ORIGINAL ARTICLE

Association study of interleukin-1 receptor associated kinase 1 rs3027898 A/C gene polymorphism and preeclampsia in Pakistani population

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Abstract

Objective: To find the association between interleukin-1 receptor-associated kinase 1 rs3027898 gene polymorphism and preeclampsia.

Methods: The case-control study was conducted from October, 2018 to September, 2019 at the Railway General Hospital and the Department of Biochemistry, Islamic International Medical College, Rawalpindi, Pakistan, and comprised patients diagnosed with preeclampsia and healthy controls. The interleukin receptor-associated kinase-1 polymorphism was determined using multiplex tetra primer amplification refractory mutation system polymerase chain reaction. Outcomes were determined in terms of association of interleukin receptor-associated kinase-1 with preeclampsia. Data was analysed using SPSS 22.

Results: Of the 160 subjects, 80(50% were cases with a mean age of 30±5.3 years and 80(50%) were controls with a mean age of 27±3.7 years. AC genotype was seen in 45(56.25%) cases and 30(37.5%) controls, AA genotype in 25(31.25%) cases and 30(37.5%) controls, while CC genotype was seen in 10(12.5%) cases and 20(25%) controls ($p>0.05$).

Conclusion: There was no significant association of interleukin receptor-associated kinase-1 genotypes with preeclampsia.

Keywords: Preeclampsia, Interleukin receptor-associated kinase-1, Gene polymorphisms. (JPMA 71: 1332; 2021)

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Introduction

Preeclampsia (PE) is defined as hypertension (HTN) with proteinuria diagnosed after 20 weeks of gestation till 2 weeks postpartum (PP). If proteinuria is absent, it is diagnosed through HTN with any of the following: platelet count 100,000/ml, serum creatinine 1.1mg/dl, or doubling of concentration of serum creatinine in the absence of other renal disease, increase in liver transaminases to twice the normal concentration, pulmonary, cerebral and visual symptoms.[1] It affects 2-8% of all pregnancies and remains a leading cause of maternal and perinatal deaths worldwide, with around 344,000 women dying from PE in the last 10 years.[2] Approximately 13-26% small-for-gestational-age infants and 16-21% of all preterm births are due to PE, and the other associated complications of prematurity are neonatal deaths as well as serious long-term neonatal morbidity.[3] PE incidence in pregnant women in Pakistan is 1.2%. The World Health Organisation (WHO) estimates that PE is responsible for 12% maternal mortality in Asia. Ethnicity can be a risk factor for developing PE. The prevalence of PE is different in every country; 3.5% in the United States (US), 9% Brazil, 3% Australia, 12.5% Bangladesh, 3% India, and 5% in Thailand.[4]

The mechanisms involved in development of disease are not clearly understood. The development of preeclampsia is related to placenta, it disappears soon after delivery.[5]

The improper invasion of trophoblast early in pregnancy is the start of the pathological process of PE, causing an increase in oxidative stress (OS), which, in turn, leads to endothelial dysfunction in the second stage of the disease. In case of normal development of placenta, cytotrophoblast cells form extravillous trophoblast which penetrates the decidua and the first three parts of the myometrium. This causes remodelling of spiral vessels into low-resistance vessels which causes increase in blood flow. It is essential for foetus growth.[6] If the placenta develops abnormally, leading to impaired invasion of trophoblast, there is decrease in uteroplacental blood flow. It is the start of PE.[7] The state of low blood perfusion is the cause of hypoxia in placenta, local OS, endothelial dysfunction and systemic inflammatory response. The first stage of improper invasion of trophoblast is difficult to diagnose, while high resistance in circulation is a clinical syndrome.[8]

The known risk factors are very young or advanced age of the mother, low socioeconomic status (SES), smoking,

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obesity, family history of PE, history of PE in previous pregnancy, parity and type of pregnancy whether single or multiple, family history of diabetes mellitus (DM) and HTN.[9]

