Study of Urotensin II gene and serum levels in relation to pre-eclampsia

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Summary

Objective: To verify the relationship between Urotensin II (UII) gene and serum levels and pre-eclampsia (PE). *Study Design:* Prospective case control study. *Setting:* Tertiary Obstetric centre and university hospital. *Subjects:* A total of 80 pregnant women at their third trimester were included, 30 of which were with mild PE, 30 with severe disease and 20 age- and BMI-matched normotensive pregnant women (controls). *Materials and Methods:* UII gene polymorphism as well as UII serum levels were assessed and compared in patients vs. control. *Results:* No difference was seen between the groups in terms of age or parity at the time of recruitment. A statistically significant difference in the Urotensin II genotype frequencies between patients and control groups was found. The mean serum UII, also showed a significant difference between the studied groups, and control group. Comparing the observed and expected values of UII genotype frequencies in mild, severe PE, and in controls, no significant difference was noted in the homo-mutant, the hetero-mutant or the wild genotypes. *Conclusions:* Elevation of UII in the serum of PE patients could be correlated to the severity and/or progression of the disease. The UII genotype frequencies between patients and control groups showed a significant difference, which implies a potential benefit for UII gene or level in serum as a diagnostic or prognostic indicator in pre-eclampsia.

Key words: Pre-eclampsia; Urotensin II.

Introduction

Pre-eclampsia (PE) is a disease of pregnancy resulting from a maternal physiological response to abnormal placentation. It is a multisystem disorder affecting approximately two to seven percent of all pregnancies in the United Kingdom and is a significant cause of maternal and fetal morbidity and mortality [1]. The onset and clinical course are unpredictable. The disease occurs after the 20th week of gestation and is characterized by: hypertension, proteinuria, and /or edema [2]. It has been suggested that PE might originate from an abnormally shallow endovascular cytotrophoblast invasion in spiral arterioles which may lead to relative placental ischemia and increased inflammatory response [3]. A reliable early marker of PE would permit patient identification and possible prophylaxis if available, or at least predicting / avoiding complications. A large number of tests have been proposed to predict PE, beginning from blood pressure measurement and proteinuria determination, to Doppler ultrasound evaluation and/or blood and urine biochemical markers [4]. Urotensin II (UII) is a cyclic peptide of 11 amino-acids that was initially isolated from fish urophysis and subsequently discovered in mammals including humans [5]. The human isoform was identified in 1998.A cyclic hexapeptide region of the molecule is responsible for the biological activity and is absolutely conserved between species [6,7]. The gene for UI is located on chromosome 1 p36- p32 and encodes a peptide that is considered the most potent endogenous vasoconstrictor discovered to date [8]. The UII receptor, also referred to as the hypocretin receptor, is a G-protein coupled receptor which binds the peptide hormone UII [9]. It was originally isolated as an orphan receptor, expressed in neural and sensory tissues and named GPR14, or sensory epithelial neuropeptide-like receptor (SENR), which is widely expressed in cardiovascular, pulmonary, central nervous, renal, and metabolic systems [10]. UII has emerged as a contributor to cardiovascular physiopathology. [11, 12] Higher circulating levels of U-II have been observed in patients with PE, inferring a possible role for this ligand in this disease [13, 14].

In this study, the role of UII gene and serum levels in the development / progression of PE was evaluated.

Materials and Methods

A total of 80 pregnant women were recruited from the hospital's outpatient clinic during the period of January 2012 to July 2012 and were subdivided on the basis of presence/severity of PE into three groups, mild PE (n=30), severe PE (n=30), and healthy pregnant women as control (n=20). Full history taking and thorough physical examination was done for all subjects. Those with any past or present history of a medical condition (e.g. chronic hypertension, cardiac disease, diabetes mellitus) were excluded. A full informed consent was taken from all subjects before commencement. The medical ethics committee of the faculty of medicine approved the study design. All enrolled subjects promptly received the appropriate treatment according to the standard protocols.

