

The role of interleukin-6 promoter polymorphism -174G/C in Saudi women with hypertensive disorders of pregnancy

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ABSTRACT

الأهداف: دراسة دور طفرة -174G/C بجين الانترلوكين-6 في حالات اضطرابات ارتفاع ضغط الدم المصاحبة للحمل (HDP) عند النساء السعوديات.

الطريقة: أجريت هذه الدراسة على حالات ارتفاع ضغط الدم المصاحبة للنساء السعوديات اللاتي يتابعن بمستشفى الملك خالد الجامعي، الرياض، المملكة العربية السعودية خلال الفترة من أبريل 2010م إلى ديسمبر 2011م. وتمت دراسة 109 مريضة وقورنت بمائة امرأة تتمتع بحمل طبيعي (المجموعة الشاهدة). تكونت مجموعة الدراسة من 60 امرأة تعاني من ارتفاع ضغط الدم الحاملي (GH) و 49 امرأة مصابة بمقدمات الارتعاج (PE). واستخدمت طريقة تفاعل البلمرة المتسلسل بالوقت الفعلي لتحديد الطفرة -174G/C بجين الانترلوكين-6.

النتائج: أظهرت نتائج الدراسة أن توزيع الطرازات الجينية للطفرة -174G/C بجين الانترلوكين-6 لدى المرضى كانت (58.5% GG، 31.1% GC، و10.4% CC)، بينما بالمجموعة الضابطة كانت (30.9% GC، 67% GG) و (2.1% CC). كما أظهرت الدراسة زيادة نسبة الإصابة بمرض HDP في حالات زيجوت متماثلة الألائل CC [نسبة الأرجحية 5.76، 95% فاصل الثقة -27.06، 1.23 (p=0.03)]. وتوضح هذه العلاقة بحالات GH فقط مع [نسبة الأرجحية 7.65، 95% فاصل الثقة -38.03، 1.54 (p=0.01)]. في حين لم يظهر هذا الارتباط في حالات PE [نسبة الأرجحية 3.48، 95% فاصل الثقة -21.99، 0.55 (p=0.19)].

خاتمة: تشير النتائج إلى وجود علاقة إيجابية بين طفرة -174G/C بجين الانترلوكين-6 واحتمال الإصابة باضطرابات ارتفاع ضغط الدم أثناء الحمل لدى النساء السعوديات. ووجد أن النساء ذوات النمطين الجينيين CC وGC أكثر عرضة للإصابة ب GH ولكن ليس مع PE مما يشير إلى اختلافهما من حيث الاستعداد الوراثي / الفسيولوجيا المرضية.

Objectives: To study the relationship between -174G/C interleukin-6 single nucleotide polymorphism and hypertensive disorders of pregnancy (HDP) in Saudi women.

Methods: In this case-control study, 109 HDP patients and 100 women with normal pregnancy as a control group were studied. The HDP study group constituted of 60 women with gestational hypertension (GH) and 49 women with preeclampsia (PE). All women were randomly selected from the antenatal clinic and the prenatal and postnatal wards at the Antenatal Clinic and the Obstetric Ward of King Khalid University Hospital, Riyadh, Kingdom of Saudi Arabia from April 2010 to December 2011. The -174 GC of IL-6 SNP was determined using real time polymerase chain reaction, allele discrimination technique.

Results: Distribution of -174 GC of IL-6 genotype in HDP patients was GG (58.5%), GC (31.1%), and CC (10.4%), while in the control group was GG (67%), GC (30.9%), and CC (2.1%). The CC homozygosity was significantly associated with HDP (odds ratio [OR] = 5.76; 95% confidence interval [CI] 1.23 – 27.06, p=0.03). This association only manifested with GH (OR = 7.65; 95% CI = 1.54-38.03, p=0.01). However, no significant association was found with PE (OR = 3.48; 95% CI = 0.55-21.99, p=0.19).

Conclusion: The results indicate a positive association between -174 GC of IL-6 genotype and the risk of HDP. Genotypes CC and GC are associated with increased risk of GH but not with PE, suggesting that they are of differing genetic predisposition/pathophysiology.