The definite cause of PE is not known, but it is obvious that genes play a definite role. Disorders with genetic elements interfere with mother's vascular responses, affect trophoblastic function, or increase the placental mass, causing foetal demands to increase in relation to supply.[10] The genes involved will not cause PE straightaway, but will lower a mother's threshold at which she will develop the disease. Since the diagnostic criterion of PE is based on the continuous distribution of blood pressure (BP) and proteinuria, it is likely that no single aetiology or genetic marker will account for all PE cases. There has been evidence of paternal and foetal genetic effects. The observation that PE is common in molar pregnancies in which all the foetal chromosomes are derived from the father is evidence for the role of paternal genes.[10] PE is a common but often serious disease for mother or child or both, and a large percentage of modern cases are due to new mutations without a direct familial pattern.[11]

A study on the genetic association of PE evaluated 350 preeclamptic mother-offspring pairs and 600 control pairs, showing 775 single nucleotide polymorphisms (SNPs) in 190 genes. It detected six genes with significant maternal-foetal genotype interaction related to insulin growth factor-1 (IGF1), interleukin 4 receptor (IL4R), insulin growth factor 2 receptor (IGF2R), gene protein subunit beta 3 (GNB3), colony stimulating factor-1 (CSF1) and thrombospondin 4 (THBS4).

A 2006 study reported that seven genes cover 70% of literature regarding PE genetics.[12] These genes were angiotensinogen (AGT), angiotensin receptors 1 and 2 (AGTR1, AGTR2), coagulation factor V (FV), methylenetetrahydrofolate reductase (MTHFR), nitric oxide synthase 3 (NOS3) and tumour necrosis factor-alpha (TNF-α). The interest in these genes can be explained by their involvement in the underlying aetiologies in the pathogenesis of PE.[12]

IL1-receptor-associated kinase 1 (IRAK-1) is a serine and threonine protein kinase involved in the signalling mechanisms of the toll and IL1 receptor (TIR) family.[13] On toll-like receptor (TLR) stimulation, IRAK-1 is recruited and phosphorylated into the TLR signalling complex. IRAK-1 is an important intracellular signalling protein that is activated by ligands of TLRs and recognises many different varieties of ligands, including pathogens, and is involved in pathogen-mediated inflammation.[14]

The current study was planned to assess the association of IRAK-1 rs3027898 gene polymorphism and preeclampsia in the local population.

Materials and Methods

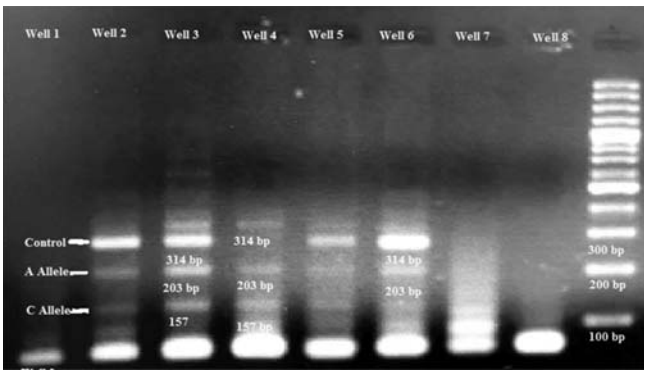
The case-control study was conducted from October, 2018 to September, 2019 at the Railway General Hospital (RGH) and the Department of Biochemistry, Islamic International Medical College (IIMC), Rawalpindi, Pakistan. After approval from the IIMC ethics review committee, the sample size was calculated using the formula: $z^2(pq)/e^2$, where, $z = 1.96$ deviant error for 0.05, p = prevalence, q = 1- p, and e = tolerance error[5]. The sample was raised using non-probability convenience sampling technique from among women at the RGH Department of Gynaecology and Obstetrics.

Those included were pregnant females regardless of age and parity who were diagnosed with PE. Also included were age-matched controls who were pregnant with no diagnosis or personal and familial history of PE.

