54.97	23	9	1.0
36 wks of gestation			
Q1: ≤36.97	8	9	6.5 (1.6–26.9)
Q2–4: >36.97	23	4	1.0

Quartiles (Q) were determined on the basis of the distribution among women with normotensive pregnancies (controls). For the odds ratios shown in the lowest quartile, the reference category was the higher 3 quartiles.
OR = odds ratio, CI = confidence interval.

pronounced as the severity of preeclampsia increased, regardless of gestational age, suggesting that changes in Ang II concentration effectively may reflect disease severity and clinical outcomes in patients with preeclampsia. These findings agree with previous studies, including that employing a method that combining separation of plasma angiotensin peptides by high-performance liquid chromatography with a sensitive radioimmunoassay of Ang II.[6,16,17]

It is clearly established that despite the known reduction in circulating volume during preeclampsia, most components of the circulating RAAS are downregulated compared with normal pregnancy.[23] However, no clear agreement exists about Ang II levels during normal pregnancy or in preeclampsia. Although we and others have found that low circulating levels of Ang II are associated with preeclampsia ([6,15–17], present study), other studies have failed to demonstrate such an association.[11–14] Possible explanations for these apparent discrepancies include differences in selection criteria (i.e., inclusion of women with a misdiagnosis of preeclampsia according to new guidelines[21]) and the relatively small number of preeclampsia patients.

On the other hand, in the present nested case-control study, we found that serum Ang II levels in women who eventually developed preeclampsia behaved distinctly during pregnancy than in healthy controls. Even more important was the observation that the dynamics of changes in concentrations values of Ang II preceded the appearance of preeclampsia. Women who developed preeclampsia exhibited significantly lower Ang II concentrations as early as at week 12 of gestation. More vividly, decreased Ang II concentrations were more pronounced in women who subsequently developed preeclampsia from 24 weeks onward than in HP women. In this vein, a longitudinal study has also shown that in HP women, circulating

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Ang II levels gradually increasing with gestational age, with a significant rise seen in the second and third trimester of pregnancy.[6,24]

Women whose values for Ang II fall within the lowest quartile showed an increased risk for preeclampsia. When the same analysis based on gestational age was performed, it was found that the risk for developing preeclampsia progressively increased throughout pregnancy.

To date, measurements of Ang II in serum or plasma samples at diagnosis of preeclampsia have only been performed employing immunoassay techniques. In this vein, and to our best knowledge, the present report represents the first that has prospectively examined the relationship between circulating Ang II concentrations and the risk to subsequently develop preeclampsia.

It is well known that normal pregnancy is to be a state of relative resistance to intravenous infusion of Ang II, conversely pregnant women who later developed preeclampsia are sensitive to the pressor response of Ang II, even this sensitivity occurs prior to the clinical signs and symptoms of preeclampsia; therefore, the pressor response to Ang II has been proposed as a diagnostic tool for predicting hypertensive disorders of pregnancy.[9,25] Several hypotheses have been proposed to explain the increased sensitivity to Ang II, including increased AT_1R expression,[26] increased heterodimerization of the AT_1R with the bradykinin type 2 receptor,[27] increased neutrophil infiltration, resulting in excessive ROS production and consequent increase vascular reactivity,[28] increased circulating sFlt-1, which acts by inhibiting eNOS function,[29] and the presence of AT_1R-AA.[30] Regarding the latter hypothesis, it is improbable since antibodies are virtually absent in our studied population as we reported previously.[18] In addition, a recent study showed that antibodies are not sensitive for preeclampsia and hence not useful as a biomarker [31].

In the classical study in 1973 by Gant et al,[9] it was shown that in HP women the Ang II doses required to elicit the pressor response (to get a 20 mm Hg increase in diastolic blood pressure) increased until 28 weeks pregnancy, decreasing thereafter. In contrast, patients destined to develop pregnancy-induced hypertension exhibited a maximal resistance at 15 to 18 weeks of gestation (similar to HP women), decreasing progressively the resistance thereafter until the end of pregnancy. As group, patients destined to develop pregnancy-induced hypertension, the pressor dose requirements were lower throughout gestation than HP women; these differences became highly significant at 23 to 26 weeks of gestation, and the difference between the groups became progressively widened thereafter. Interestingly, the time course of Ang II hypersensitivity closely resembles the changes in serum concentrations of Ang II in our preeclampsia patients studied. We found that from week 24 onward, patients who subsequently developed preeclampsia exhibited a marked reduction of serum Ang II levels than HP women. Likewise, at these times, the difference in responsiveness to Ang II between HP women and patients destined to develop pregnancy-induced hypertension was marked. The observation of a temporal relationship between circulating Ang II levels and Ang II sensitivity suggests that Ang II concentrations may directly influence the increased sensitivity to Ang II in preeclampsia. Collectively, these data provide for the first time the notion that alterations in circulating Ang II concentrations are present in women who subsequently developed preeclampsia and that these changes are apparently related to Ang II sensitivity.

Our data for Ang II disagree with those obtained by immunometric assays, but they also appear to be inconsistent with one another. Nevertheless, our values for Ang II during pregnancy are considerably higher than data in previous studies.[6,15,16,24] This difference may be explained by a sub-estimation caused by losses of peptide during extraction process in these earlier studies, or due to the impurity or relative decreased bioactivity of the Ang II standard employed in our study.

The strengths of our study are the following: the study was designed to determine whether, when, and how the circulating concentrations of Ang II change in pregnancies who subsequently developed preeclampsia; as the levels of Ang II change depending on the gestational age, measurements at regular (every 4 weeks) time intervals were performed in the same participants; and to rule out the effects of potential confounders, cases and controls were matched for maternal age, BMI, nulliparity, previous history of preeclampsia, and smoking status. Nonetheless, it is important to acknowledge that our study has some limitations. First, it did not include patients with other obstetrics conditions sharing similarities with preeclampsia, such as gestational hypertension or pregnancies complicated by isolated intrauterine growth restriction. Second, it may not be possible to generalize our findings to pregnant women at high risk for preeclampsia, such as chronic hypertension, pregestational diabetes, renal diseases, autoimmune disorders, or other health conditions.

In summary, the results presented herein employing a specific bioassay to measure Ang II concentrations showed that Ang II levels are markedly diminished in preeclampsia at diagnosis and are closely associated with the severity of preeclampsia. Further, we present evidence that circulating Ang II concentrations are associated with the potential to develop preeclampsia and that changes in circulating levels of Ang II precede the onset of clinical disease and also are closely related to Ang II sensitivity. In particular, measurement of Ang II has potential relevance as a diagnostic and predictive biomarker for preeclampsia. Further studies are still needed to elucidate whether the changes in the levels of Ang II are a physiological response to the increased blood pressure or an active contributor to the pathophysiology of preeclampsia.

Author contributions

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Formal analysis: Alfredo Leaños-Miranda, María Guadalupe Berumen-Lechuga.

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Writing – original draft: Alfredo Leaños-Miranda, María Guadalupe Berumen-Lechuga.

Writing – review & editing: Alfredo Leaños-Miranda.

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