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### The genetic predisposition to produce high levels of TGF-1 impacts on the severity of eclampsia/pre-eclampsia

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ORIGINAL ARTICLE

## The genetic predisposition to produce high levels of TGF- $\beta$ 1 impacts on the severity of eclampsia/pre-eclampsia

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### Abstract

**Objectives.** To investigate the hypothesis that women who are genetically programmed to produce higher levels of transforming growth factor-beta 1 are more likely to develop severe eclampsia/pre-eclampsia. **Design.** Case-control study. **Methods.** Blood samples from women whose pregnancy was complicated by eclampsia ( $n=37$ ) or pre-eclampsia ( $n=49$ ) and healthy controls ( $n=86$ ) were analyzed for the presence of polymorphisms at codons 10 and 25 of the transforming growth factor-beta 1 gene. The polymorphisms are thought to determine whether an individual produces low, medium, or high levels of the cytokine. The analysis was carried out using the ARMS-PCR technique. **Results.** Women who developed eclampsia/pre-eclampsia with severe renal and neurological complications or had neonatal deaths/still births were more likely to have the high-producer allele T in codon 10 of the transforming growth factor-beta 1 gene than healthy controls. By contrast, the transforming growth factor-beta 1 producer genotype and allele frequency as determined by gene polymorphisms at codon 25 were comparable in cases and controls. The cytokine producer status *per se* appears to have had no bearing on whether a patient developed eclampsia/pre-eclampsia. **Conclusions.** Our findings suggest that women who experience eclampsia/pre-eclampsia with severe maternal and/or fetal complications are more likely to have a genetic predisposition to produce high levels of transforming growth factor-beta 1 as defined by polymorphisms at codon 10. While it is recognized that eclampsia/pre-eclampsia has heterogeneous pathomechanisms, we have demonstrated a strong relationship between poor maternal and pregnancy outcomes and codon 10 polymorphisms. The characterization of the immunogenetic make-up of the women may be an additional tool in the differentiation of component pathologies and/or prediction of severity of the syndrome.

**Key words:** Eclampsia/pre-eclampsia, TGF- $\beta$ 1 gene polymorphism, Zimbabwe

The eclampsia/pre-eclampsia syndrome comprises a heterogeneous group of disorders occurring in pregnancy. The syndrome involves both the mother and her fetus. The common denominator in eclampsia/pre-eclampsia is insufficient trophoblast invasion of maternal spiral arteries, impaired placental perfusion, and widespread endothelial cell dysfunction (1–5).

The abnormal placentation reflects an immune pattern, which is influenced by the proinflammatory cytokine imbalance (6,7). Each individual is genetically preprogrammed to produce low, medium, or

high levels of cytokines (8). These genetic predispositions can explain familial associations of pre-eclampsia and predict disease severity (9,10).

Polymorphisms in the cytokine genes of tumor necrosis factor-alpha (11–14), interleukin-10 (15), interleukin-1 beta (16), and interleukin-6 (17) have previously been studied in the context of pre-eclampsia. In this paper we focused on transforming growth factor-beta 1 (TGF- $\beta$ 1). TGF- $\beta$ 1 has the potential to shift the balance between syncytiotrophoblast and the invasive cytotrophoblast, upon which normal development of pregnancy depends.

This occurs as a consequence of TGF- $\beta$ 1 inhibition of cytotrophoblast differentiation and invasion (18–20). The exposure of first-trimester trophoblast culture to TGF- $\beta$ 1 significantly inhibits the proliferation of the trophoblast in a dose-dependent manner (21). TGF- $\beta$ 1 has a range of endocrine effects, which include a reduction of human placental lactogen and the inhibition of progesterone and estradiol secretion by trophoblast cell lines (22,23).

There is increasing clinical data to support the involvement of TGF- $\beta$ 1 in the pathogenesis of pre-eclampsia. Plasma and placental levels of TGF- $\beta$ 1 are significantly higher in patients with pre-eclampsia than in normotensive pregnant women (24–29). Although a majority of studies suggest a role for TGF- $\beta$ 1 in the pathogenesis of eclampsia, there are recent studies that have shown no differences in serum levels of the cytokine (30). This may be explained partly by differences in the ethnic make-up of the patients or by the influence of such cofactors as endoglin, recently reported by Venkatesha et al. (31).