For all subjects, eight ml of venous whole blood were withdrawn, where serum urea and creatinine, and enzymatic activity of alanine amino transferase (ALT) and aspartate amino transferase (AST) were measured using a clinical chemistry analyzer

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Table 1. — *Comparison between the studied groups according to age, parity, and gestational age*

	Mild PE (n = 30)		Severe PE (n = 30)		Control (n = 20)		Test of sig.
	n	%	n	%	n	%	
Age (years)							
< 25	10	33.3	5	16.7	3	15.0	
25 – 30	11	36.7	12	40.0	12	60.0	$p = 0.234$
> 30	9	30.0	13	43.3	5	25.0	
p_1			$p = 0.296$		$p = 0.215$		
p_2					$p = 0.343$		
Min. – Max.	20.0 – 40.0		20.0 – 41.0		19.0 – 40.0		
Mean ± SD	27.57 ± 6.06		29.90 ± 5.82		28.45 ± 5.42		$^{F}p = 0.299$
Median	25.50		30.0		28.50		
p_1			$^{Scheffe}p = 0.305$		$^{Scheffe}p = 0.871$		
p_2					$^{Scheffe}p = 0.690$		
Parity							
0	14	46.7	11	36.7	6	30.0	
1	10	33.3	8	26.7	9	45.0	
2	5	16.7	5	16.7	4	20.0	-
3	1	3.3	3	10.0	1	5.0	
>3	0	0.0	3	10.0	0	0.0	
Min. – Max.	0.0 – 3.0		0.0 – 5.0		0.0 – 3.0		
Mean ± SD	0.77 ± 0.86		1.33 ± 1.42		1.0 ± 0.86		0.326
Median	1.0		1.0		1.0		
p_1			0.160		0.305		
p_2					0.671		
Gestational age (weeks)							
Min. – Max.	34.0 – 38.0		32.0 – 38.0		32.0 – 40.0		
Mean ± SD	36.80 ± 1.10		36.20 ± 1.81		37.15 ± 1.60		0.086
Median	37.0		37.0		37.50		
p_1			0.317		0.729		
p_2					0.103		

p: p value for Chi Square test; ^{F}p: p value for F test (ANOVA);
$^{Scheffe}p$: p value of Post Hoc test (Scheffe); *: Statistically significant at $p \leq 0.05$;
p_1: p value between mild PE and each other group
p_2: p value between severe PE and control

[15]. Complete urine analysis was also done. Serum UII level was measured by enzyme-linked immunosorbent assay (ELISA) [16] and molecular studies including DNA extraction from peripheral blood leucocytes were also done. Detection of S89N polymorphism in the UTS2 gene using polymerase chain reaction / restriction fragment length polymorphism (PCR / RFLP) technique was done as follows: PCR amplification using specific primers forward primer (50-gtgcctgtctgtctgcattca-30) and reverse primer (50-gagtcctgtaaaaccagctacag-30), restriction digestion of PCR products using Afa I enzyme and 3% agarose gel electrophoresis of digested PCR products. The 89S is expected to show three bands with 161, 84, and 18 bp, while 89N shows two bands with 245 and 18 bp [17].

Statistical analysis was done using SPSS software version 20 (Statistical Package of Social Sciences, Chicago, Illinois, USA). The data of the nominal variables were summarized in the form of frequency or percentages. The Chi-Square test (χ^2 test) was used with a Monte Carlo estimate of the exact p-value. Fisher's Exact Test was also used to compare proportions of nominal clinical data variables. To compare observed frequencies of different genotypes in a group of subjects to the Hardy-Weinberg equilibrium (HWE) expected frequencies in the same group of subjects, chi-square goodness of fit test with one degree of freedom was used [18, 19].

Table 2. — *Comparison between the studied groups in terms of UII gene and serum levels.*

	Mild PE (n = 30)		Severe PE (n = 30)		Control (n = 20)		Test of sig.
	n	%	n	%	n	%	
UII Gene							
Wild	9	30.0	5	16.7	11	55.0	
Hetero	14	46.7	11	36.7	7	35.0	$^{c2}p = 0.015^{*}$
Homo	7	23.3	14	46.7	2	10.0	
p_1			$^{c2}p = 0.147$		$^{c2}p = 0.178$		
p_2					$^{c2}p = 0.005^{*}$		
Serum UII							
Min. – Max.	70.0 – 98.0		167.0 – 201.0		34.0 – 55.0		
Mean ± SD	84.10 ± 6.81		180.53 ± 11.27		44.10 ± 6.46		$^{KW}p < 0.001^{*}$
Median	85.50		176.0		46.0		
p_1			$^{MW}p < 0.001^{*}$		$^{MW}p < 0.001^{*}$		
p_2					$^{MW}p < 0.001^{*}$		

p: p value for Chi Square test; ^{KW}p: p value for Kruskal Wallis test
^{MW}p: p value for Mann Whitney test; ^{c2}p: p value for Chi Square test
p_1: p value between mild PE and each other group
p_2: p value between severe PE and control
*: Statistically significant at $p \leq 0.05$