Those excluded were non-pregnant females as well pregnant females diagnosed with HTN, gestational DM or with known autoimmune disorders.

After obtaining informed written consent from the subjects, demographic data was recorded, followed by 4ml blood samples collected by venipuncture after proper antiseptic measures. The samples were labelled with proper name and number, and were transported to lab in K3 ethylenediaminetetraacetic acid (EDTA)-containing vacutainers and were preserved at -80O Celsius until further analysis.

Genomic deoxyribonucleic acid (DNA) was extracted using chelex method from the whole blood and stored at -80O Celsius. IRAK1 polymorphism was determined by tetra-primer amplification refractory mutation system (ARMS) polymerase chain reaction (PCR). Two external primers forward outer 5'CCATGCCTGGCTAATTTTTGTACTTTTT-3 and reverse outer 5'GTCTTCAGAAGCAAGTCAGGTTTCA TGT-3' and two internal primers, forward inner 5'AGACCCTGGACGCTCAAGAATCA-3 and reverse inner 5'TGAGCTTTCCTGATGCCTTAC ACTTTAG-3, were used.[15] The product sizes were 203bp for the A allele, 157bp for the C allele and 314bp for the control band. PCR reaction ingredients were 25µl of DreamTaq Green master mix (2x) (Thermoscientific) which consists of 1. DreamTaq DNA polymerase, 2.2x DreamTaq Green buffer, 3.4mM magnesium chloride ($MgCl_2$), 4.0.4Mm each of deoxyadenosine triphosphate (dATP), deoxycytidine triphosphate (dCTP), deoxyguanosine triphosphate (dGTP), deoxythymidine triphosphate (dTTP), 14.5µl



Control band at 314 bp
C allele at 157 bp
A allele at 203 bp

Figure: Gel Electrophoresis bands for interleukin receptor-associated kinase-1(IRAK-1).

distilled water, 2µl of each inner forward and reverse primer (10uM) and 2µl of each outer forward and reverse primer (12uM) (M/s Macrogen), 1.5µl of DNA, and 14.5µL distilled water. PCR (Major Science Thermocycler) cycling conditions were initial denturation at 95°C for 5min, followed by loop 1 of 20 cycles: 30sec of denaturation at 95°C, 30sec of annealing at 66°C and 30 sec of extension at 72°C. It was followed by loop 2 of 20 cycles: 30sec of denaturation at 95°C, 30sec of annealing at 64°C, and 30sec of extension at 72°C. Final common extension step was at 72°C for 10min. PCR products were visualised on 2% agarose gel and estimated in comparison to 100bp DNA ladder. Electrophoresis was done at ~100 volts for 40-50 minutes. Gel documentation was done by placing the gel on ultra-violet (UV) trans-illuminator. The amplified DNA fragments were seen as whitish bands (Figure).

Data was analyzed using SPSS 22. Chi-square test was used to find significance among different variables in the study groups and genotypes of IRAK1 that were AA, AC and CC. Odds ratio (OR) with 95% confidence interval (CI) was calculated for genotypes of IRAK1 to find their association with cases and controls. P<0.05 was taken as significant.

Results

Of the 160 subjects, 80(50% were cases with a mean age of 30±5.3 years and 80(50%) were controls with a mean age of 27±3.7 years (p>0.05). Family history of HTN, weight and parity were significantly different between the groups (Table-1).

AC genotype was seen in 45(56.25%) cases and 30(37.5%)

Table-1: Demographic characteristics of cases and controls.

Parameter		Groups		p-value
		Cases (N=80) n(%)	Controls (N=80) n(%)	
Mean Age in Years		30±5.3	27±3.7	0.07
Family History Negative For Hypertension		21(26.25)	33(41.25)	0.02
Family History Positive For Hypertension		59(73.75)	47(58.75)	
Weight (kg)	Below 65	15(18.75)	35(43.7)	0.00
	Above 65	65(81.25)	45(56.25)	
Parity	Primigravida	47(58.75)	42(52.5)	0.05
	Multigravida	33(41.25)	38(47.5)	

Table-2: Association of demographic characteristics of cases with genotypes of interleukin receptor-associated kinase-1(IRAK1).