TGF- $\beta$ 1 is also involved in the pathogenesis of hypertension and proteinuric renal damage (32–37), which, in part define the eclampsia/pre-eclampsia syndromes. This study investigates our hypothesis that women who carry gene polymorphisms that encode for the production of higher levels of TGF- $\beta$ 1 are more likely to develop the eclampsia/pre-eclampsia syndrome. This, to our knowledge, is the first study that investigates polymorphism in codons 10 and 25 of the TGF- $\beta$ 1 gene, which are associated with high, medium, or low TGF- $\beta$ 1 synthesis (38,39) in patients with eclampsia/pre-eclampsia in Africa.

## Patients and methods

We included 86 consecutive women presenting with eclampsia or severe pre-eclampsia to Harare Maternity Hospital, Harare, Zimbabwe. Thirty-seven developed eclampsia and the remaining 48 had severe pre-eclampsia. Severe pre-eclampsia was diagnosed in patients whose diastolic and systolic blood pressures were respectively at least 110 and 160 mmHg on two separate occasions. All pre-eclamptic patients had a minimum proteinuria of two pluses (++) as detected using urine dipsticks. Twenty-four-hour urinary protein measurement is not routinely performed at the study center. Eighty-six normotensive, healthy women, who had uneventful pregnancies, consented to serve as study controls. The healthy women were enrolled from the postnatal ward of Harare Maternity Hospital. All patients and controls were indigenous black Zimbabweans from the relatively homogenous Shona ethnic groups that

inhabit Northern Zimbabwe. None of the cases and controls were related to each other.

Five milliliters of EDTA anti-coagulated blood was collected from patients and controls and stored at  $-80^{\circ}\text{C}$  until required for analysis.

The study was conducted with the approval of the Medical Research Council of Zimbabwe and all participating women gave their informed consent.

### *DNA extraction and ARMS-PCR assay*

Blood samples were thawed at room temperature and 200  $\mu\text{l}$  was used to extract genomic DNA. The QiAmp DNA purification kit (Qiagen Inc. Valencia, CA, USA) was used to purify DNA in accordance with the manufacturer's instructions. The detection of the alleles at codons 10 and 25 of the TGF- $\beta$ 1 gene was carried out using the ARMS-PCR assay (38). Polymorphism in the amplified products was detected by gel electrophoresis on 2% agarose gels in the presence of ethidium bromide. The DNA bands were visualized under UV light on a transilluminator and gels were photographed using Polaroid type 667 film.

### *Statistics*

The program for Apple Macintosh (GraphPad Software Inc., Sorrento Valley Rd., San Diego, CA) was used for data analysis. We used Yates' corrected  $\chi^2$  test to compare proportions and Fisher's exact test (2-tailed) for expected frequencies of less than 5.

## Results

### *Patient profile*

The median age of patients was 23 (95%CI 22.6–25.1) years and that of the healthy women was 24 years (95%CI 23.4–27.5). The median age of eclamptic patients was 21 years (95%CI 20–24) while pre-eclamptic patients had a median age of 25 years (95%CI 24–27,  $p=0.006$ ). The parity of eclamptic patients ranged from 0 to 3 (20/32 were nulliparous). The respective parity of women in the pre-eclampsia group ranged from 0 to 2 (20/48 were nulliparous). The mean gestational age for eclamptics was 35 weeks (range 24–40) and the respective gestational age for pre-eclamptics was 33 weeks (range 27–40).

### *Arterial blood pressure*

The mean maximum systolic blood pressure for eclamptics and pre-eclamptics was 184 and

182 mmHg respectively, and the corresponding diastolic blood pressure readings were 126 and 128 mmHg. Twenty-one women had diastolic blood pressure readings of 140 mmHg or greater. The highest blood pressure, mean systolic 205 (range 170–260) and mean diastolic 147 (range 110–200) were recorded in the six patients with hemolysis elevated liver enzymes and low platelets (HELLP) syndrome. Nine mothers had histories of proteinuria and hypertension in previous pregnancies. Five of these had two or more previous pregnancies complicated by these symptoms.

#### *Disease course and pregnancy outcomes*

We identified a subgroup of 25 women with severe maternal or neonatal outcomes. One was excluded from analysis because of incomplete genotyping data. Twelve women with eclampsia had a severe disease course and prolonged recovery. All had acute renal failure and fluctuating levels of consciousness. Two developed hemiplegia and one had multiple generalized seizures before and after delivery. Thirteen women (15%), six of them with eclampsia, had poor pregnancy outcome (3 neonatal deaths (NND), 3 fresh still-births (FSB), and 7 macerated still births (MSB)) (Table II). Intrauterine growth retardation (IUGR) was present in 10 cases (9%). Six patients developed HELLP syndrome.