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Hypertensive disorders of pregnancy (HDP) are a group of conditions that include gestational hypertension (GH), preeclampsia (PE)/eclampsia and chronic hypertension, with or without superimposed PE.¹ They are a major worldwide problem affecting 5-14% of pregnancies and their prevalence is on the increase. These disorders can expose both mother and fetus to serious complications.²⁻⁴ Furthermore, affected mothers are at an increased risk of developing cardiovascular diseases later on in life.^{5,6} As complex diseases they are of multifactorial etiology with both genetic and environmental factors.⁷ Several body systems, including blood pressure regulation, metabolic, homeostasis and inflammatory systems have been implicated in the pathogenesis HDP.⁸ There is also mounting evidence supporting that cytokines are involved in the development of HDP, and PE in particular.⁹⁻¹⁴ Interleukin-6 (IL-6) is a multifunctional pleiotropic cytokine with pro- and anti-inflammatory actions, plays an important role in the hemostatic and immune systems.¹⁵ While, some studies found elevated IL-6 in PE, others found no association with the disease.¹⁶⁻¹⁸ In humans, the IL-6 gene is located on short arm of chromosome 7 (7q21). It encodes for the proinflammatory cytokine, IL-6, secreted mainly by neutrophils, granulocytes, and macrophage. The IL-6 is the main stimulant of the acute phase response, it stimulates T lymphocytes, differentiation of B lymphocytes, and the production of C reactive protein (CRP).¹⁹⁻²¹ Various polymorphisms in the promoter region of the IL-6 gene was reported to influence IL-6 transcription.^{12,13,22} In an attempt to determine whether IL-6 is a contributing factor to the pathogenesis of the disorders or a consequence, the associations of several IL-6 polymorphisms with the risk of disease were examined. One of these single nucleotide polymorphisms (SNP) is the functional C-174G, which has been studied in PE patients mainly in Caucasians and it has been shown that the allelic distribution and genotype frequency differ between ethnic groups.²³⁻²⁵ Furthermore, this promoter SNP was found to increase the risk of cardiovascular diseases.²⁶⁻²⁸ However, its relation with the risk of GH has not been investigated. The current study was carried out to determine the allele frequency of -174 GC of IL-6 SNP variant in a sample

of healthy pregnant Saudi women and those with HDP (and compare with other populations) to test whether this SNP is associated with the risk of GH and PET.

Methods. Subjects. This case-control study was conducted at the Antenatal Clinic and the Obstetric Ward of King Khalid University Hospital, Riyadh, Kingdom of Saudi Arabia. The study groups were 109 women with HDP selected according to the definitions of the American College of Obstetrics and Gynecology.¹ The control group is 100 women with normal pregnancy. Patients and controls were recruited as non-probability consecutive sample. The clinical characteristics of patients and controls are shown in Table 1. There were 51% antenatal among the HDP patients and 50% among normal pregnancy. The postnatal samples were all collected 24 hours after delivery. The study was in accordance with the principles of Helsinki Declaration and was approved by the Institutional Review Board. Informed consent was obtained from patients and healthy controls prior to enrollment. All subjects underwent complete physical examination and routine biochemical blood testing, including fasting blood glucose and lipid profile. Height, weight, and waist circumference were measured to calculate body mass index (BMI). Patients received one or more of antihypertensive drugs and magnesium sulphate, as required.

DNA extraction and genotyping. Blood was collected for genomic DNA extraction in 5 ml (Becton Dickinson Pty Ltd) tubes containing 15% EDTA. The DNA was extracted using the commercially available High Pure polymerase chain reaction (PCR) Preparation Kit (Roche Diagnostics GmbH, Germany) and stored at -20°C. Determination of -174 GC of IL-6 polymorphism was performed by real-time PCR using Roche Light Cycle Machine-version 2 in a reaction volume of 20 mL containing 2 mL of DNA (20-80 ng), 0.5 mM each of the primers (sense, 5'-TTA CTC TTT GTC AAG ACATGC CA-3'; anti-sense, 5'-ATG AGCCTC AGA CAT CTC CAG-3') (Tim mol Biol), 2 mL of reaction buffer [LightCycler FastStart DNA Master Hybridization Probes; Roche Diagnostics], one mL of MgCl₂ (final concentration; 2.25 mM), and 0.2 mM each of the labeled probes. The fluorescein labeled anchor probe: 5'-CTA AGC TGC ACT TTT CCC CCT AGT-3'. The LightCycler Red 640 labeled sensor probe: 5'-GTG TCT TGCGAT GCT AAA GGA-3'. The PCR conditions were as follows: initial denaturation at 95°C for 10 minutes, followed by 45 cycles of denaturation (95°C for 5 seconds), annealing (60°C for 10 seconds), and extension (72°C for 15

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seconds). Melting curve analysis was performed based on the derivative melting curves and samples were classified as the IL-6 genotype GG, CG, or CC.

