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FAS and FAS ligand gene polymorphisms in Egyptian females with preeclampsia

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ABSTRACT

We aimed to evaluate the association of Fas polymorphism and the Fas ligand with preeclampsia, investigating whether the G 670 Fas gene variant and the Fas Ligand INV2nt 124 G variant had a differential distribution in patients with preeclampsia. The preeclamptic group consisted of 50 pregnant women who developed preeclampsia, while the control group consisted of 50 age-matched pregnant women with uncomplicated pregnancies. Fas and Fas ligand gene polymorphisms were tested using polymerase chain reaction–restriction fragment length polymorphism. Regarding the Fas 670 A > G polymorphism, statistically significant differences were found between the two groups regarding the AA and GG/AG genotypes as well as the A, G allele frequency, while no statistically significant differences were found regarding AG or GG genotypes. Regarding the FasLG IVS2nt 124 A > G polymorphism, no statistically significant differences were found between the two groups studied. Concerning the Fas 670 A > G gene, no statistically significant differences between the severe and mild preeclampsia groups regarding the A allele frequency were found. Concerning the FasLG IVS2nt 124 A > G gene, there were no statistically significant differences between the severe and mild preeclampsia groups regarding the A allele frequency or the G allele frequency. The presence of the Fas gene polymorphism Fas A670G is associated with an increased risk of preeclampsia, while the presence of FasLG IVS2nt 124 A > G gene may be protective against preeclampsia.

1. Introduction

Preeclampsia is a major human pregnancy-specific disorder that occurs in at least 5–10% of pregnancies and leads to maternal and fetal morbidity and mortality (Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy, 2000). It is characterized by the onset of proteinuria and hypertension after the 20th week of gestation. Infants are at increased risk of growth restriction and the adverse effect of preterm delivery (Stella and Sibai, 2006; Ilekis et al., 2007). The etiology remains poorly understood, although the involvement of immune, angiogenic, metabolic, and genetic factors is suggested (Sibai et al., 1997; Roberts and Cooper, 2001; Tyurin et al., 2001; Kaufmann et al., 2003; Maynard et al., 2003; Bdolah et al., 2004; Chaouat et al., 2004; Redman and Sargent, 2005). In preeclampsia, there is incomplete invasion of the uterine spiral arteries by endovascular trophoblasts. As a result, decidual vessels, but not myometrial vessels, become lined with endovascular trophoblasts (Meekins et al., 1994). The magnitude of defective trophoblastic invasion of the spiral arteries is likely associated with the severity of the hypertensive disorder (Madazli et al., 2000). It is thought that these changes

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cause placental perfusion to be pathologically diminished, which eventually leads to the pre-eclampsia syndrome (Redman and Sargent, 2003).

Increased placental trophoblastic apoptosis has been suggested as a possible cause (Huppertz and Kingdom, 2004). The Fas–Fas ligand system is the major signal transduction pathway involved in apoptosis. Fas and FasL (CD95) are classical trans-membrane proteins that belong to the tumor necrosis factor receptor super family (TNFRSF) of proteins. Fas is ubiquitously expressed, while FasL is limited to certain leukocytes (activated T lymphocytes, natural killer [NK] cells) and tissues with immune privilege, including the human trophoblast throughout gestation (Sziller et al., 2005).

As the Fas and FasL might represent candidate genes involved in the pathogenesis of preeclampsia, the presence of a genetic variant might increase susceptibility to the development of the pathology (Ashton et al., 2005). The Fas 670 G variant was found to be associated with a decreased Fas production in activated T lymphocytes (Pinti et al., 2002; Lai et al., 2003). Thus, we and others have hypothesized that genetic polymorphisms affecting Fas or Fas ligand might increase the risk of preeclampsia.

The aim of the present work was to evaluate the association of Fas A670G and FasL INV2nt A124 G polymorphisms with the risk of preeclampsia in the Egyptian women, their relation to the severity of the disease and fetal complication.

2. Patients and methods

We performed a case–control study at the Obstetrics and Gynecology Department of Kasr Al Aini School of Medicine In Cairo, Egypt between July 2011 and November 2012. The study cases and control subjects were recruited from the outpatient antenatal care clinic. They were all Egyptian women living in Cairo. Approval was obtained from the local ethics committee, and all subjects gave written informed consent prior to their participation in the study. We identified and enrolled 50 preeclamptics and 50 apparently healthy, normotensive controls, matching the age and parity of the preeclamptics. Women with essential hypertension, diabetes, or autoimmune diseases were excluded from the research. Venous blood samples were collected on EDTA vacutainers. Samples were collected from pregnant women in their first trimester (between 10 and 14 weeks of gestation) as a part of a first-trimester screening program and stored. Patients were followed until the time of delivery to see who developed preeclampsia and who completed a normal healthy pregnancy.

