Original Article

Misfolding Linked Mutations of SERPINA1 Gene are Uncommon in Preeclampsia

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Abstract

Background: Alpha-1 antitrypsin (A1AT) is a protease inhibitor that plays an important role in regulating oxidative stress in preeclampsia (PE). Recent studies have shown that A1AT is misfolded in PE. However, the cause of A1AT misfolding is not known. Mutations in *SERPINA1* gene is an established source of A1AT deficiency. PiS and PiZ are the two common misfolding-associated mutations in the *SERPINA1* gene. **Objective:** The purpose of this study was to evaluate the frequency of PiS and PiZ mutations in the *SERPINA1* gene in preeclamptic women. **Materials and Methods:** We carried out a cross-sectional study by including 200 preeclamptic pregnant women. PiS and PiZ mutations in the *SERPINA1* gene were genotyped by using polymerase chain reaction-restriction fragment length polymorphism method. **Results:** PiS and PiZ mutations were absent both in homozygous and heterozygous conditions in the preeclamptic women. **Conclusion:** PiS and PiZ mutations in the *SERPINA1* gene may not be associated with A1AT misfolding in PE.

Keywords: Genetics, preeclampsia, serpins

Introduction

Preeclampsia (PE) is a complication of pregnancy characterized by *de novo* hypertension and proteinuria on or after the 20th week of gestation. PE complicates 2%–8% of all pregnancies globally and constitutes a leading cause of maternal and perinatal morbidity and mortality.[1,2] The cardinal pathogenetic events in PE are defective remodeling of the uterine spiral artery, placental ischemia, endothelial dysfunction, and oxidative stress.[3] The origin of these pathogenetic modifications is not clearly understood, and hence, PE continues to be called a disease of theories.

Recent studies have shown that PE is a proteopathy, i.e., involves aberration in protein folding.[4-9] One of the important proteins found to be misfolded in the placenta of preeclamptic women is alpha-1 antitrypsin (A1AT).[6] A1AT is a serine protease inhibitor that plays a critical role in the regulation of proteolytic tissue damage and anti-inflammatory process.[10] A1AT appears to regulate the release of inflammatory cytokines particularly *via* the activation of mitogen-activated protein kinase pathways as well as nuclear factor-κB pathways.[11] One of the activators of this pathway is the oxidative stress generated by reoxygenation subsequent to hypoxia.[12] Hypoxia-induced oxidative stress is a hallmark of PE.[13] The importance of A1AT in PE is further supported by animal model studies, wherein administration of exogenous A1AT was found to prevent the development of PE.[14] These evidence indicate that A1AT plays an important role in the pathogenesis of PE and also holds therapeutic value. However, the origin of A1AT misfolding in PE is not known.

A1AT is highly prone to misfolding due to mutation of the corresponding *SERPINA1* gene.[15] PiS and PiZ alleles are the two common mutations in the *SERPINA1* gene that increase the propensity of A1AT to misfold.[16] PiS and PiZ mutations result in the substitution of glutamic acid with valine

Access this article online

Quick Response Code:

Website: www.amhsjournal.org

DOI: 10.4103/amhs.amhs_79_19

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Received : 10 June 2019 **Revised :** 20 October 2019
Accepted : 06 November 2019 **Published :** 16 December 2019

How to cite this article: Nagarajappa C, Rangappa SS, Balakrishna S. Misfolding linked mutations of SERPINA1 gene are uncommon in preeclampsia. Arch Med Health Sci 2019;7:177-80.

at position 288 and glutamic acid with lysine at position 366. The wild-type allele is denoted as PiM. We hypothesized that mutations in the *SERPINA1* gene may be the cause for A1AT misfolding in PE. To test our hypothesis, we undertook this study, wherein the primary objective was to determine the frequency of PiS and PiZ mutations in the *SERPINA1* gene in a cross section of preeclamptic women.

Materials and Methods

Study design

We carried out this cross-sectional study by including pregnant women diagnosed with PE. The blood sample was collected from each patient and used to determine the presence of the *SERPINA1* gene mutation. The study was approved by the Institutional Ethics Committee. The patients were enrolled during the period of November 2016–February 2018. All the patients provided informed consent at the time of enrollment.