Statistical analysis. Collected data were analyzed using the Statistical Package for Social Sciences version 18 (SPSS Inc, Chicago, IL, USA). Continuous variables were expressed as means and standard deviations (SD). Allele frequencies, genotype distributions, and odds ratio (OR) were compared using 2x2 contingency table analysis. A 2-sided $p < 0.05$ was considered significant.

Results. The study population included 60 GH, 49 PE patients, and 100 controls and the percentage

of primigravida were 29 (for GH), 32 (for PE), and 17 (for controls). The mean age and gestational age were comparable between the different study groups, except for the gestational age of postnatal group, which was significantly less than controls ($p < 0.001$). All patients and controls (antenatal group) were in their third trimester. Hematological and biochemical parameters for patients and controls are shown in Tables 2 & 3. In the antenatal group, the BMI, systolic, and diastolic blood pressure were significantly higher in the GH and PE than normal pregnancy ($p = 0.001$). Genotyping was available for 97 controls and 106 HDP patients (59 GH and 47 PE cases). The distribution of -174 GC of IL-6

Table 1 - Characteristics of the control (normal pregnancy) and study groups (gestational hypertension [GH] and preeclampsia [PE]) of subjects included in a study at the Antenatal Clinic and the Obstetric Ward of King Khalid University Hospital, Riyadh, Kingdom of Saudi Arabia.

Characteristics	Antenatal group				Postpartum group			
	Control (n=50)	GH (n=30)	PET (n=21)	P-value	Control (n=50)	GH (n=30)	PET (n=27)	P-value
Age (years)	29 ± 6	32 ± 7	30 ± 7	0.19	28 ± 5	32 ± 5	29 ± 6	0.06
BMI (Kg/m ²)	32.4 ± 4.9	36.9 ± 7.0*	37.6 ± 5.5*	<0.001	32.9 ± 5.9	35.3 ± 6.1	34.5 ± 3.6	0.15
SBP (mm Hg)	114 ± 9	141 ± 10*	153 ± 9*	<0.001	112 ± 9	136 ± 10*	150 ± 13*	< 0.001
DBP (mm Hg)	68 ± 9	93 ± 9*	95.4 ± 9*	<0.001	67 ± 7	94 ± 6*	93 ± 10*	< 0.001
Gestational age (weeks)	34.8 ± 3.8	34.2 ± 5.3	33.9 ± 3.3	0.64	38.5 ± 1.6	37.9 ± 0.9	34.8 ± 2.7*	< 0.001

Data are presented as mean ± standard deviation. *Significant at $p \leq 0.05$ in comparison with normal pregnancy. In the postpartum group, gestational age is at delivery. BMI - body mass index, SBP - systolic blood pressure, DBP - diastolic blood pressure

Table 2 - Hematological parameters of the control (normal pregnancy) and study groups (gestational hypertension [GH] and preeclampsia [PE]) of subjects included in a study at the Antenatal Clinic and the Obstetric Ward of King Khalid University Hospital, Riyadh, Kingdom of Saudi Arabia.

Characteristics	Antenatal group (n=101)				Postpartum group (n=107)			
	Control (n=50)	GH (n=30)	PET (n=21)	P-value	Control (n=50)	GH (n=30)	PET (n=27)	P-value
WBC (x10 ⁹ /L)	10.1 ± 2.9	9.4 ± 2.5	9.3 ± 3.3	0.47	11.5 ± 2.7	9.3 ± 1.7'	12.9 ± 3.5'	<0.001
RBC (x10 ⁹ /L)	4.1 ± 0.5	4.1 ± 0.4	4.2 ± 0.5	0.53	3.8 ± 0.4	3.8 ± 0.2	3.8 ± 0.6	0.96
Hemoglobin (g/L)	105.7 ± 32.0	119.5 ± 11.2	118.0 ± 34.0	0.07	106.4 ± 13.7	106.0 ± 8.5	106.2 ± 17.2	0.99
Hematocrit (%)	33.7 ± 3.5	34.9 ± 3.0	36.8 ± 5.1'	0.02	31.3 ± 3.8	31.6 ± 3.0	31.5 ± 4.7	0.93
Platelets (x10 ⁹ /L)	225.7 ± 60.8	214.6 ± 48.4	177.7 ± 65.7'	0.03	220.4 ± 60.9	238.8 ± 79.1	201.4 ± 64.1	0.18

Data are presented as mean ± standard deviation. *Significance at $p \leq 0.05$ in comparison with normal pregnancy. WBC - white blood cell, RBC - red blood cell

Table 3 - Biochemical parameters of the control (normal pregnancy) and study groups (gestational hypertension [GH] and preeclampsia [PE]) of subjects included in a study at the Antenatal Clinic and the Obstetric Ward of King Khalid University Hospital, Riyadh, Kingdom of Saudi Arabia.