The diagnosis of preeclampsia was established before 37 weeks of gestation in cases using (1) blood pressure of 140/90 mmHg (twice on separate occasions >6 h apart) and urinary protein >300 mg/l, or ≥2+ on dipstick urine analysis. Severe preeclampsia was defined as BP ≥160/110 mmHg with proteinuria >5 g in 24 h, with or without other features like oliguria (<500 ml/24 h), cerebral or visual disturbances, pulmonary edema or cyanosis, epigastric or right upper quadrant pain, HELLP syndrome, or intrauterine growth restriction.

The fetal outcomes assessed were intrauterine growth retardation (IUGR), intrauterine fetal death (IUFD), and prematurity (delivery <37 weeks' gestation). IUGR was diagnosed in neonates when their birth weight was below the 10th percentile value for the given gestational age (Sziller et al., 2005).

2.1. Genotyping

Genomic DNA was isolated from peripheral blood cells extracted from the whole blood using a DNA extraction kit, (QIAamp Blood Kit (Cat. No. 51106; Qiagen Inc., Valencia, CA), and following the manufacturer's instructions. Fas and FasL polymorphisms were identified by the PCR-RFLP method. The target fragments containing these two polymorphisms were amplified by the primers: 5-ATAGCTGGGGCTATGCGATT-3 (forward) and 5-CATTTGACTGGGCTGTCCAT-3 (reverse) for the Fas 670 A>G and 5-GCAGTTCAGACCTACATGATTAGGAT-3 (forward) and 5-CCAGATACAGACCTGTTAAATGGGC-3 (reverse) for FasLG IVS2nt 124 A>G (Sun et al., 2004). The reaction mixture for PCR amplification consisted of a DNA template, 0.5 μM of each primer, 10× PCR buffer, 1.5 mM $MgCl_2$, 0.5 U of Taq DNA polymerase, 0.2 mM of each dNTP. PCR-grade water was added to a final volume of 20 μl. PCR master mix and specific primers were supplied by (Fermentas, Hanover, MD, USA). The ScrFI and FokI restriction enzymes were used to distinguish the 670 A>G and IVS2nt 124 A>G polymorphisms respectively (supplied by New England Biolabs, Ipswich, MA, USA).

Concerning the Fas 670 A>G gene polymorphism, the wild genotype (AA) produces a single band at 193 bp, the heterozygous genotype (AG) produces three bands (193, 136, and 57 bp), and the presence of homozygous mutation (GG) produces two bands at 136 and 57 bp.

Concerning the FasL IVS2nt 124 A>G gene polymorphism, the wild genotype (AA) produces a single band at 230 bp, the heterozygous allele (AG) produces three bands (230, 180, and 50 bp), and the presence of a homozygous mutation (GG) produces two bands at 180 and 50 bp.

2.2. Statistical analysis

Statistical calculations were performed using Microsoft Excel version 7 (Microsoft Corp., Redmond, WA, USA) and SPSS for Windows version 16 (SPSS Inc., Chicago, IL, USA) software. Results were reported as mean ± standard deviation (±SD) or frequency (%) when appropriate. Comparison of categorical data was carried out using the Chi-squared test (2×), while for the numerical data the independent *t*-test and ANOVA test were used. Odds ratios were used to assess the risk conferred by a particular allele and genotype. A *P*-value less than 0.05 was considered statistically significant, and less than 0.01 was considered highly statistically significant. Multiple logistic regression was performed to analyze the simultaneous effect of the polymorphisms studied on the risk of preeclampsia by the use of odds ratios (used to assess the risk conferred by a particular allele and genotype). OR <1 and 95% confidence interval range less than 1 indicates a decreased risk, while OR >1 and 95% confidence interval range more than 1 indicates a increased

Table 1
Comparison of clinical and laboratory data from patients with preeclampsia and controls.

