Gene polymorphisms and risk of preeclampsia in Egyptian women

Original Article

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ABSTRACT

Aim: The aim of this study was to evaluate the possible association between ACE I/D, AT1 receptor 1166 A:C, AT2 receptor-1332 A:G, and MMP-9-1562 C:T polymorphisms and risk of preeclampsia in Egyptian women.

Materials and Methods: This case-control study included 108 pregnant women was allocated into two groups, 54 pre-eclamptic women group and control group which included 54 normotensive pregnant women. Genotyping of AT1 1166 A:C and AT2 −1332 A: G were performed by duplex polymerase chain reaction-restriction fragment length polymorphism PCR-RFLP. Genotyping of I/D polymorphism of ACE was carried out by PCR and genotyping of MMP-9 −1562C/T was performed by tetra-primer amplification refractory mutation system T- ARMS–PCR.

Results: The DD genotype of ACE gene was significantly associated with increased risk of preeclampsia [OR (95% CI) = 2.47 (0.72–8.5), *p = 0.02*] and the D allele was significantly associated with an increased risk of preeclampsia [OR (95% CI) = 1.95 (1.08–3.54), *p = 0.02*]. The AT2 GG genotype frequency was significantly higher in preeclampsia [OR (95% CI) = 3.24 (1.25–8.41), *p = 0.002*] and the G allele [OR (95% CI) = 2.41 (1.39–4.18), *p = 0.002*]. However, the AT1 CC and MMP9 TT genotypes frequency were insignificantly associated with preeclampsia.

Conclusion: ACE gene I/D and -1332A/G of AT2 receptor polymorphisms, but not AT1 receptor gene A1166C and MMP-9 (-1562 C/T) polymorphisms, could be related to the risk of preeclampsia in Egyptian women.

Key Words: Angiotensin converting enzyme, angiotensin II type 1-2 receptors, matrix metalloproteinase 9, preeclampsia

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INTRODUCTION

Preeclampsia (PE), a pregnancy complication, affects 10% of pregnancies worldwide[1]. Development of PE has been attributed to multiple genetic variations. Among them are genes encoding the renin angiotensin system (RAS) enzymes and their receptors, and Matrix metalloproteinases (MMPs) enzymes[2,3].

Renin angiotensin system (RAS) plays a central role in regulating blood pressure during pregnancy. Angiotensin converting enzyme (ACE) catalyzes the conversion of angiotensin I into angiotensin II (ANG II), ANG II is a vasoconstrictor octa-peptide that elevates the blood pressure. Moreover, in preeclampsia, there is increased responsiveness to ANG II. ANG II binds to numerous types of receptors, binding to angiotensin type 1 receptor (AT1) mediates vasoconstrictor and the proliferative function of ANG II, while binding to type 2 receptor (AT2) suppresses its proliferative function[4].

The AT1 receptor gene localizes on chromosome (3q21–q25). The AT1 receptor 1166 A:C polymorphism (rs5186) localizes in 3′-untranslated region of AT1 receptor gene and could cause the post transcriptional modification of AT1 receptor mRNA [5]. The AT2 receptor gene localizes on chromosome (Xq23–26) and has two introns and three exons. AT2 receptor gene −1332A: G polymorphism (rs14035430) localizes in intron 1, 29 bp just before the beginning of exon 2, close to transcriptional activity region[6].

ACE (I/D) polymorphism (rs1799752) has been reported. The existence of D allele of ACE I/D polymorphism has been linked to preeclampsia in several populations[7-9].

Matrix metalloproteinases (MMPs) enzymes play a central role in reconstruction of extracellular matrix[10]. Polymorphism of −1562 C:T (rs3918242) in MMP-9 promoter increases MMP-9 levels[11, 12]. Altered MMP

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levels in preeclampsia may affects trophoblastic invasion[10]. Indeed, MMPs may be involved in endothelial dysfunction in preeclampsia by their interaction with inflammatory mediators and increased oxidative stress[10].

Genetic involvement in development of preeclampsia has been reported.

AIM OF THE STUDY

The aim of this study was to evaluate the possible roles of ACE I/D, AT1 receptor 1166 A:C, AT2 receptor-1332 A:G, and MMP-9-1562 C:T polymorphisms in the risk of preeclampsia.

