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NF-κB and SOD expression in preeclamptic placentas

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Background/aim: Preeclampsia is a leading cause of maternal death in the developing world. Our aim was to quantify and compare messenger (mRNA) expression of nuclear factor-kappa beta (NF-κB) and superoxide dismutase (SOD) in control patients with preeclampsia and without preeclampsia with or without familial hereditary background.

Materials and methods: Four groups of patients were formed depending on the presence or absence of preeclampsia and presence or absence of familial history for preeclampsia. NF-κB and SOD were measured in human placentas by real-time quantitative polymerase chain reaction. The 2–ΔΔct analysis method was used to measure the difference in the relative expression of the target genes in each group of patients.

Results: In NF-κB expression, there was an increase of 23.35% in the group of women with preeclampsia versus women with preeclampsia without familial history. Regarding SOD, there was a reduction of about 33.33% in the expression in women with preeclampsia with familial history versus women with preeclampsia without familial history.

Conclusion: Familial presence of preeclampsia could predispose to altered expression in SOD and NF-κB.

Key words: Genetic background, preeclampsia, nuclear factor-kappa beta, superoxide dismutase

1. Introduction

Preeclampsia is a multisystemic and multifactorial disease and complicates 2%–8% of pregnancies after 20 weeks of gestation, being the leading cause of maternal death in developing countries and a major contributor to maternal and perinatal morbidity. Globally, preeclampsia and eclampsia account for 10%–15% of maternal deaths. The majority of these deaths in developing countries result from eclampsia, while in developed countries, complications of preeclampsia are more often the cause (1). In Mexico, preeclampsia represents up to 34% of all maternal deaths, being the leading cause of death-associated pregnancy complications (2,3). There is a certain type of complex inheritance in this disease: some studies postulate that persons with a familial history of preeclampsia are more susceptible to this disease. For example, Darcy et al. described that women with preeclampsia were 2.3 times more likely to have a female sibling who also had preeclampsia (4). A study carried out by Esplin et al. also found that both men and women who were the product of a pregnancy complicated by preeclampsia were significantly more likely than control men and women to have a child who was the product of a pregnancy complicated by preeclampsia (5). The greater likelihood of preeclampsia among relatives with a previous preeclamptic pregnancy is consistent with the pathophysiological role for genetics and/or behavioral factors that cluster in families.

Normal pregnancy exhibits an increase in free radical (FR) production; in parallel, antioxidant defenses also increase the maintenance of balance in the redox state (6). In contrast to normal pregnancy, there is an imbalance in the redox state in preeclampsia, i.e. women with preeclampsia exhibit an increased production of FRs and, at the same time, a decrease in several important antioxidants, generating oxidative stress (OS) with consequent damage to the entire class of important molecules such as DNA, proteins, unsaturated fatty acids, etc., and overstimulation of lipid peroxidation (7,8). During a preeclampsia-complicated pregnancy, catalase activity increases and superoxide dismutase (SOD) and glutathione reductase (GR) activity decreases compared with the same parameters during physiological pregnancy (9).

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Recently, it is has come to be known that, in addition to the direct damage that FRs can inflict on the molecules, imbalance in the redox state can alter the normal expression of various signaling pathways that comply with the physiological functions necessary to maintain the organisms' homeostasis (10,11). One of these signaling pathways is the nuclear factor-kappa beta (NF-κB) pathway (12). This transcription factor is critical for a wide range of physiological processes, such as immunity, cell development, growth, and survival; however, aberrant activation of NF-κB could lead to morbid states (13). The aim of this study was to compare *NF-κB* and *SOD* gene expression in women who had suffered preeclampsia or not with or without familial history.

2. Materials and methods

This was a cross-sectional, prospective, nonrandomized, and descriptive study developed from March 2012 to February 2013, approved by the Ethics Committees of the Maternal-Perinatal Hospital "Mónica Pretelini Saénz" (HMPMPS) (2010-12-156), Health Institute of the State of Mexico (ISEM), and the Medical Sciences Research Center (CICMED), Autonomous University of the State of Mexico (UAEMex) (2010/02), and performed according to the ethical standards of the Helsinki Declaration of 1964. Written informed consent was obtained from all patients.

2.1. Patients

Pregnant women were recruited at the HMPMPS, ISEM, Toluca, Mexico. All subjects with chronic medical disorders such as diabetes mellitus, cardiovascular disease, autoimmune disease, chronic renal disease, chronic hypertension, collagen disorder, and other chronic disease were excluded.

Four groups were formed as follows: a) normal pregnancies; b) normal pregnancies with familial history of preeclampsia; c) women with preeclampsia with familial history of preeclampsia, and d) women with preeclampsia without familial history of preeclampsia. The definition of and the criteria for preeclampsia were based on the diagnostic criteria outlined by the American College of Obstetrics and Gynecology (14). The sample was considered at convenience during the year of follow-up.