Parameter	Genotypes			p-value
	AA n(%)	AC n(%)	CC n(%)	
Mean age in years				
Less than 30 years	5(16.66)	20(66.66)	5(16.66)	0.03
Above 30 years	15(30)	25(50)	10(20)	
Family history negative for hypertension	8(38.09)	10(47.61)	3(14.28)	0.02
Family history positive for hypertension	10(16.94)	25(42.37)	24(40.67)	
Weight in kg				
Below 65 kg	10(66.66)	5(33.33)	0(0)	0.00
Above 65 kg	5(7.69)	50(76.92)	10(15.38)	
Parity				
Primigravida	2(4.25)	35(74.46)	10(21.27)	0.00
Multigravida	20(60.60)	10(30.30)	3(9.09)	

Table-3: Genotypic frequencies of cases and controls.

Genotypes	Cases N=80 n(%)	Controls N=80 n(%)	Odds ratio (95% CI)	p-value
AA	25(31.25)	30(37.5)	1.8(0.8-3.6)	0.10
AC	45(56.25)	30(37.5)		
CC	10(12.5)	20(25)	0.48(0.19-1.18)	0.10

CI: Confidence interval.

controls, AA genotype in 25(31.25%) cases and 30(37.5%) controls, while CC genotype was seen in 10(12.5%) cases and 20(25%) controls. The difference between the groups was significant (Table-2).

Genotypic frequency of IRAK1 between the cases and the controls was not significant (Table-3).

Discussion

Genetic association of IRAK1 have been studied in many autoimmune disorders, like rheumatoid arthritis (RA),[16] cerebral infarction,[17] systemic lupus erythematosus (SLE),[18] psoriatic arthritis,[19] Sjogern syndrome (SS)[20] and septic shock.[21]

The current study showed significant association of maternal age of cases with genotypes of IRAK1. Studies have concluded that PE risk increases with increasing maternal age.[22] The current study showed significant association of family history of HTN of cases with IRAK1 genotypes. This finding is in agreement with studies conducted in Brazil,[23] Sudan[24] and Uganda.[23,25] This is due to genetic factors that contribute to the physiological predisposition of PE. Maternal risk factors, like family history of HTN, badly affect the capability of the placenta to cater with decreased placental perfusion, resulting in a reduction in the supply of nutrients to the foetus.[26] The current study showed significant association of weight of the cases with IRAK1genotypes. It is a well established fact that increased maternal weight is related to increased pregnancy complications, like PE. A study showed that obesity before pregnancy gives a 30% risk for PE in pregnant women with body mass index (BMI) >35.[27] Also, there was a significant association between IRAK1 genotypes with parity. Some studies have not shown significant association between parity and PE.[28]

The current study is first in Pakistan to explore the association of genetic polymorphism of IRAK1 with PE.

Polymorphisms of rs3027898 and rs1059702 of IRAK1 gene were associated with SLE in Chinese Han population; the C allele of rs3027898 was associated with a decreased risk for patients with oral ulcers.[29]

The IRAK1 gene is recognised as a risk gene in autoimmune disorders. Three SNPs in IRAK1, namely, rs3027898, rs1059702 and rs1059703, are considered to be associated with risk of autoimmune disorders.[30]

Polymorphisms of IRAK1 gene rs3027898 and rs1059703 did not have any association with juvenile autoimmune arthritis in India.[19]

In China, IRAK1 gene was found to be a crucial risk factor for autoimmune thyroid diseases.[31]

Conclusion

There was no significant association of IRAK1 genotypes with preeclampsia among the studied subjects.

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