PATIENTS AND METHODS

2.1: Study design and patients selection:

A case control study was conducted on pregnant women recruited from Department of Obstetrics and Gynecology, Faculty of Medicine, Zagazig University, Egypt from November 2018 to July 2019. All patients were of Egyptian nationality. All participants signed informed consent form. The study proposal was approved by the Institutional Review Board of Faculty of Medicine, Zagazig University, Egypt.

A total sample size of 108 patients of equal group sizes was calculated to achieve a 95% confidence interval and 80% power as ACE (I/D) DD frequency was (58.3 %) in preclampsia while it was (30%) in control, the estimated sample was 54 in each group (Open Epi). The subjects included 108 pregnant women who were allocated into two groups: (54 preeclamptic patients and 54 normotensive pregnant women). Preeclamptic women were further subdivided into (27 mild and 27 severe).

Preeclampsia was diagnosed using the criteria of the National High Blood Pressure Education Program Working Group[13] with systolic and diastolic blood pressure ≥140 mm Hg and ≥90 mm Hg, respectively, and presence of proteinuria ≥ 300 mg in 24-hour urinary collection, or ≥30 mg/dl protein in random urine sample (1+ reaction on a standard urine dipstick) which occurring after 20 weeks gestation in a woman with previously normal blood pressure. Women with a past history of chronic hypertension, diabetes, cardiac and renal diseases, pregnancies with deformed fetuses, multiple pregnancies were excluded from the study.

Severe preeclampsia was diagnosed by having one or more of the following: blood pressure ≥160110/ mm Hg on 2 separate occasions at least 6 h apart with patient on bed rest, proteinuria ≥ 2 g in 24-hour urinary collection ≥ 3+ on 2 random urine samples at least 4 hour apart, headache, upper abdominal pain, visual disturbances, thrombocytopenia , serum creatinine and transaminase elevation, and fetal-growth restriction.

The control group included 54 normotensive, age and parity-matched pregnant women with no hypertension or protein urea with the same inclusion and exclusion criteria of preeclamptic group.

2.2. Genotype analysis :

2.2.1 DNA extraction:

From whole blood treated EDTA using commercially available G-spin TM Total DNA Extraction Kit (iNtRON Biotechnology, Seongnam, Korea).

2.2.2:Genotyping of I/D polymorphism (rs1799752) of ACE gene:

Polymerase chain reaction (PCR) assay was used for detection of ACE I/D (rs1799752) polymorphism according to Serdaroglu et al.[14] . ACE I/D polymorphism was genotyped as a 190 bp for D allele and a 490 bp for I allele. DD genotype samples were confirmed by the use of a pair of primers producing an amplified product only in the presence of the insertion.

2.2.3 Genotyping of AT1 receptor 1166 A:C (rs 5186) and of AT2 receptor −1332 A: G (rs14035430) polymorphisms

Duplex Polymerase chain reaction- restriction fragment length polymorphism (PCR- RFLP) assay was used for detection of AT1 and AT2 receptors polymorphisms as described by Zˇivkovic et al. [15] The PCR product was digested by enzyme HaeIII (Haemophilus aegypticus); which cuts the AT1 receptor gene polymorphic site) and EcoRI (Escherichia coli); which cuts the AT2 receptor gene polymorphic site (New England Biolabs, UK). Genotyping of AT1 receptor polymorphism was as a 233 bp band for the C allele, a 256 bp band for the A allele and the AT 2 receptor was genotyped as a 91 and 29 bp for G allele and a 120 bp for A allele.

2.2.4 Genotyping of MMP-9 (−1562C/T) polymorphism (rs3918242)

Analysis of MMP-9 polymorphism was done by using tetra-primer amplification refractory mutation system–PCR (T- ARMS–PCR) as described by Zˇivkovic *et al.*[16] and Chiranjeevi *et al.*[17]. Genotyping of MMP 9 polymorphism was as a 220 bp for C allele and 296 bp for T allele with a common band of 436 bp.

The separation of PCR products of all genes were done in 3% agarose electrophoresis system (Maxicell, EC 360 Electrophoretic Gel System, Thermo Scientific) then Visualization under ultraviolet trans-illumination (Heralab GmbH laborgeratetransilluminator, Germany) with ethidium bromide staining with 100 bp-SizerTM DNA marker (iNtRON Biotechnology) and photographed.