	Preeclampsia($N=50$)	Controls($N=50$)	P-value
Maternal age (years)	26.3 ± 6.12	28.6 ± 5.9	0.058
Parity	1.7 ± 1.1	1.5 ± 1.1	0.365
Blood pressure (third trimester)			
Systolic blood pressure (mmHg)	153.8 ± 14.4	114.6 ± 6.13	<0.001
Diastolic blood pressure (mmHg)	99.4 ± 11.8	74.3 ± 6.38	<0.001
Hematocrit value	33.1 ± 5.5	34.9 ± 4.34	0.072
Platelet count ($\times 10^6$/l)	218.3 ± 68.06	224.4 ± 64.39	0.645
Total bilirubin (mg/dl)	1.33 ± 0.4	1.03 ± 0.35	<0.001
AST	31.7 ± 14.4	21.2 ± 9.56	<0.001
ALT	26.3 ± 10.2	23.2 ± 7.83	0.091
Creatinine	1.15 ± 0.45	0.95 ± 0.32	0.012
Urinary proteins (g/24 h urine)	1.4 ± 0.5	Nil	

Table 2
Fas 670 A > G and FasLG IVS2nt 124 A > G alleles and genotypes in patients with pre-eclampsia and controls.

	Pre-eclampsia $N=50$ (%)	Controls $N=50$ (%)	P-value
Genotype frequency			
1Fas 670 A > G			
-AA	8 (16.0)	18 (36.0)	0.040
-AG	30 (60.0)	25 (50.0)	0.421
-GG	12 (24.0)	7 (14)	0.307
-AA/AG	38 (76.0)	43 (86.0)	0.307
-GG/AG	42 (84.0)	32 (64.0)	0.040
-A allele	46 (46)	61 (61)	0.047
-G allele	54 (54)	39 (39)	0.047
2 FasLG IVS2nt 124 A > G			
-AA	39 (78.0)	31 (62.0)	0.126
-AG	7 (14.0)	15 (30.0)	0.091
-GG	4 (8.0)	4 (8.0)	0.712
-A allele	85(85)	77 (77)	0.207
-G allele	15 (15)	23 (23)	0.207

Table 3
Results for allele frequencies comparing severe preeclamptics with mild preeclamptic cases.

	Severe preeclampsia $N=36$ Alleles = 72 (100%)	Mild preeclampsia $N=14$ Alleles = 28 (100%)	P-value
1. Fas 670 A > G			
-A allele	34 (47.22%)	14 (50%)	0.978
-G allele	38 (52.73%)	14 (50%)	0.992
2. FasLG IVS2nt 124 A > G			
-A allele	64 (88.8%)	24 (85.7%)	0.931
-G allele	8 (11.12%)	4 (14.3%)	0.923

Table 4
Summary of findings using a multiple logistic regression model for analyzing the simultaneous effect of the polymorphisms studied on the risk of preeclampsia.

Polymorphism	OR (95% CI)	P-value
Fas gene	3.8 (2.1–10.4)	0.023
FasLG IVS2nt 124 A > G gene	0.328 (0.122–0.469)	0.042

risk. Correlation between various variables was obtained using Pearson's moment correlation equation for linear relation. Correlation was considered strong if between 1.0 and 0.5, medium if between 0.5 and 0.3, weak if between 0.3 and 0.1, and no correlation if between 0.1 and 0.0.

3. Results

Table 1 shows selected clinical features of the cases and controls. There were no differences in mean maternal age or parity between the two groups. As expected, the systolic and diastolic blood pressures were significantly higher in preeclamptic women compared with the control group ($P<0.001$ for both). There were no statistically significant differences between the two groups regarding hematocrit ($P=0.072$), platelet count ($P=0.645$) or ALT ($P=0.091$). Statistically significant differences were found regarding creatinine ($P=0.012$), and regarding total bilirubin and AST.

The results of our analysis of Fas alleles, Fas 670 A > G, FasL alleles, and FasL IVS2nt 124 A > G for cases and controls are shown in Table 2. For Fas 670, we found statistically significant differences between the two groups regarding the AA genotype ($P=0.040$) and GG/AG ($P=0.040$) as well as the A and G allele frequency ($P=0.047$ for both), while no statistically significant differences were found regarding AG or GG genotypes ($P=0.421$, 0.307 respectively; Table 2). For FasL, there were no significant differences between the cases and controls regarding allele or genotype frequencies.

Table 3 shows the results for allele frequencies comparing severe preeclamptics with mild preeclamptic cases. No significant differences were found.