2.2. Clinical measurements

Weight (kg), height (m; Seca, GmbH, Germany), and waist circumference (cm) of all participants were measured in the morning. Body mass index was calculated as weight (kg) divided by height (m) squared. Blood pressure was recorded at each patient visit using a standard sphygmomanometer (Riester Big Ben® Square, Germany) with an appropriately sized cuff.

2.3. Laboratory analysis

All patients underwent standard care, including routine laboratory tests, with an 8-h fasting period: albumin (mg/dL); cholesterol (mg/dL); creatinine (mg/dL); glucose (mg/dL); triglycerides (mg/dL); uric acid (mg/dL); liver profile (Dimension R × L Max; Dade Behring, USA), and hematological parameters (Advia 120; Bayer Health, Germany). All these tests were measured at the HMPMPS according to standardized procedures recommended by the International Federation of Clinical Chemistry and Laboratory Medicine.

2.4. Placental samples

Placental tissue samples were isolated a few minutes after delivery. Tissues were dissected from the maternal side in areas free of visible infarction, calcification, hematoma, or tears. The dimensions of each sample were approximately 1–3 cm^3 and 100 mg wet weight. The basal and chorionic plates were removed, and each sample was washed three times with ice-cold calcium and magnesium-free phosphate-buffered saline solution. Immediately afterward, the tissue was stored at –80 °C until analysis.

2.5. mRNA extraction

The samples were homogenized using a Bullet Blender Standard BBX24 instrument (Next Advance, Inc., USA). Messenger RNA (mRNA) was extracted using the Magna Pure LC RNA isolation kit III (Roche, Germany) in the Magna Pure LC 2.0 Instrument. The $A_{260}/_{280}$ nm absorbance ratio was >1.8 (quality) and total RNA concentration was calculated by determining absorbance at 260 nm established with the NanoPhotometer (Implen GmbH, Germany).

2.6. cDNA synthesis

A total of 200–400 ng of total mRNA was reverse-transcribed to complementary DNA (cDNA) using a

Table 1. Primer sequences.

Gene	Forward	Reverse
GAPDH	5'-ctttggtatcgtggaaggactc-3'	5'-gtagaggcagggatgatgttct-3'
NF-κB	5'-tgggaatccagtgtgtgaag-3'	5'-cacagcattcaggtcgtagt-3'
SOD2	5'-gcctacgtgaacaacctgaa-3'	5'-gccgtcagcttctccttaaa-3'

GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; NF-κB: nuclear factor-kappa-beta, SOD2: superoxide dismutase 2.

Transcriptor High Fidelity cDNA Synthesis Kit (Roche). The samples were measured with a NanoPhotometer (Implen GmbH), and the extracts were then adjusted to a concentration of 20 µg of DNA for the PCR reaction.

The primers used in the experiments were designed using the Primer Quest web tool (Integrated DNA Technologies, Inc., USA) and synthesized at the Synthesis and DNA Sequencing Unit of the National Autonomous University of Mexico (UNAM) Institute of Biotechnology (Cuernavaca, Morelos, Mexico). A BLAST search was performed on primer sequences to ensure oligohybridization of only specific products. The sequences of the primers used in the analysis are depicted in Table 2.

Real-time quantitative polymerase chain reaction (qPCR) was performed with the 7500 Fast Real Time PCR System (Applied Biosystems, Applera UK, Cheshire, UK). The final reaction volume (20 µL) contained 10 µL of SYBR Advantage qPCR premix (2X), 0.4 µL of each forward and reverse oligonucleotide, 0.4 µL of ROX Reference Dye LSR or LMP (50X), 2 µL of template, and 6.8 µL of dH_2O (SYBR Advantage q-PCR Premix; Clontech, USA). The final concentration of each gene was optimized according to the final results calculated with the Taguchi method. The thermal cycling conditions were as follows: 10 min at 95 °C followed by 45 cycles of denaturation at 95 °C for 15 s and annealing/extension at 60 °C for 1 min.

The fold change in NF-κB and SOD was normalized against glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and then compared with the untreated controls (calibration sample) with the delta-delta-(CT) method ($2^{-\Delta\Delta CT}$) as follows: = (CT-target – CT-reference) treated-sample – (CT-target – CT-reference) calibrator-sample, where CT = threshold cycle. Calibrator-sample refers to the expression level (1×) of the target gene normalized to the constitutive gene. The calibrator was chosen from the group of women with normal pregnancies without familial history of preeclampsia.

