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CYP1A1, *GSTT1*, *IL-6* and *IL-8* transcription and IL-6 secretion on umbilical endothelial cells from hypertensive pregnant women: Preliminary results

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ABSTRACT

The impact of pregnancy hypertension in the offspring endothelia remains unknown. We evaluated the transcriptional expression of four genes that participate in the process of endothelial dysfunction using umbilical vein endothelial cell cultures (HUVEC) from healthy pregnant women (PW) and those with hypertensive disorders (HD). The cytochrome P450 1A1 (*CYP1A1*), gluthathione *S*-transferase subtype T1 (*GSTT1*), interleukin 6 (*IL-6*) and 8 (*IL-8*) mRNA and IL-6 protein levels were assessed. *IL-6* and *IL-8* transcripts were significantly reduced in HUVEC obtained from HD women. Our results suggest that a hypertensive environment *in utero* modifies the transcriptional expression of key inflammatory molecules in the newborn.

1. Introduction

HD and their complications during pregnancy are main causes of maternal morbidity and mortality [1]. The vascular endothelium plays a crucial role in the pathogenesis of hypertension. When endothelium is subjected to oxidative and inflammatory conditions develops a dysfunctional phenotype characterized by inflammation, oxidative stress and increased cell adhesion [2,3], losing the vasodilation and vasoconstriction balance.

Hypertension during pregnancy increases the risk for fetal complications such as prematurity, low birth weight and fetal death [4]. In the long term, the risk for cardiovascular diseases in offspring increases [5], which display augmented blood pressure and a higher risk for hypertension [6]. Therefore, we assessed the transcription levels of genes associated with oxidative and inflammatory pathways that may be responsible of triggering an endothelial dysfunctional phenotype in the fetal endothelia. Using HUVEC from normotensive and hypertensive PW, we evaluated the transcript levels of the pro-oxidant metabolizing enzyme, *CYP1A1*, the phase II antioxidant enzyme, *GSTT1,* and the inflammatory mediators, *IL-6* and *IL-8;* genes associated with endothelial dysfunction and hypertension [3,7–9]. IL-6 secretion was determined in the supernatant HUVEC media to confirm mRNA findings.

2. Methods

Umbilical cords came from PW in labor attending the *Hospital General Ticomán (Secretaría de Salud, Ciudad de México)*. All patients were classified following the physician's criteria at hospital admission. Inclusion criteria for the HD group were systolic blood pressure ≥ 140 mm Hg or diastolic blood pressure ≥ 90 mm Hg. Proteinuria, assessed with a dipstick test, was expressed as 1(+), 2(+) or 3(+) (30,

Abbreviations: CYP1A1, cytochrome P450 1A1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GSTT1, glutathione *S*-transferase T1; HD, hypertensive disorders; HUVEC, human umbilical vein endothelial cells; IL, interleukin; PW, pregnant women

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100 or 300 mg albumin/dl, respectively). Unfortunately, we were unable to obtain the participant's clinical history from the HD women for further classification, therefore this group may encompass patients with chronic hypertension, gestational hypertension, preeclampsia and preeclampsia superimposed on chronic hypertension. Samples were collected from 53 controls and 30 HD patients. The Ethic Committee of the Hospital approved this protocol (Reference E1/230/2012).

HUVEC cultures were obtained as described [10] and the endothelial phenotype confirmed by the endothelial marker CD144 (96% positive cells). Confluent cultures were harvested at passage 1 to 3 and preserved in Trizol (Invitrogen) at −70 °C. Supernatant media was collected and stored at −20 °C. All experiments were performed in the same passage including those using the supernatant culture media.

Total RNA was isolated using Trizol reagent's protocol. RNA was digested with DNase I and cDNA was synthetized with the SuperScript III First-Strand Synthesis System (Invitrogen). TaqMan probes for qPCR were: Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH)* (Hs02758991_g1), *IL-6* (Hs00153120_m1), *IL-8* (Hs00174103_m1), *CYP1A1* (Hs01054797_g1) and *GSTT1* (Hs00184475_m1) (Applied Biosystems). qPCR was performed using the TaqMan Gene expression Master Mix containing 100 ng cDNA in a StepOnePlus Real-Time PCR System (Life Technologies). Amplification efficiencies for *GAPDH, IL-6, IL-8* and *GSTT1* were 93.3%, 104.6%, 96.8% and 98%, respectively. Transcript's amplification was validated for the ΔΔCt comparative method using GAPDH as housekeeping. A pooled cDNA was loaded into each PCR assay for interplate calibration. For *CYP1A1*, ΔΔCt validation was unapproved and the standard curve method was used for its quantification [11]. Briefly, *CYP1A1* and *GAPDH* PCR products were purified (GenElute PCR Clean-Up kit, SIGMA) and quantified (Quant-iT Pico Green, Molecular Probes), standard curves were prepared from the purified amplicons and transcripts abundance was determined by interpolation [11,12]. An enzyme-linked immunosorbent assay (ELISA) was used for human IL-6 determination in the supernatant culture media (R&D Systems).