#### *Polymorphisms of the TGF- $\beta$ 1 gene in codon 25*

Taken as a group, the distribution of polymorphisms that confer an ability to produce high (G/G), intermediate (G/C), or low (C/C) producer genotypes as described by mutations at codon 25 of the TGF- $\beta$ 1 were comparable in patient with eclampsia, pre-eclampsia, and healthy controls. None of the 156 subjects (patients or controls) studied had the low producer genotype (Table I).

#### *Polymorphisms of TGF- $\beta$ 1 in codon 10*

Compared to healthy controls, the frequency of the polymorphisms that code for the increased production of TGF- $\beta$ 1 as determined by mutations at codon 10 was higher in those patients who experienced severe maternal complications (renal failure and neurological involvement, HELLP syndrome) or fetal/neonatal demise. Two thirds (17/24) of patients with severe complications were high producers of TGF- $\beta$ 1 as defined by mutations at codon 10. Six had the heterozygous (medium cytokine producer) genotype and only one was a low producer. The difference in the incidence of the genotypes

(T/T, T/C, and C/C) between patients with both severe maternal and fetal complications was analyzed using the  $\chi^2$  contingency test. The results show a significant difference ( $p=0.0367$ ) relative to controls. If the subgroup that developed the HELLP syndrome is included, the  $\chi^2$  contingency  $p$ -value increases to 0.0184. Similarly, when we only looked at the allele representation, the difference in the allele frequency between patients with severe maternal and/or fetal complications and controls was highly significant ( $p=0.0023$ ). This suggests that the increased prevalence of high-producer genotype is a systematic change, which influences disease severity.

#### *Hypertension in pregnancy*

The severity of hypertension does not appear to be associated with mutations of the TGF- $\beta$ 1 gene at codons 10 or 25. The distribution of genotypes and alleles was comparable to that in healthy controls. However, recurrent pre-eclampsia often develops in the presence of high-producer allele in codon 10 genotype (Table II).

### **Discussion**

We have employed molecular biology techniques to investigate the relevance of a genetic predisposition to producing different levels of TGF- $\beta$ 1 to the pathogenesis of eclampsia/pre-eclampsia and its associated clinical symptoms. We have four broad conclusions that emanate from the study.

Firstly, there was no difference in the distribution of polymorphisms at codon 25 between patients and controls. This polymorphism is therefore unlikely to have an impact on the pathogenesis of eclampsia or pre-eclampsia. The biological significance of the striking absence of low producers of TGF- $\beta$ 1 as described by polymorphisms at this codon in this population is open to speculation.

Secondly, the cytokine producer status in patients with severe hypertension was similar to that seen in healthy controls. From this observation we can conclude that the inheritance of high, low, or medium producer genotypes for the TGF- $\beta$ 1 cytokine does not influence severity of hypertension in our study population.

Thirdly, all patients with renal complications were high producers of TGF- $\beta$ 1, as described by polymorphisms at codon 10. This suggests significant involvement of this cytokine in renal disease. This is in agreement with other reports of the involvement of TGF- $\beta$ 1 in the pathogenesis and progression of nephropathy albeit in nonpregnant subjects

Table I. A comparison of the inheritance pattern of the genetically programmed ability to produce high, intermediate, or low levels of TGF- $\beta$ 1 between subsets of patients and healthy controls. The inheritance of the allele (T) at codon 10 confers a high producer while inheritance of the C allele at this codon confers a low cytokine producer genotype. The high producer allele at codon 25 is the G.