Sample size

The sample size was calculated using OpenEpi version 3.01 statistical web tool (http://www.openepi.com/Menu/OE_Menu.htm). The sample size for 99% confidence limit was found to be 196 preeclamptic patients based on the prevalence of PE in the Indian population which is ~8%.[17]

Patient selection

The study population included pregnant women diagnosed with PE. Diagnosis criteria for PE were as follows: (i) new-onset hypertension (two readings of systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg measured 4 h apart while the patient is on bed rest; (ii) ≥20 weeks of gestation; (iii) new-onset proteinuria (>300 mg protein for 24 h of urine or + 1 on dipstick); and (iv) in the absence of proteinuria, other symptoms such as hemolysis elevated liver low platelet (HELLP) syndrome, edema, thrombocytopenia, impaired liver function, new-onset cerebral or visual disturbances, and renal insufficiency, and in the absence of other renal diseases, nausea, severe headache, and convulsions.[2] Inclusion criteria were as follows: (i) pregnant women with PE, (ii) superimposed eclampsia, (iii) singleton and multiple gestations, and (iv) primigravida and multigravida conditions. Exclusion criteria were: (i) pregnant women with chronic hypertension and (ii) comorbidities such as diabetes mellitus, epilepsy, respiratory diseases, and heart diseases.

Genotyping of PiS and PiZ alleles of *SERPINA1* gene

Blood samples were collected in an ethylenediaminetetraacetic acid vacutainer used for the isolation of genomic DNA by salting-out method.[18] Quantity and purity of the DNA samples were checked by a spectrophotometer. PiS and PiZ alleles of the *SERPINA1* gene were determined by polymerase chain reaction (PCR)-restriction fragment length polymorphism method. PCR conditions are summarized in Table 1. PCR was performed in 25-µl final volume containing 1 pM of each primer, 1-mM deoxynucleoside triphosphates, 1.5-mM $MgCl_2$, 100-ng genomic DNA, and 1 unit of Taq DNA polymerase. An aliquot of the amplicon in each case was incubated at 65°C with ten units of Taqα1 restriction enzyme for 16 h, and the digestion pattern was analyzed on 3% agarose gel.

Results

A total of 200 preeclamptic pregnant women meeting the inclusion criteria were included in the study. Baseline parameters and clinical outcome of the study participants enrolled in the study are listed in Table 2. The cohort included patients of all the major clinical subtypes. Almost 56.5% of the preeclamptic women were primigravida and 43.5% were multigravida. This is in agreement with the general trend of PE being more common in primigravida than in multigravida. Nearly 47% of the preeclamptic women presented with mild type, whereas the remaining 53% showed severe type. Overall 15% of the preeclamptic patients were superimposed with eclampsia. HELLP syndrome was a rare complication that

Table 1: Polymerase chain reaction-restriction fragment length polymorphism parameters used for the genotyping of PiS and PiZ alleles of *SERPINA1* gene

Parameter	PiS allele	PiZ allele
PCR primers		
Forward	5' TGA GGG GAA ACT ACA GCA CCT C 3'	5' TAA GGC TGT GCT GAC CAT CGT C 3'
Reverse	5'AGG TGT GGG CAG CTT CTT GGT CA 3'	5' GGA GAC TTG GTA TTT TGT TCA ATC 3'
PCR conditions (35 cycles)		
Initial denaturation (min)	94°C (5)	94°C (3)
Cycle denaturation (s)	94°C (30)	94°C (30)
Annealing (s)	60.4°C (30)	61.3°C (30)
Extension (s)	72°C (30)	72°C (30)
Final extension (min)	72°C (5)	72°C (5)
PCR amplicon size (bp)	121	144
RFLP pattern (bp)		
Wt/Wt	100+21	123+21
Wt/mut	121+100+21	144+123+21
mut/mut	121	144

bp: Base pairs, Wt: Wild-type allele, mut: Mutant allele, PCR: Polymerase chain reaction, RFLP: Restriction fragment length polymorphism

Table 2: Baseline parameters and clinical outcome of the study participants (*n*=200)

Parameters	Observation	
Age (years)	24.8±3.5	
Gravida, *n* (%)		
Primigravida	113 (56.5)	
Multigravida	87 (43.5)	
Severity, *n* (%)		
Mild	94 (47)	
Severe	106 (53)	
Blood pressure (mmHg)	Mild PE	Severe PE
Systolic blood pressure	137.3±8.5	175.3±15.7
Diastolic blood pressure	95.0±5	116.5±8
Dipstick proteinuria, %		
1+	48	
2+	20	
3+	32	
Gestational age of onset (weeks), *n* (%)		
Early onset (<34)	75 (37.5)	
Late onset (≥34)	125 (62.5)	
Comorbidities, *n* (%)		
Eclampsia	30 (15)	
IUGR	21 (10.5)	
Stillbirth	8 (4)	
HELLP syndrome	2 (1)	

IUGR: Intrauterine growth restriction, HELLP: Hemolysis elevated liver low platelet, PE: Preeclampsia

was observed only in 1% of the patients. Fetal complications in the form of intrauterine growth restriction and stillbirth were seen in 10.5% and 4% of the patients, respectively. Nearly 37.5% of the patients showed the early-onset type of PE, whereas the remaining 62.5% showed the late-onset type. All the preeclamptic patients were genotyped for PiS and PiZ mutations in the *SERPINA1* gene. PiS and PiZ mutations were not observed in any of the patients either in homozygous or heterozygous conditions.