Results

There was no difference in terms of age among the studied groups (mild PE: 27.57 (SD 6.06) years, severe PE group: 29.90 (SD 5.82) years, and control: 28.45 (SD 5.42) years). No difference was shown in terms of parity (mild PE: 0.77 (SD 0.86), severe PE group: 1.33 (SD 1.42), and control group: 1.0 (SD 0.86)) nor regarding gestational age at the time of recruitment (mild PE: 36.80 (SD 1.10) weeks, severe PE group: 36.20 (SD 1.81) weeks, and control group: 37.15 (SD 1.60) weeks) (Table 1).

A statistically significant difference in the UII genotype frequencies between patients and control groups was found. (*$p = 0.0211$**) The mild PE patients had a higher frequency of the homo-mutant genotype "CC" than controls (23.3 % vs 20 %), a higher frequency of the hetero-mutant genotype "AC" than controls (46.7 % vs 40 %) and a lower frequency of the wild genotype "AA" than controls (30 % vs 40 %). Meanwhile, patients with severe disease had a higher frequency of the homo-mutant genotype "CC" than controls (50 % vs 20 %), a lower frequency of the hetero-mutant genotype "AC" than controls (33.3 % vs 40 %), and a lower frequency of the wild genotype "AA" than controls (16.7 % vs 40 %). (Table 2, Figure 1)

The mean serum UII, however, showed a significant difference between the studied groups (mild PE: 84.10 (SD 6.81) pg/ml, severe PE group: 180.53 (SD 11.27) pg/ml, and control group: 44.10 (SD 6.46) pg/ml). (Table 2, Figure 2)

Comparing the observed and expected values of UII genotype frequencies in patients with mild, severe PE and in controls, no significant difference was noted whether in the homo-mutant, hetero-mutant or the wild

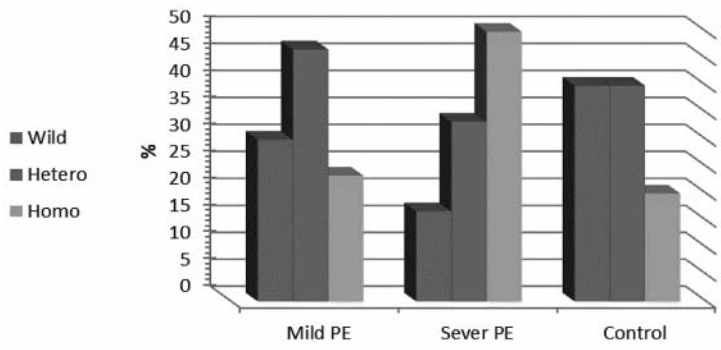

Figure 1. — UII gene frequencies in studied groups.

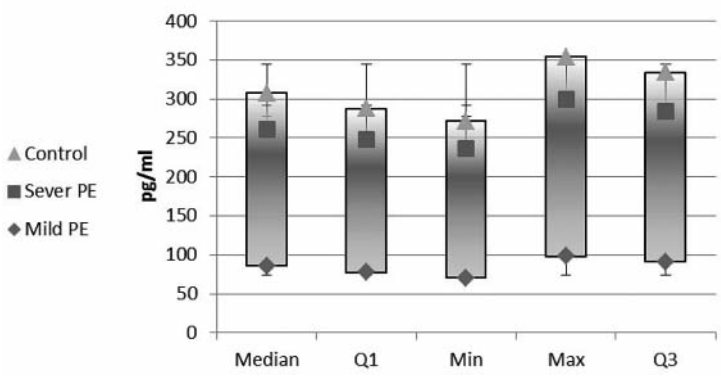

Figure 2. — Serum UII in studied groups.