STATISTICAL ANALYSIS:

Processing of data were done using the Statistical Package for Social Science version 13 (SPSS Inc., Chicago, IL). All numerical parameters were compared using the Student t test and non-numerical parameters were compared using chi-square test, odds ratios (ORs) and their 95% confidence intervals (95% CIs). A value of $p<0.05$ was considered statistically significant.

RESULTS

The characteristics of patients and controls are demonstrated in Table 1. Systolic and diastolic blood pressure was significantly higher in both groups of cases (mild and severe) than control ($p< 0.001$), but there was no significant difference regarding blood pressure in severe preeclampsia compared to mild preeclampsia. Comparing platelet count between severe cases and control, there was a significantly lower level ($p<0.001$) and also platelet count is significantly lower in severe preeclampsia compared to mild preeclampsia ($p=0.02$). Also, proteinuria was significantly higher in severe preeclampsia than in mild preeclampsia ($p <0.001$). Moreover there was a significant difference in past history of preeclampsia and family history of both groups of cases than control ($p= 0.04$).

The ACE I/D (rs1799752) genotypes distribution was consistent with the Hardy–Weinberg equilibrium (HWE) in control and in patients ($p=0.73$ and $p=0.11,$ respectively). As regard the risk of development of preeclampsia, DD genotype was significantly higher in preeclamptic patients than control (OR, 2.47; 95% CI = 0.72–8.5 and $p = 0.02$) (Table 2). Regarding the preeclampsia subtypes, the genotype frequencies of II, ID and DD were 7.4 %, 25.9 % and 66.7 % in mild cases 11.1%, 29.6 % and 59.3% in severe cases. The carriage of D allele was significantly associated with mild preeclampsia (OR 2.29, 95% CI = 1.07–4.96 and $p = 0.03$) (Table 3).

The A1166C AT1 receptor genotypes distribution was consistent with the HWE in controls ($p = 0.60$) and in patients ($p = 0.66$). The frequencies of A and C alleles in preeclamptic women were 74.1 and 25.9 % and in control were 77.8 and 22.2%, respectively. The occurrence of the AT1 receptor CC genotype was insignificantly associated with preeclampsia ($p = 0.67$) and the C allele was also insignificantly associated with preeclampsia ($p = 0.63$) (Table 2). As regard preeclampsia subtype, the genotype frequencies of AA, AC and CC were in 51.9%, 40.7% and 7.4% in mild preeclampsia and 55.6%, 40.7% and 3.7% in severe preeclampsia. The carriage of C allele was insignificantly associated with preeclampsia subtypes ($p = 0.43$ and $p = 0.79$) (Table 3).

The frequencies of AT2 receptor −1332 A: G genotypes were consistent with the HWE among the control ($p = 0.22$) and the patients ($p = 0.09$). As regard the risk of development of preeclampsia, the GG genotype was significantly associated with an increased risk of preeclampsia. The carriage of G allele was significantly associated with an increased risk of preeclampsia (OR = 2.41, 95% CI = 1.39–4.18, and $p = 0.002$) (Table 2). As regard preeclampsia subtypes, the genotype frequencies of AA, AG and GG were 11.1%, 59.3 % and 29.6% in mild preeclampsia; 18.5%, 63.0% and 18.5% in severe preeclampsia. The carriage of G allele was significantly associated with mild preeclampsia (OR 2.91, 95% CI = 1.48–5.71 and $p = 0.002$) and marginally associated with severe preeclampsia (OR 2.00, 95% CI = 1.03–3.89 and $p = 0.04$) (Table 3). Table 4 showed the association of the significant polymorphisms and the clinical characteristics of the preeclampsia. No significant association was detected ($p >0.05$).

The MMP-9 (-1562 C/T) genotypes distribution was in accordance with the HWE in control and in patients ($p = 0.38$ and $p = 0.78,$ respectively). There was insignificant association of TT genotype and T allele with preeclampsia ($p = 1.00$ and $p = 0.51$, respectively) (Table 2). As regard preeclampsia subtypes, the genotype frequencies of CC, CT and TT were in 74.1%, 22.2% and 3.7% in mild cases 81.5%, 18.5% and 0% in severe cases. The T allele was insignificantly associated with both mild preeclampsia and severe preeclampsia ($p=0.29$ and $p=1.00$, respectively) (Table 3).