Circulating levels	Antenatal group				Postpartum group			
	Control (n=50)	GH (n=30)	PET (n=21)	P-value	Control (n=50)	GH (n=30)	PET (n=27)	P-value
RBS (mmol/L)	4.8 ± 1.2	5.2 ± 1.2	5.9 ± 1.7	0.06	4.3 ± 0.5	4.6 ± 1.0	4.6 ± 1.1	0.55
Urea (mmol/L)	2.2 ± 0.9	2.9 ± 1.1*	4.0 ± 1.6*	<0.001	2.7 ± 0.9	2.6 ± 0.9	3.6 ± 2.2*	0.01
Creatinine (Umol/L)	44.6 ± 9.6	52.9 ± 15.0*	70.9 ± 29.9*	<0.001	52.1 ± 11.0	58.2 ± 15.4	67.4 ± 17.4*	0.007
Sodium (mmol/L)	136.4 ± 1.6	135.8 ± 2.0	136.6 ± 1.9	0.31	135.7 ± 2.0	135.9 ± 2.7	136.0 ± 3.9	0.92
Potassium (mmol/L)	3.9 ± 0.4	4.1 ± 0.3	4.2 ± 0.5*	0.01	3.9 ± 0.3	3.8 ± 0.3	4.2 ± 0.4*	0.04
Total protein (g/L)	65.0 ± 5.2	64.7 ± 4.2	58.3 ± 5.2*	0.003	54.5 ± 0.7	55.7 ± 6.2	54.6 ± 6.6	0.93
Albumin (g/L)	25.67 ± 1.16	26.21 ± 3.08	23.91 ± 3.33	0.16	21.50 ± 0.71	22.0 ± 3.83	21.24 ± 3.80	0.90

Data are presented as mean ± standard deviation. *Significant at $p \leq 0.05$ on comparison with normal pregnancy. RBS - random blood sugar

polymorphism and allele frequencies in the study groups are presented in Tables 4 & 5. The Hardy-Weinberg equilibrium was performed using Pearson's chi² test (degrees of freedom) and all genotype distributions were in accordance with the expected distribution among the control ($p=0.49$), HDP ($p=0.051$), and PE ($p=0.72$) groups. However, it showed deviation in GH cases ($p=0.03$). The genotypic frequencies in HDP patients were GG (58.5%), GC (31.1%) and CC (10.4%), while in the controls it was GG (67%), GC (30.9%) and CC (2.1%). Genotype CC was significantly more frequent in HDP patients than controls ($p=0.03$). Allele C was significantly more frequent than G in HDP patients compared to controls (OR - 1.69, 95% CI - 1.02-2.07). Hence, indicating a significant positive association between allele C of -174 GC of IL-6 SNP and the risk of HDP ($p=0.04$). However, the frequency of allele C did not significantly differ between GH (OR - 1.38, 95% CI - 0.74-3.57, $p=0.27$) and PE patients (OR - 1.44, 95% CI - 0.79-2.63, $p=0.23$) in relation to controls. Studying the genotype distributions in GH showed that GG (57.6%), GC (28.8%) and CC (13.6%), and that

genotype CC was significantly more frequent in GH patients than in controls ($p=0.01$). As for PE patients the distribution was GG (59.6%), GC (34%), and CC (6.4%), and the frequency CC homozygote did not significantly differ from controls ($p=0.19$).

Discussion. Hypertension is one of the most common medical conditions complicating pregnancy, with significant implications on maternal and perinatal morbidity and mortality. However, the etiology of this complex health problem remains undetermined. Impaired maternal immune tolerance plays a major role in the pathogenesis of HDP. In addition, the role of genetic predisposition is well-recognized but not yet defined.^{29,30} The results of our study revealed that -174 GC of IL-6 SNP is significantly associated with increased risk of HDP in Saudi women. This functional SNP has been shown to influence the levels of inflammatory markers; IL-6 and CRP.^{13,22,31,32} Increased concentrations of both inflammatory markers are commonly reported with the risk of HDP.^{16,18,33-38} In this study of -174 GC IL-6 SNP in Saudi population, the genotype distribution in women with normal pregnancy was GG (67%), GC (30.9%), and CC (2.1%). Upon comparison with HDP, CC was significantly ($p<0.03$) more frequent. Also, the C allele was significantly more frequent with HDP ($p<0.04$). Women CC homozygotes had an over 5 folds higher risk of HDP (OR = 5.76; 95% CI = 1.23-27.06) ($p=0.03$) than those with GG genotype. This positive association of -174 GC of IL-6 SNP also manifested with GH but not in PE patients. Similarly, women with CC genotype had over 7 folds higher risk of GH (OR = 7.65; 95% CI = 1.54-38.03) ($p=0.01$) than those with GG genotype. Whereas, no significant difference was found on PE risk between the 3 genotypes ($p=0.19$). This is in keeping with the findings of a recent meta-analysis, reporting on