No significant correlation was found between patients' genotypes and fetal complications. We did not find any statistically significant correlation between Fas 670 gene polymorphism and intrauterine growth retardation ($r=0.091$, $P=0.082$), intrauterine fetal death ($r=0.087$, $P=0.072$), and prematurity ($r=0.075$, $P=0.061$). Also, we did not find any statistically significant correlation between FasL gene polymorphism and intrauterine growth retardation ($r=0.085$, $P=0.071$), intrauterine fetal death ($r=0.073$, $P=0.062$), and prematurity ($r=0.086$, $P=0.074$).

Table 4 summarizes our findings using a multiple logistic regression model for analyzing the simultaneous effect of the polymorphisms studied on the risk of preeclampsia. After adjusting for age and parity, we found that the presence of the Fas gene polymorphism was associated with an increased risk of preeclampsia (OR = 3.8, CI = 2.1–10.4), while the presence of the FasL IVS2nt 124 A > G gene

polymorphism was associated with a decreased risk of preeclampsia (OR = 0.328, CI = 0.122–0.469).

4. Discussion

Our findings confirm an association of the Fas 670 A > G gene polymorphism with pre-eclampsia in that we found the GG/AG genotype in 84% of women with pre-eclampsia versus 60% of normal women. Moreover, the AA genotype was found in 36% of normotensive women and only 16% of preeclamptic women. Finally, the G allele frequency was higher in the preeclamptic group. Logistic regression analysis showed that the Fas A670G polymorphism could be considered an independent risk factor for preeclampsia and is associated with an increased risk of nearly 4-fold. Our analysis also suggests that the presence of the FasL IVS2nt 124 A > G gene polymorphism is associated with a decreased risk of preeclampsia.

Our data are consistent with those of Ciarmela (2010); who observed the Fas 670 G gene variant in 42 Italian pre-eclamptic patients (84%) versus 96 members of the general population control group (67.6%; $P = 0.029$). They also noted the Fas 670 AA genotype in 33 normotensive pregnant women (37.1%) compared with only 5 out of 31 preeclamptic pregnant women (16.1%).

Both our results and those of Ciarmela et al. contrast with the findings of Sziller et al. (2005); who found 82% of Hungarian pre-eclamptics with GG/AG and 62.9% of controls with GG/AG and that the carriage rate of the Fas 670 G allele variant was higher among preeclamptics (59.7%) than among controls (42.1%; $P = 0.01$). Hu et al. (2005) and Polavarapu et al. (2013) found no differences in the Fas or Fas ligand genotype/allele frequency between preeclamptic and normotensive placentas. We speculate that the populations studied by these investigators might be genetically distinct from ours.

Sziller et al., 2005; noted that the Fas 670 G allele increases the risk of preeclampsia and preeclampsia-associated IUGR in women who deliver at <37 weeks. We did not find the latter, although this could be due to our small sample size.

We recognize that our study has limitations. Among these is our relatively small sample size. On post hoc analysis, we found that our study was powered to detect a minimal difference of 20% between groups (alpha = 0.05 and power = 80%).

In successful pregnancies, binding of trophoblast-associated FasL to Fas-expressing activated maternal T lymphocytes that invade the trophoblast during implantation induces apoptosis in Fas-bearing maternal T cells, allowing fetal trophoblast to invade the myometrium while escaping immune recognition (Abrahams et al., 2004). The Fas-expressing invading trophoblasts may also undergo apoptosis from FasL-expressing maternal T cells, limiting the extent of myometrial invasion (Frangsmyr et al., 2005). The disturbed Fas-mediated apoptosis resulting from genetic polymorphism could be involved in the pathogenesis of preeclampsia and HELLP syndrome (Miko et al., 2009).

Our results are consistent with the hypothesis that reduced Fas-mediated apoptosis in maternally activated T-lymphocytes could result in insufficient trophoblast invasion of the spiral arteries as the activated T-lymphocytes become able to enhance the destruction of the cytotrophoblasts. A reduced number of invading cytotrophoblasts may lead to inadequate modification of the spiral arteries. This, in turn, predisposes to placental ischemia and vascular endothelial damage (Gerretsen et al., 1981; Roberts and Redman, 1993; Ness and Roberts, 1996).

5. Conclusion

The presence of the Fas gene polymorphism Fas A670G is associated with an increased risk of pre-eclampsia, while the presence of the FasLG IVS2nt 124 A > G gene may be protective against preeclampsia.

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Conflict of interest

None declared.

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