Table 1: Characteristics of study patients

Variables	Controls (n=54)	Mild-PE (n=27)	Severe-PE (n=27)	*p1*	*p2*	*p3*
Age (years):	26.31 ± 5.2	25.78 ± 4.99	24.74 ± 4.2	0.69	0.17	0.38
Systolic BP (mmHg):	107.59 ± 9.75	148.52 ± 9.07	147.59 ± 8.48	<0.001*	<0.001*	0.72
Diastolic BP (mmHg):	68.43 ± 6.13	92.59 ± 6.56	94.63 ± 5.71	<0.001*	<0.001*	0.23
Platelets count: (mm3x103)	279.17 ± 61	260.81 ± 70.18	220.74 ± 49.89	0.2	<0.001*	0.02*
Hemoglobin (g/dL):	10.79 ± 0.91	10.51 ± 0.98	10.37 ± 1.18	0.25	0.07	0.66
INR:	1.01 ± 0.04	1.02 ± 0.06	1.04 ± 0.10	0.37	0.06	0.38
Parity:PG/MP	23/31 (42.6/57.4)	7/20 (25.9/74.1)	9/18 (33.3/66.7)	0.22	0.57	0.76
History of preeclampsia:	0 (0)	3 (11.1)	2 (7.4)	0.01*	0.04*	0.64
Family history:	0 (0)	3 (11.1)	3 (11.1)	0.01*	0.01*	1
Proteinuria:						
Negative	54 (100)	0 (0)	0 (0)			
+	0 (0)	3 (11.1)	0 (0)			
++	0 (0)	24 (88.9)	0 (0)	<0.001*	<0.001*	<0.001*
+++	0 (0)	0 (0)	22 (81.5)			
++++	0 (0)	0 (0)	5 (18.5)			

PE: Preeclampsia; BP: Blood pressure; PG/MP: primigravida/multipara
*: Significant
p1: Control versus mild PE, p2: Control versus severe PE, p3: Mild PE versus Severe PE

Table 2: Genotype distributions and allelic frequencies of the studied polymorphisms

Polymorphism	Preeclampsia (n=54)	Controls (n=54)	OR (95% CI)	*p*
rs1799752				
II	5 (9.3)	8 (14.8)	1	
ID	15 (27.8)	24 (44.4)	1 (0.3-3.63)	0.62
DD	34 (62.9)	22 (40.8)	2.47 (0.72–8.5)	0.02*
Alleles				
I	25 (23.1)	40 (37.0)	1	
D	83 (76.9)	68 (63.0)	1.95 (1.08–3.54)	0.02*
rs5186				
AA	29 (53.7)	32 (59.3)	1	
AC	22 (40.7)	20 (37.0)	1.21 (0.55–2.67)	0.69
CC	3 (5.6)	2 (3.7)	1.65 (0.26–10.62)	0.67
Alleles				
A	80 (74.1)	84 (77.8)	1	
C	28 (25.9)	24 (22.2)	0.82 (0.44–1.53)	0.63
rs14035430				
AA	8 (14.8)	22 (40.7)	1	
AG	33 (61.1)	28 (51.9)	3.24 (1.25–8.41)	0.02*
GG	13 (24.1)	4 (7.4)	8.94 (2.24–35.61)	0.002*
Alleles				
A	49 (45.4)	72 (66.7)	1	
G	59 (54.6)	36 (33.3)	2.41 (1.39-4.18)	0.002*
rs3918242				
CC	42 (77.8)	45 (83.3)	1	0.47
CT	11 (20.3)	8 (14.8)	1.47 (0.54–4.02)	0.45
TT	1 (1.9)	1 (1.9)	1.00 (0.07–17.68)	1.00
Alleles				
C	95 (88.0)	98 (90.7)	1	
T	13 (12.0)	10 (9.3)	1.34 (0.56–3.21)	0.51

OR: Odds ratio; CI: confidence interval.
*: Significant

Table 3: Allelic frequencies and distributions of the studied polymorphisms in control and different preeclampsia subtypes