2.7. Statistical analysis

Anthropometric and laboratorial results were presented as median (range), and those of genetic expression were presented as mean ± standard error (SE). The Mann–Whitney U test was used for variables that were not normally distributed. The normality hypothesis was tested utilizing the Kolmogorov–Smirnov test. Statistical analysis was performed using SPSS 20.0. In all cases, $P \leq 0.05$ was considered statistically significant.

3. Results

3.1. General patient characteristics

We studied 31 pregnant women with mean age of 22.3 ± 3.4 years: eight with normal pregnancies, eight with normal pregnancies but with familial antecedents of preeclampsia, seven with preeclampsia without familial history, and seven with preeclampsia with familial history.

Taking into account all women with a previous case of preeclampsia in their family (N = 15), the mothers

Table 2. Clinical and laboratorial characteristics.[1]

	Group			
Variable	Healthy pregnancies	Healthy pregnancies[a]	Preeclampsia	Preeclampsia[a]
Age (years)**&	20.5 (17–25)	24 (17–25)	25 (22–29)	22 (16–26)
BMI (kg/m^2)	26.2 (19.3–40)	26.6 (23.2–33.3)	28.6 (22.5–32.9)	31.2 (22.5–33.1)
Pregnancies	2 (1–4)	2.5 (1–4)	1 (1–2)	1 (1–5)
Prenatal consultations	6.5 (5–9)	6 (5–8)	6 (5–7)	5 (2–7)
Baby weight (kg)	3 (2.9–3.8)	2.8 (2.2–3.7)	3.6 (2–4)	2.9 (2.4–3.8)
SBP (mmHg)**t	111.5 (104–125)	121 (88–145)	138 (100–152)	133 (110–150)
DBP (mmHg)*t	68 (59–85)	73 (49–88)	81 (67–106)	80 (70–90)
MBP (mmHg)*t	83.3 (74–98.3)	89.8 (69–107)	100 (78.7–121.3)	96 (90–110)
Leukocytes (cells/mm^3)	7500 (6000–15,300)	9000 (6100–12,300)	8900 (4500–14,000)	7700 (6300–10,200)
Erythrocytes (cells[b]/mm^3)	4.5 (3.8–5.3)	4.5 (4.1–4.7)	4.4 (3.7–4.9)	4.2 (4–4.9)
Hb (g/dL)	13.5 (9.7–15.2)	13.3 (12.1–14.3)	13.2 (8.9–14.9)	12.7 (10.9–14.9)
Platelets (cells[c]/mm^3)	235 (108–269)	216 (165–376)	145 (122–263)	181 (147–313)

[1]Median (range); BMI: body mass index, Hb: hemoglobin, SBP: systolic blood pressure, DBP: diastolic blood pressure, MBP: median blood pressure. a: With family history for preeclampsia, b: millions, c: thousands. &: Difference between healthy women and preeclamptic women with familial history; t: difference between healthy women and preeclamptic women without familial history.
*: $P < 0.05$; **: $P < 0.01$.

represented 40% (N = 6) and their female siblings 60% of the affected relatives (N = 9).

Primi- or multiparous women with a new sexual partner constituted 50% (4 of 8) of the healthy group of pregnancies without a familial history of preeclampsia, 37.5% (3 of 8) of the healthy group of pregnancies with familial history, 71.4% (5 of 7) of the group of women with preeclampsia without familial history, and 85.7% (6 of 7) of the group of women with preeclampsia with familial history.

There were statistical differences in systolic ($P < 0.01$), diastolic ($P < 0.05$), and mean blood pressure ($P < 0.05$) between healthy women and women with preeclampsia without familial history. The only difference between healthy women and pregnant women with preeclampsia with familial history was in age ($P < 0.01$). Among laboratory data, there were no statistical significant differences in the hematological variables (Table 2).

When comparing the two groups with preeclampsia, women with affected relatives showed lower levels of uric acid ($P < 0.05$). Lactate dehydrogenase exhibited a tendency to be higher in the group with affected first-degree relatives ($P = 0.053$).

3.2. Gene expression

Upon analyzing the normalized CT values with the Kruskal–Wallis test, there were no statistically significant differences in either the expression of NF-κB or SOD among the four groups (Table 3) (Figures 1A and 1B). Notwithstanding the lack of statistical difference, there was a reduction of about 33.33% in SOD expression in women with preeclampsia versus women with preeclampsia without familial history. Regarding NF-κB, there was an increase of 23.35% in the group of women with preeclampsia versus women with preeclampsia without familial history. When including all of the patients, only in two groups, that is, normal pregnancies and those of women with preeclampsia, was there also no difference in genetic expression.

4. Discussion

A large amount of investigation has shown a genetic association between a multifactorial polygenic inheritance and the development of preeclampsia (15). The risk of developing preeclampsia is increased five times in women with first-degree relatives with this disease, while those with second-degree relatives with the disease have their risk doubled (16). However, paternal genes may also play an important role in the development of preeclampsia, because the risk of this disease is increased in women with the pregnancies of men who have previously been involved in pregnancies complicated by preeclampsia (17).