Data was corrected by amplification efficiency, interplate calibration, and average between replicates with the GenEx Software Version 6 (MultiD Analyses AV). Relative expression of *IL-6*, *IL-8* and *GSTT1* was expressed as $2^{-\Delta\Delta Ct}$, while *CYP1A1* was calculated as $CYP1A1_{sample}/GAPDH_{sample}$ ratio divided by the mean of the $CYP1A1_{control}/GAPDH_{control}$ ratio [11]. Wilcoxon rank sum test was used for comparison between populations. The outliers' exclusion criterion was applied to samples that exceeded four standard deviations from the mean. The association between variables was calculated with Spearman's rank correlation test (STATA v.13, StataCorp, Texas, USA).

Table 1
Characteristics of the population under study.

	Normotensive	*HD*[a]	*p value*
n	53	30	
Age (years)	24.22 ± 6.16	25.25 ± 7.36	0.70
Systolic blood pressure (mm Hg)	**110.50 ± 7.19**	**146.16 ± 16.67**	**< 0.01**
Diastolic blood pressure (mm Hg)	**71.15 ± 6.75**	**94.25 ± 12.37**	**< 0.01**
Proteinuria (dipstick test)	–	2 ± 1.21	
Gestational age (weeks)	39.08 ± 1.38	39.41 ± 1.07	0.49
Newborn's weight (kg)	3.02 ± 0.43	2.84 ± 0.69	0.65

Results are mean ± SD. Statistically significant data differences in bold. HD, Hypertensive disorders.

[a] Clinical data was obtained from 12 HD women.

3. Results and discussion

No significant differences were found between mother's age, weeks of gestation and newborn's weight between normotensive and HD women (Table 1). Congruent with the inclusion criteria, the difference in systolic and diastolic blood pressure between the studied groups was statistically significant. Proteinuria was evaluated in the HD group and 2+ and 3+ values were obtained.

Despite PW exposure to environmental pollutants in Mexico City [13], *CYP1A1* levels were low and remained unmodified in HUVEC from HD and normotensive PW (Fig. 1A). Since HUVEC, once isolated, are no longer exposed to the maternal stimuli, it is possible that *CYP1A1* transcriptional activation ceased at the time of cell harvesting. Constitutive *GSTT1* expression was found in HUVEC; nonetheless, no-significant differences were observed between normotensive and HD conditions (Fig. 1B). The lack of significant differences in *GSTT1* levels among groups may relate to the large expression variations between *GSTT1* positive homozygotes and heterozygotes, as previously reported [14]. Additionally, four subjects were *GSTT1*-null, as suggested by a negative PCR amplification. Previous studies determined that, in Mexicans, the prevalence of this genotype is between 9.3% and 9.7%, [15,16], frequencies higher than our estimation of 4.9%. Altogether, it seems that the *CYP1A1* and *GSTT1* transcriptional behavior is not modified in HUVEC exposed to a hypertensive environment *in utero* in a long-term fashion.

The levels of *IL-6* and *IL-8* transcripts were lower in HD than in control (Fig. 1C and D), and the IL-6 protein was no significantly decreased (Fig. 1E). At the protein level, others observed non-significant IL-6 reductions in cord blood from preeclamptic women [17,18]. Moreover, in placental explants cultures, the production rates of IL-6 are lower in preeclamptic placentas than in the normotensive ones [19]. Additionally, we found association between *IL-6* and *IL-8* transcripts ($r = 0.71$, $p < 0.001$); result corroborated by others at the protein level [20,21]. Furthermore, *CYP1A1* and *IL-6* mRNAs were inversely correlated in HUVEC ($r = -0.45$, $p < 0.001$). That correlation has been observed in other cell models such as in hepatocyte cultures stimulated with IL-6 [22] and in dendritic cells treated with a CYP1A1 inductor [23]. No more associations were found between the evaluated genes (data not shown).

The present study presented limitations due to the lack of clinical data of the volunteers, which impede an accurate sample classification and data analysis. Moreover, outcomes obtained with an *in vitro* cellular model, such as HUVEC primary cultures, may not be extrapolated to *in vivo* vascular endothelia, due to HUVEC embryonic origin with their specific biochemical, physiological, molecular and genetic features, along with the variability associated between different donors. Nevertheless, results presented in this study provide valuable information to perform similar investigations in larger and fully characterized populations.