Polymorphism	Controls (n=54)	Mild-PE (n=27)	OR (95% CI)	*P*	Severe-PE (n=27)	OR (95% CI)	*P*
rs1799752							
I	40 (37.0)	11 (20.4)	1		14 (25.9)	1	
D	68 (63.0)	43 (79.6)	2.29 (1.07-4.96)	0.03*	40 (74.1)	1.68 (0.82-3.46)	0.16
rs5186							
A	84 (77.8)	39 (75.9)	1		41 (75.9)	1	
C	24 (22.2)	15 (27.8)	1.35 (.64-2.85)	0.43	13 (24.1)	1.11 (0.51-2.4)	0.79
rs14035430							
A	72 (66.7)	22 (40.7)	1		27 (50)	1	
G	36 (33.3)	32 (59.3)	2.91 (1.48-5.71)	0.002*	27 (50)	2 (1.03-3.89)	0.04*
rs3918242							
C	98 (90.7)	46 (85.2)	1		49 (90.7)	1	
T	10 (9.3)	8 (14.8)	1.7 (0.63-4.6)	0.29	5 (9.3)	1 (0.32-3.09)	1

PE: Preeclampsia; OR: Odds ratio; CI: confidence interval.
*: Significant

Table 4: The association of the significant polymorphisms and the clinical characteristics of the preeclampsia

Parameters	rs1799752			rs14035430		
	DD (n=34)	II & ID (n=20)	*p*	GG (n=13)	AA & AG (n=41)	*P*
Age (years):	25.6 ± 4.79	24.75 ± 4.25	0.54	24.74 ± 4.2	24.92 ± 4.82	0.76
Systolic BP (mmHg):	155.2 ± 4.58	149.5 ± 8.87	0.59	154.5 ± 41.6	148.5 ± 114.4	0.61
Diastolic BP (mmHg):	93.2 ± 6.84	94.3 ± 4.94	0.57	92.8 ± 5.92	96.15±6.5	0.09
Platelets count: (mm3x103)	245.7 ± 60.96	232.4 ± 68.7	0.46	242.9 ± 61.6	234.1±71.84	0.67
Hemoglobin (g/dL):	10.37±1.15	10.45±0.97	0.79	10.41±0.89	10.34±1.58	0.83
INR:	1.04± 0.08	1.02 ± 0.08	0.38	1.04 ± 0.082	1.01 ± 0.08	0.37
Parity:PG/MP	23/11 (67.7/32.3)	15/5 (75/225)	0.57	8/5 (61.5/38.5)	30/11 (73.2/26.8)	0.42
History of preeclampsia:	4 (11.8)	1 (5)	0.41	0 (0)	5 (12.2)	0.19
Family history:	0 (0)	2 (10)	0.84	2 (15.4)	4 (9.8)	0.57
Proteinuria:						
+	2(5.9)	1 (5)		1 (7.7)	2 (4.9)	
++	16 (47.1)	8 (40)		7 (53.8)	17 (41.4)	
+++	12(35.3)	10(50)	0.69	4 (30.8)	18 (43.9)	0.82
++++	4(11.7)	1(5)		1 (7.7)	4 (9.8)	

BP: Blood pressure; PG/MP: primigravida/multipara

DISCUSSION

Preeclampsia is a condition with extensive endothelial dysfunction, accompanied by inflammation, vasoconstriction, and platelet activation. In preeclampsia, the mother blood flow decreases that may be caused by signalling events reduction of ANG II. At present, little is known about the exact cause for RAS dysfunction occurring in preeclampsia, although numerous patho-physiological mechanisms have been suggested including autoimmunity, oxidative stress, and endothelial damage[18]. Human linkage and several genetic studies have found gene polymorphisms in RAS components that explain variance in activity of this system[19]. Studying these polymorphisms showed both positive[20, 21] and negative linkage with preeclampsia[22, 23].

