CORRESPONDENCE

DESCRIPTION OF A STRONG RELATIONSHIP AMONG TOTAL CELL FREE DNA LEVELS, LDH VALUES, AST VALUES AND PLATELET COUNT IN PATIENTS WITH HELLP SYNDROME

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Preeclampsia (PE) is a hypertensive disorder of the third trimester of pregnancy, typically characterized by hypertension and proteinuria, although the last is not required in the new definition of PE. The disease is caused by an ineffective placentation resulting in placental ischemia.[1,2] The most severe form of PE is the so-called hemolysis, elevatedliver enzymes and low platelet count (HELLP) syndrome resulting from microan- giopathic hemolytic anemia, thrombocytope- nia and hepatolysis. HELLP syndrome is associated with 1% maternal mortality and aperinatal mortality of about 20%, and the only known cure is delivery of the placenta. Several important complications have been observed in ~ 38–44% of HELLP syndrome, including disseminated intravascular coagulation, acute renal failure, subcapsular liver hematoma and liver rupture, heart failure, placental abruption, retinal detachment, intracranial hemorrhage, posterior reversible encephalopathy, and many other clinical manifestations.[2–7]

The diagnosis of PE is mainly based on the measurement of blood pressure and proteinuria. However, the sensitivity and specificity of these parameters are low.[8] Therefore, there is an urgent demand for widely applicable and affordable tests that allow accurately identifying women at risk for developing PE or for developing a HELLP syndrome from a preexisting PE.

It has recently been observed that circulating cell-free DNA (cfDNA) levels rise in pathologies involving ischemia, such as acute coronary syndrome, ischemic heart failure, stroke and mesenteric ischemia, as well as in patients who have sufferedcardiac arrest outside the hospital. Similarly, increases in circulating cfDNA have been documented in situations involving hypoxia, such as experimental acute pulmonary thromboembolism or obstructive sleep apnea/hypopnea syndrome. In PE, as a result of placental ischemia, we have also documented increased levels of cfDNA in patients having the HELLP syndrome with the highest values.[9–11]

In this paper, we studied a sample of consecutive cases of 16 patients with HELLP syndrome diagnosed and recruited at the Women's Hospital of the Virgen del Rocio University Hospital, Seville, Spain since January 2002–December 2017. The HELLP syndrome was diagnosed according to the criteria described by Sibai.[12]

Maternal peripheral blood was centrifuged for 10 min at 3500 r.p.m. and the serum was frozen at − 80 °C for later DNA extraction. Total cfDNA was extracted automatically from the stored serum samples (1 ml) using a Compact MagnaPure Instrument (Roche Diagnostics, Basel, Switzerland). The DNA was resuspended in a final volume of 90 µl of RNase/DNase-free cryotubes, and the serum DNA template was amplified in a total reaction volume of 20 µl using a real-time-PCR assay for the β-globin sequence on a Light-Cycler-480 (Roche Diagnostics),according to the manufacturer's instructions. The β-globin Taqman system uses the follow-ing primers: beta-globin-354F (5′-GTGCA CCTGACTCCTGAGGAGA-3′); beta-globin-455R (5′-CCTTGATACCAACCTGCCCAG-3′); and a dual-labeled fluorescent probe beta-globin-402 T (5′-(FAM)AAGGTGAACGTGG ATGAAGTTGGTGG(TAMRA)-3′). Amplification was carried out over 48 cycles at 95 ° C for 5 min and at 62 °C for 20 min. The quantification standard curve is referred to base 10 dilutions of a human genomic DNA control sample initiated at 50 ng μl^{-1} (ref: 11 691 112 001; Roche Diagnostics). All samples were analyzed in triplicate. In each analysis,

Table 1 Maternal and neonatal demographic, and at-enrollment characteristics

HELLP syndrome (n = 16)	
Age (years)	32.1 ± 1.3
Primipara, *n* (%)	11 (66.7)
IVF, *n* (%)	5 (31.3)
Singleton pregnancy, *n* (%)	13 (81.3)
Pregestational BMI, (kg m^{-2})	24.7 ± 2.1
Previous medical history	
Chronic hypertension (%)	2 (12.5)
Diabetes mellitus, *n* (%)	1 (6.2)
At enrollment/onset	
Gestational age (weeks)	32.8 ± 0.7
Gestational weight (kg)	1866 ± 168.1
Gestational diabetes, *n* (%)	0
IGR, *n* (%)	0
Highest SBP (mm Hg)	159 ± 6.2
Highest DBP (mm Hg)	98.2 ± 2.9
Antihypertensive use	
1 drug, *n* (%)	6 (40)
2 drugs, *n* (%)	8 (53.3)
⩾3 drugs, *n* (%)	2 (12.5)
Clinical and biochemical characteristics	
Schistocytosis, *n* (%)	12 (85.7)
Placental abruption, *n* (%)	3 (18.7)
LDH (U l^{-1})	340 ± 34.5
Hb (g l^{-1})	103.2 ± 3.5
AST (UI l^{-1})	123.19 ± 33.6
Platelet (10^9 per l)	103.8 ± 7.0

Abbreviations: AST, aspartate transaminase; BMI, body mass index; DBP, diastolic blood pressure; HELLP, hemolysis, elevated liver enzymes and low platelet count; IGR, intrauterine growth restriction; IVF, *in vitro* fertilization; LDH, lactate dehydrogenase; PE, preeclampsia; SBP, systolic blood pressure.
Categorical variables are represented by absolute frequencies and percentages (*n*, %). Non-categorical variables are represented by mean ± s.e.m.