Over 70 biological candidate genes have been examined, representing pathways involved in various pathophysiological processes, including vasoactive proteins, thrombophilia, hypofibrinolysis, OS and lipid metabolism, endothelial injury, and immunogenetics (18,19). These findings and others suggest a multifactorial polygenic inheritance with a genetic component in the development of this disease (20). In analyzing OS, SOD probably possesses a tendency to be decreased in women with a familial history of preeclampsia.

Even more, several microarray studies have analyzed the transcriptome of preeclamptic placentas for identifying genes that could be involved in placental dysfunction. Some of the promoters of upregulated genes are enriched in putative binding sites for NF-κB (21), which determines an inflammatory response (22). Contrariwise, specific inhibitors of p38 mitogen-activated protein kinase and NF-κB, but not protein kinase C signaling pathways, can reduce the stimulatory effect of interleukin-1 beta (23).

It has only been in recent years, as susceptibility genes for other complex disorders have been reported, that the effect of the small size of individual genetic variants have become apparent, the majority increasing the risk for disease by <50% (24). In this study, we compared *NF-κB* and *SOD* expression in pregnant women with or without preeclampsia and familial history of this disease. Although there were expected changes in the relative expression of both genes when there was familial history for preeclampsia, these modifications were not sufficiently strong to achieve statistical difference.

A limitation of this study is the small sample size of each group; however, investigation concerning preeclampsia is time-consuming and, similar to other studies (25,26), we

Table 3. Relative gene expression.[1]

	Group			
Gene	**Healthy pregnancies**	**Healthy pregnancies[a]**	**Preeclampsia**	**Preeclampsia[a]**
NF-κB	4.84 (2)	3.29 (1.1)	6.55 (3.1)	8.08 (1.7)
SOD	3.17 (2.8)	6.68 (3.3)	2.82 (1.2)	1.88 (0.7)

[1]Median (standard error [SE]). NF-κB: Nuclear factor-kappa-B, SOD: superoxide dismutase. a: With family history for preeclampsia.

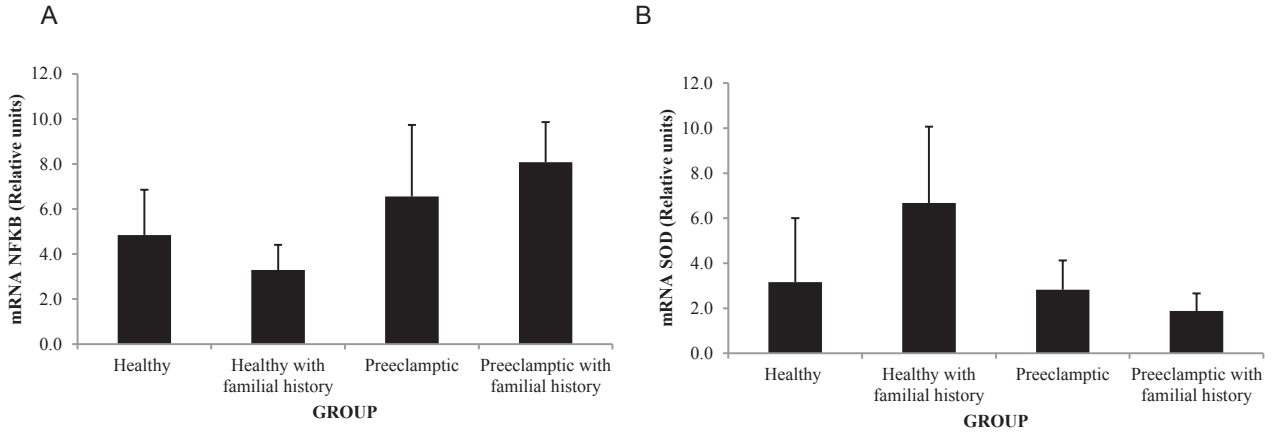

Figure 1. A) Messenger RNA (mRNA) expression of NF-κB, B) mRNA expression of SOD. NF-κB: Nuclear factor kappa-beta; SOD: superoxide dismutase.

think that analysis of novel molecules is valid in a small population as an initial approach.

While it is noteworthy that no one gene will be identified as the sole risk factor for preeclampsia, interactions among numerous pathways activated by several genes and/or single nucleotide polymorphisms, in addition to predisposing environmental factors, are most likely to underscore the genetic component of this disorder

Our study implies that the familial presence of preeclampsia could predispose to an altered expression in *SOD* and *NF-κB*, partially explaining the genetic background that predisposes to a higher risk of this disease.

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