Table 3. — *The observed and expected values of the genotype frequencies among UII gene in mild PE group.*

	Observed	Expected		2	*p*
Homozygote reference (wild)	9	p2n	8.5	0.117	0.732
Heterozygote (hetero)	14	2pqn	14.9		
Homozygote variant (homo)	7	q2n	6.5		

Table 4. — *The observed and expected values of the genotype frequencies among UII gene in severe PE group.*

	Observed	Expected		2	*p*
Homozygote reference (wild)	5	p2n	3.3	1.875	0.171
Heterozygote (hetero)	10	2pqn	13.3		
Homozygote variant (homo)	15	q2n	13.3		

Table 5. — *The observed and expected values of the genotype frequencies among gene in control group*

	Observed	Expected		2	*p*
Homozygote reference (wild)	8	p2n	7.2	0.556	0.456
Heterozygote (hetero)	8	2pqn	9.6		
Homozygote variant (homo)	4	q2n	3.2		

genotype. This agrees with hardy Weinberg equilibrium model (Tables 3, 4, 5).

Discussion

PE and eclampsia may occur in as many as eight percent of pregnancies and remain a leading cause of maternal and fetal morbidity and mortality [20]. It could be argued that screening for hypertensive disorders should be given a higher priority, than the currently screened-for conditions at routine antenatal care visits.[21] The maternal and fetal short- and long-term outcomes, however, remain uncertain, thus advocating the search for a reliable, fairly accurate method for early detection and/or prediction of the disease. High-dose UII eventually lead to a series of clinical symptoms of PE. Therefore, the UII could be used as a serologic indicator of disease progression and severity.

The results of this study suggested that UTII single gene (S89N) polymorphism is related to the development of PE, which may be, according to the authors'knowledge, the first report on this gene polymorphism's involvement in the development of hypertension with pregnancy. Further studies are needed to investigate the prevalence of other single nucleotide gene polymorphisms in PE [17, 22].

Moreover, UII was also reported to be a proangiogenic agent and, thus, a potential partner in disease pathogenesis. Balat *et al.* reported a significant increase in the circulating

levels of UII in PE, whereas Cowley *et al.* reported no differences between PE and normal pregnancy [23, 24].

Despite the last two decades of research on this condition, the ability of clinicians to predict PE prior to the onset of symptoms has not improved remarkably. A serum test that directly predicts an impending need for delivery, allowing targeted prenatal care, could offer huge clinical benefits as delivery is currently the only cure for PE [25]. UII, a potent hypertensive agent, has significantly elevated serum levels in numerous disease conditions, including essential hypertension, atherosclerosis, heart failure, diabetes, renal failure, and metabolic syndrome. As such, serum UII may be a useful biomarker in detecting PE onset or progression and the UII gene receptor is emerging as a promising target for therapeutic intervention [26].

In agreement with the present study, Liu *et al.* verified UII serum levels in severe PE to be significantly higher than control group [27].

Yanyan *et al.* reported a significant difference between PE patients and control groups regarding serum UII levels [28]. Sakamato *et al.* concluded that UII in serum increases as pregnancy advances, decreases rapidly after delivery, and its concentration during pregnancy correlates with gestational week, thus confirming that UII is derived mainly from the placenta [29]. Our results also agreed with a report by Tan *et al.* who pointed out an association between the UTS-II 143 G⁄A polymorphism and gestational diabetes in pregnant Chinese women. Because PE is frequently associated with gestational diabetes, they hypothesized that the UTS-II 143 G⁄A polymorphism may be associated with PE [30]. Another study done by Gould PS *et al.* showed that there is an upregulation of UII receptor in PE that causes in vitro placental release of soluble vascular endothelial growth factor [31]. Dikensoy *et al.* concluded no significant association between the UTS-II S89N polymorphism and PE [32].

Conclusion

UII gene polymorphism and UII serum level may be among very important biomarkers sharing in the pathogenesis, progression, and severity of PE. All results of current and previous studies confirm a promising role of UII in detection of severity, or prediction of the disease occurrence. The combined screening for both gene polymorphism and serum levels of UII in pregnant women at risk of developing the disease would be a real value in the management of such a life-threatening condition. Further research is still needed to verify these results.

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