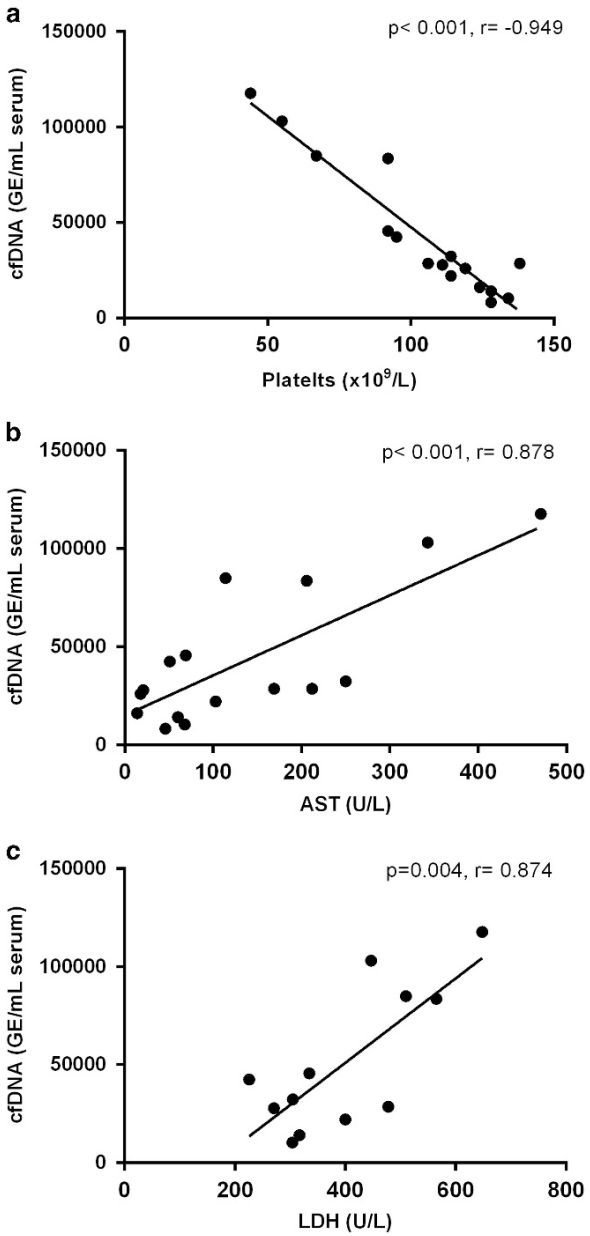

Figure 1 Relationship between total cell-free DNA (cfDNA) and platelets (a), aspartate transaminase (AST) (b) and lactate dehydrogenase (LDH) (c) in the HELLP syndrome. GE, genome equivalents.

a negative reaction and blank control were included to minimize the risk of contamination. A conversion factor of 6.6 pg of DNA per cell was used to express theresult as copy number or genome equivalents (GE per ml).

Finally, serum AST, LDH and the platelet count were measured by standard methods.

Maternal and neonatal demographic, and at-enrollment characteristics are shown in Table 1. At delivery, all of the newborn were preterm, five were males (26.3%), on average the newborn weighed 1907 ± 149 g and oneof them was complicated by the intrauterine death (5.3%).

Regarding cfDNA and the biochemical parameters that define the syndrome, we observed an inverse relation of cfDNA with the platelet count ($r = -0.949$, $P<0.001$), and a positive relation to levels of AST and LDH ($r = 0.878$, $P<0.001$ and $r = 0.874$, $P = 0.004$, respectively) (Figure 1).

Our finding might be of interest since hemolysis (LDH values), hepatolysis(AST values) and thrombocytopenia are the elements that define the syndrome. We did not find a similar description in the literature in patients with HELLP syndrome. However, Lazar *et al.*[13] measured cfDNA levels together with several clinical characteristics and laboratory parameters in a group of controls ($n = 70$) and a group of patients with PE ($n = 67$). They found a significant relationship between cfDNA levels and serum AST ($r = 0.31$, $P = 0.012$) values, as well as between cfDNA and serum ALT levels ($r = 0.46$, $P<0.001$). In the Methods section, it was mentioned that pregnant women with eclampsia or HELLP syndrome were not enrolled. Nevertheless, ALT was significantly different between women with PE and controls (respectively, 15 (6–233) *vs.* 12 (7–32), $P<0.05$). Although AST was not significantly different between groups, the range of variation in the PE group (10–148) *vs.* the control group (10–35) leads us to suspect that perhaps some patients with incomplete forms of HELLP syndrome were enrolled in the PE group. As far as we know, our study is the first report describinga relation among cfDNA levels and the parameters that name the syndrome. Further studies are needed to evaluate the usefulness of this determination at early diagnosis of hypertension in pregnancy or at debut of PE before diagnosis of HELLP in the third trimester. Also the predictive value of cfDNA for diagnosis of PE in the first trimester remains unknown. Finally, its applicabilityon the differential diagnosis with other acute liver diseases in the third trimester also remains unknown.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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