Discussion

Protein folding is a process by which the linear polypeptide chain assumes a thermodynamically stable and a functional form.[19] Folding ensures that the hydrophobic groups are buried away in the core, whereas the charged groups are freely exposed to the surrounding aqueous medium. The collapse of the stable folding pattern leads to the loss of protein function and exposes the core hydrophobic groups to the aqueous medium. Hydrophobic interactions between the exposed core groups result in the formation of aggregates called amyloid deposits that build up in tissues.[20] Misfolded proteins are commonly observed in neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and Huntington's disease; it is also associated with conditions such as Type II diabetes and medullary carcinoma of thyroid.[21]

Emerging evidence indicates that PE is a proteopathy.[4-9] Misfolded proteins have been reported in the placenta, urine, and plasma of preeclamptic women.[4-9] This is further supported by the demonstration of elevated activation of the "unfolded protein response (UPR)" pathway in the placenta of preeclamptic women.[22] UPR promotes protein folding by activating chaperone expression and induces apoptosis if the restoration is insufficient. We have recently shown urinary misfolded proteins equally represented in both early- and late-onset PE.[9] Major proteins identified in the urinary misfolded proteome of preeclamptic women include A1AT, ceruloplasmin, immunoglobulin-free light chains, albumin, interferon-inducible protein 6-16, and Alzheimer's β-amyloid.[6]

At present, there is little information on the origins of protein misfolding in PE. Genetic involvement in the misfolding of A1AT protein is a classical textbook example.[23] Therefore, we chose to evaluate the involvement of common mutations in the *SERPINA1* gene with PE. *SERPINA1* gene codes for A1AT protein. A1AT is an important plasma serine protease inhibitor whose main function is to neutralize the proteolytic effect of neutrophil elastase. This enzyme facilitates infiltration of neutrophils into tissues through proteolytic degradation of extracellular matrix proteins such as elastin, proteoglycan, collagen, and fibrinogen present in the vascular basement membrane. Reduction in A1AT level disturbs the homeostatic balance between A1AT and elastase, and the excess elastase activity leads to tissue damage. Elastase-mediated tissue damage arising due to A1AT deficiency is well documented in the case of chronic pulmonary obstructive disease.[24]

A1AT deficiency arises due to point mutations that lead to misfolding of the mutant enzyme.[25] However, the clinical manifestation of A1AT deficiency involves both genetic predisposition and environmental modifiers. The active oxygen intermediates produced due to cigarette smoking accelerate the severity of clinical manifestations by functionally inactivating the residual AIAT enzyme.[25] PiS and PiZ are the common mutations in the *SERPINA1* gene that render A1AT prone to misfolding. These mutations cause severe A1AT deficiency when present in recessive condition. However, under the heterozygous condition, they produce borderline deficiency with little clinical manifestation.[24] Individuals who carry PiS and PiZ mutations under heterozygous conditions are at an increased risk of developing severe A1AT deficiency when exposed to compounding risk factors such as oxidative stress. For instance, heterozygous individuals *per se* may not develop chronic pulmonary obstructive disease but are at an increased risk when habituated to tobacco smoking.[26] We assumed that a similar interaction may be involved in the pathogenesis of PE because the involvement of oxidative stress is already well established.[27] Therefore, women who carry PiS and PiZ mutations under heterozygous conditions may not manifest any symptoms of A1AT deficiency but may be prone to A1AT misfolding during pregnancy due to oxidative stress. Another line of evidence that motivated us to choose A1AT is the consistent observation from several studies that its level is deficient in the plasma and placenta of preeclamptic

women.[14,28,29] Furthermore, animal studies on preeclamptic mouse model have shown that intravenous injection with exogenous A1AT ameliorates PE.[14]

Conclusion

In this study, we evaluated the frequency of PiS and PiZ mutations in the *SERPINA1* gene among preeclamptic women. We found that the frequency of these mutations was zero in these women. This encourages us to conclude that common mutations in the *SERPINA1* gene may not be associated with the risk of developing PE.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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