We conclude that long-term HUVEC primary cultures obtained from hypertensive mothers showed an *IL-6* and *IL-8* transcripts expression deregulation with respect to those from normotensive women. We suggest that a decreased inflammatory cytokine release from HUVEC may protect the newborn against the increased mother's inflammatory response [2,3]. This is supported by a newborn's reduced immunological activity [24], and an augmented susceptibility for nosocomial infections [25] in offspring from hypertensive and preeclamptic mothers.

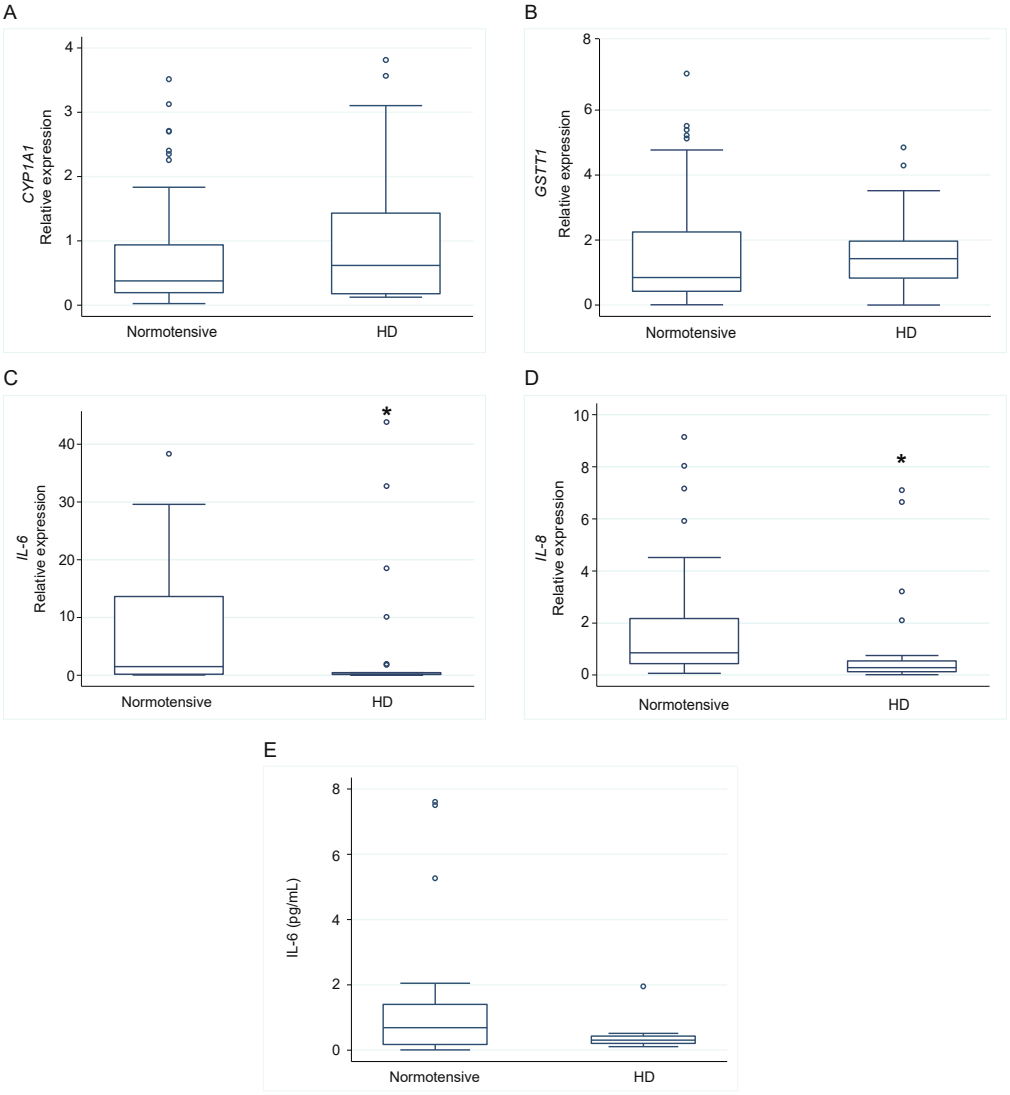

Fig. 1. Relative mRNA expression and IL-6 protein in HUVEC from normotensive and HD pregnant women. Relative transcript abundance of A) *CYP1A1*, B) *GSTT1*, C) *IL-6* and D) *IL-8* were determined by qRT-PCR. IL-6 protein was evaluated in supernatant cell media obtained from normotensive and HD HUVEC cultures with an ELISA assay. Panel B, 52 normotensive samples were evaluated; panels C and D, one outlier excluded in the normotensive group; panel E, 46 normotensive and 13 HD samples analyzed. Wilcoxon rank sum test. $*p < 0.05$. HD, hypertensive disorders.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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