Study group	Genotypes			Alleles		<i>p</i> value
	High	Intermediate	Low	High	Low	
Codon 10 of TGF- $\beta$ 1 gene	T/T (%)	T/C (%)	C/C (%)	Allele T (%)	Allele C (%)	
Controls ( <i>n</i> =86)	34 (40)	35 (41)	17 (19)	103 (60)	69 (40)	
All cases ( <i>n</i> =86)	33 (38)	34 (40)	19 (22)	100 (58)	72 (42)	NS
Pre-eclampsia ( <i>n</i> =49)	18 (37)	18 (37)	13 (26)	54 (55)	44 (45)	NS
Eclampsia ( <i>n</i> =37)	15 (40)	16 (43)	6 (17)	46 (62)	28 (38)	NS
Codon 25 of TGF- $\beta$ 1 gene	G/G (%)	G/C (%)	C/C (%)	Allele G (%)	Allele C (%)	
Controls ( <i>n</i> =76)	66 (87)	10 (13)	0	142 (93)	10 (7)	
All cases ( <i>n</i> =80)	63 (79)	17 (21)	0	143 (89)	17 (11)	NS
Pre-eclampsia ( <i>n</i> =44)	33 (75)	11 (25)	0	77 (88)	11 (12)	NS
Eclampsia ( <i>n</i> =36)	30 (83)	6 (17)	0	66 (92)	6 (8)	NS

(40–44). The contribution of TGF- $\beta$ 1 to renal disease is interlinked with angiotensin II, an important mediator in eclampsia/pre-eclampsia (45,46).

Finally, subsets of patients whose pregnancies were complicated by severe maternal or fetal sequelae overwhelmingly tended to have genotypes and allele frequencies that conferred an ability to produce higher levels of TGF- $\beta$ 1 as described by polymorphisms at codon 10.

It was striking that 83% of women with eclampsia/pre-eclampsia-associated pregnancy loss were homozygous for high producer allele (T) in codon 10 of the TGF- $\beta$ 1 gene. Two-thirds of women with recurrent pre-eclampsia were also homozygous for the high-producer allele in codon 10 compared to 40% of healthy women. This observation suggests that the high TGF- $\beta$ 1 (codon 10) producer status influences unfavorable maternal and neonatal outcomes. This conclusion differs somewhat from that

reported by Dahar et al. (47). This Brazilian group examined polymorphisms in five cytokine genes, including the TGF- $\beta$ 1 gene. Their conclusions were drawn from analyzing 56 white and 95 non-white women with pre-eclampsia. There were no cases with eclampsia and no description of maternal and fetal outcome was presented. The study population was heterogeneous. These factors could potentially dilute any effect that this polymorphism may have had. In the light of these considerations, their conclusions regarding TGF- $\beta$ 1 are not unexpected. What we have observed is that disease severity matters and that ethnicity may play some role.

In conclusion, in the ethnically homogeneous group of patients and controls that we studied, we demonstrated for the first time a possibility that the homozygosity for the T allele in codon 10 of the TGF- $\beta$ 1 gene could contribute to severe outcomes

Table II. Polymorphism of codon 10 of TGF- $\beta$ 1 gene of women in different clinical categories.

Study subgroup	Genotypes			<i>p</i> -value*	Alleles		<i>p</i> -value**
	High	Intermediate	Low		High	Low	
Codon 10 of TGF- $\beta$ 1 gene	T/T (%)	T/C (%)	C/C (%)		T (%)	C (%)	
Severe maternal <sup>a</sup> and fetal <sup>b</sup> complications ( <i>n</i> =24) <sup>c</sup>	17 (71)	6 (28)	1 (1)	0.0184	40 (83)	8 (17)	0.0023
Recurrent pre-eclampsia ( <i>n</i> =9)	6 (67)	2 (22)	1 (11)	NS	14 (77)	4 (23)	NS
HELLP syndrome ( <i>n</i> =6)	4 (67)	2 (33)	0	NS	10 (83)	2 (17)	NS
Severe hypertension ( <i>n</i> =21) <sup>***</sup>	9 (43)	10 (48)	2 (9)	NS	28 (67)	14 (33)	NS
Healthy controls ( <i>n</i> =86)	34 (40)	35 (41)	17 (19)	103 (60)	69 (40)		

<sup>a</sup>Severe maternal complications included acute renal failure, fluctuating levels of consciousness, hemiplegia, and multiple seizures before and after delivery, as well as the HELLP syndrome.

<sup>b</sup>Severe fetal complications include 3 macerated still births (1,190, 1,120, 1,135 g), one fresh still birth (1,500 g), one neonatal death (885 g), and one spontaneous abortion (200 g).

<sup>c</sup>Severe hypertension refers to patients with diastolic blood pressure readings of 140 mmHg or higher.

\**p*-value  $\chi^2$  contingency test; \*\*two-tailed *p*-value (Fisher's exact test).

of eclampsia/pre-eclampsia. Polymorphisms in codon 10 underlie the severity but not the occurrence of disease.

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