In the present study, the primary aim was to detect if the main components of RAS; ACE, AT1 receptor, AT2 receptor and MMP-9 genes polymorphisms in mothers were associated with preeclampsia. The secondary aim was to detect whether the association, if present, related the degree of severity of preeclampsia of AT1 receptor and AT2 receptor. First, we investigated the hypothesis that ACE influences the occurrence of preeclampsia. The current study indicated that frequency of DD genotype and D allele in preeclamptic and control groups was significantly difference and ACE I/D polymorphism in the presence of DD genotype was associated with a 2.47-fold increase in the risk of preeclampsia. In line with these results were Mello *et al.* 2003[24] who found an increased risk of recurrent preeclampsia and fetal growth restriction in association with DD genotype. In addition, Velloso *et al.*[25] reported an increase of preeclampsia with ACE DD genotype in Brazil. However, numerous studies in multiple countries did not support such association in Poland[26], Finland[27], Brazil[28], Korea[29], South Africa [30] and in Japan[31].

The present study found no evidence of association between AT1 receptor A1166C polymorphism and preeclampsia. C allele frequency of the AT1 receptor gene was 22.2 % and 25.9% in control and preeclamptic women, respectively. Moreover, there was insignificant difference between both groups as regard preeclampsia subtypes when the control group was compared. Other studies goes hand in hand with these results, in Western Iran, Asian, Afro-Caribbean, and Caucasian populations did not found an association between AT1 receptor A1166C polymorphism and risk of preeclampsia[8, 32]. Li *et al.*[33] found no difference in AT1 receptor genotypes in normal pregnancy and preeclampsia. In contrary, Seremak-Mrozikiewicz et al.[34] reported an association between A1166C polymorphism and risk of preeclampsia.

AT2 receptor is the most enigmatic component of RAS. It has been showed that the high concentration of AT2 receptor in relation to AT1 receptor at late pregnancy may clarify the uterine arteries refractoriness to ANG II[35]. In the current study, a significant association between the GG genotype and G allele of AT2 receptor −1332 A: G and preeclampsia were noted in Egyptian women. Moreover, a significant increased risk of both mild and severe preeclampsia was associated with G allele of the AT2 receptor polymorphism. To our knowledge, this is the first report of such association in Egyptian women hand in hand with these results, Rahimi *et al.*[36] case-control study found an increased risk of preeclampsia in the presence of the G allele of AT2 receptor −1332 A: G polymorphism. Also, epistatic interaction of G allele and each allele of the AT1 receptor C, MMP-9 T and ACE D was related to preeclampsia risk. The result of the current study can explain the risk of preeclampsia and development of manifestation of hypertension by an increase in ACE activity in the presence of D allele along with an increase of ANG II production with consequence reduction in AT2 receptor expression in the presence of G allele which lead to increased sensitivity of AT1 receptor. In contrast to these results, the study of Zhou *et al.*[37] reported that lack of association of AT2R-1332 A: G gene polymorphism with preeclampsia.

In the present study, no association was detected between overall preeclampsia and MMP-9 (-1562C/T) polymorphism. The importance of MMPs is mostly in the process of spiral arteries trophoblastic invasion. Since the trophoblasts carry fetal DNA, detection of SNPs in fetal DNA may be more important than in maternal DNA, and further studies can address such possibility. Supporting these results, Gong *et al.*[38] meta-analysis found no association between MMP9 (-1562C/T) polymorphism and preeclampsia. In contrast to the present study, Rahimi *et al.*[39] suggested that an association between MMP-9 (C-1562T) polymorphism and early-onset and severe preeclampsia.

We should consider limitations of this study; firstly, sample size and secondly, the role of environmental interactions in pathogenesis of preeclampsia. Various environmental factors may be involved, such as chemicals exposure, maternal age, weight, height, diatery habit, smoking, and folic acid supplement, use may be related to the risk of preeclampsia.

So, the results of the study cannot be generalized to the whole of the Egyptian population, due to multiple genetically heterogeneous populations in Egypt. These findings may reflect the population studied as we tried to have strict selection criteria and by taking identical ethnic subjects from a geographically limited

area. Also, separate analysis for mild and severe preeclampsia was performed. Large-scale studies are recommended to confirm these findings. Consequently, preventative strategies could be undertaken to reduce preeclampsia –related morbidity and mortality.

CONCLUSION

ACE gene I/D and -1332A/G of AT2 receptor polymorphisms, but not AT1 receptor gene A1166C and MMP-9 (1562 C/T) polymorphisms, could be associated with the risk of preeclampsia in Egyptian women.

CONFLICT OF INTEREST

There are no conflicts of interests.

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