Table 4 - Distribution of -174 GC of IL-6 genotypes and allele frequencies in hypertensive disorders of pregnancy (HDP) patients and control group of subjects included in a study at the Antenatal Clinic and the Obstetric Ward of King Khalid University Hospital, Riyadh, Kingdom of Saudi Arabia.

Genetic parameter	Controls n (%)	HDP n (%)	P-value	Odds ratio (95% CI)
<i>Genotype</i>				
GG	65 (67.0)	62 (58.5)	Reference	1.00
GC	30 (30.9)	33 (31.1)	0.75	1.15 (0.60-2.21)
CC	2 (2.1)	11 (10.4)	0.03	5.76 (1.23-27.06)
GC + CC	32 (33.0)	44 (41.5)	0.21	1.44 (0.81-2.56)
<i>Alleles</i>				
G	160 (82.5)	157 (74.1)	Reference	
C	34 (17.5)	55 (25.9)	0.04	1.69 (1.02-2.67)

Table 5 - Distribution of -174 GC of IL-6 genotypes and allele frequencies in gestational hypertension (GH) and preeclampsia (PE) patients included in a study at the Antenatal Clinic and the Obstetric Ward of King Khalid University Hospital, Riyadh, Kingdom of Saudi Arabia.

Genetic parameter	Controls (n)	GH (n)	P-value	Odds ratio (95% confidence interval [CI])	PE (n)	P-value	Odds ratio (95% CI)
<i>Genotype</i>							
GG	65	34	Reference	1.00	28	Reference	1.00
GC	30	17	0.83	1.08 (0.52-2.23)	16	0.58	1.23 (0.58-2.62)
CC	2	8	0.01	7.65 (1.54-3.03)	3	0.19	3.48 (0.55-21.99)
GC + CC	32	25 (42.3%)	0.24	0.24 (0.76-2.9)	19 (40.4%)	0.38	1.38 (0.67-2.83)
<i>Alleles</i>							
G	160	85	Reference	Reference	72	Reference	Reference
C	34	25	0.27	1.38 (0.74-3.57)	22	0.23	1.44 (0.79-2.63)

4 studies, where no association between this promoter polymorphism and the risk of PE was detected.²³ Two of the studies were in Caucasians, one Mulatto, and the fourth on a Turkish population.^{33,39-41} In addition, a recent study on Mexican women found no significant impact on the risk of PE.⁴² However, they described a difference in the allele and genotype distributions between the groups of different ethnic descendants. The frequency of the C allele reported in Caucasian women with normal pregnancy ranged from 38.8-49.1%, less in Turkey (29%), and least in Mexicans (8%).

The -174 GC of IL-6 SNP was tested in 2 Arab countries (Egypt and Tunis).^{43,44} The frequency of the C allele in the control groups of these studies were 15.5% (2/3 females) and 50.5% (1/3 females). In spite of the reported difference of -174 GC IL-6 allelic distribution and genotype frequency between ethnic groups, it has been consistently shown not to influence the risk of PE across populations. The limitation of our study include the small numbers in the subgroups and lack of phenotype measurement.

In summary, HDP with all its categories are a heterogeneous condition. Affected women are at an increased risk of cardiovascular morbidity and mortality. Considering that immune imbalance plays an important role in HDP, we showed that the functional promoter SNP -174 GC IL-6 is significantly associated with the risk of GH. Similar to other populations, the SNP had no effect of the risk of PE in Saudi women. Our results showed that women homozygotes for the C allele are more susceptible to GH with over 7 folds increased risk of than GG genotype. However, this positive association could not be detected in PE patients. This suggests that different HDP categories may have different underlying genetic contributors. However, the lack of association between the 174 G/C of IL-6 polymorphism and the risk of PE does not rule out the possibility that this locus plays a role in the its pathogenesis as it may harbor other functional polymorphisms. Further studies are required to determine the genetic predisposition of HDP in different ethnic groups.

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