Available online at www.sciencedirect.com

SciVerse ScienceDirect

Taiwanese Journal of Obstetrics & Gynecology 51 (2012) 240–244

www.tjog-online.com

Short Communication

Interleukin-1β gene is not associated with preeclampsia in Taiwanese

Lin Kang [a,b], Chung-Hwan Chen [c,d,e,f,g], Chen-Hsiang Yu [b], Chiung-Hsin Chang [b,*], Fong-Ming Chang [a,b,*]

[a] *Graduate Institute of Clinical Medicine, College of Medicine, National Cheng Kung University, Tainan City, Taiwan*
[b] *Department of Obstetrics and Gynecology, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan City, Taiwan*
[c] *Department of Orthopaedics, Kaohsiung Medical University Hospital, Kaohsiung City, Taiwan*
[d] *Department of Orthopaedics, Faculty of Medicine, Kaohsiung Medical University, Kaohsiung City, Taiwan*
[e] *Department of Sports Medicine, Faculty of Medicine, Kaohsiung Medical University, Kaohsiung City, Taiwan*
[f] *Orthopaedic Research Center, Kaohsiung Medical University, Kaohsiung City, Taiwan*
[g] *Graduate Institute of Medicine, Kaohsiung Medical University, Kaohsiung City, Taiwan*

Accepted 15 March 2012

Abstract

Objective: To identify associations between the interleukin-1β gene and preeclampsia in Taiwanese women.
Methods and Materials: We genotyped Taiwanese population (102 women with preeclampsia and 148 controls) for two polymorphisms of the interleukin-1β gene (promoter region and Exon 5) by using polymerase chain reaction and restriction fragment length polymorphism analysis. The association between the genotype and disease was examined by Chi-square tests.
Results: We found no association between the two polymorphic sites of interleukin-1β gene and preeclampsia. No significant differences were detected in genotype distributions and allele frequencies of the *Ava*I polymorphism at position −511 in the promoter region and the *Taq*I polymorphism at position +3953 within Exon 5.
Conclusion: Our data do not support a role of the interleukin-1β gene in the pathogenesis of preeclampsia in Taiwanese women.

Keywords: interleukin-1β gene; preeclampsia; polymorphism; Taiwanese

Introduction

Preeclampsia is one of the major and crucial problems in obstetrics [1]. It is characterized by the new onset of hypertension and proteinuria after the 20th week of gestation and occurs in about 6% to 10% of all pregnancies and results in substantial maternal and fetal morbidity and mortality [1]. The underlying etiology of preeclampsia remains unknown even in the 21st century despite extensive research into its pathogenesis. Several pathophysiologic abnormalities such as abnormal trophoblast differentiation and invasion, placental and endothelial dysfunction, immune maladaptation, and exaggerated systemic inflammatory response have been proposed to explain the development of preeclampsia [2,3]. Among them, a genetic component in preeclampsia cannot be neglected. Since 1968, preeclampsia has been proposed to be a genetic disease [4–8]. Chesley and Cooper [5] claimed that a possible single gene may be responsible for preeclampsia. To date, a variety of genes have been tested to be associated with preeclampsia and the results remained controversial. In 1999, Dr Broughton Pipkin advocated that it is unlikely that there is only a single gene and that a cluster of polymorphisms of genes, possibly in conjunction with environmental factors, predispose to the development of preeclampsia [9]. In other words, several candidate genes must be investigated to identify the disease-gene association in preeclampsia.

* Corresponding authors. Department of Obstetrics and Gynecology, National Cheng Kung University Medical College and Hospital, No. 138, Victory Rd., Tainan 704, Taiwan.
E-mail addresses: ahsin@mail.ncku.edu.tw (C.-H. Chang), fchang@mail.ncku.edu.tw (F.-M. Chang).

1028-4559/$ - see front matter
doi:10.1016/j.tjog.2012.04.014

In addition, although the cause of preeclampsia is not known completely, a central pathophysiologic feature is systemic inflammation, which secondarily involves widespread endothelial dysfunction [10,11]. Redman et al [11] considered preeclampsia to be an excessive maternal inflammatory response to pregnancy. Levels of plasma and placental proinflammatory cytokines are higher in pregnant women with preeclampsia, which implies that cytokines might play an important role in the pathogenesis of preeclampsia [12–20]. An exaggerated inflammatory response to pregnancy may occur in genetically susceptible women, such as those who carry cytokine gene polymorphisms that are known to upregulate cytokine production [10–20].

Interleukin-1β (IL-1β) is a proinflammatory cytokine produced by monocytes, macrophages, and epithelial cells and secretion of IL-1β leads to a proinflammatory cascade, including production of tumor necrosis factor α, interferon γ, interleukin-2, and interleukin-12 [10–20]. Many studies have investigated the influence of IL-1β on preeclampsia [10–22]. In preeclampsia, increased placental expression of IL-1β has been observed and is considered to elevate circulating cytokines levels [12–20]. However, in the previous studies examining the relationship between the IL-1β genotypes and preeclampsia, only small sample sizes were used [21], and no significant differences were found [21,22]. To the best of our knowledge, no study had investigated the relationship between IL-1β genotypes and preeclampsia in Taiwanese women. Since the prevalence of polymorphisms varies in different populations, we tested the hypothesis that the polymorphisms within the IL-1β gene are an important risk factor for preeclampsia in Taiwanese women.

Materials and methods

Subjects

This study was approved by the National Cheng Kung University Hospital (NCKUH) Institutional Review Board (IRB No: HR-95-43 and HR-100-066), and all participants gave written informed consent.

From August 2006 to July 2011 at the Department of Obstetrics and Gynecology of NCKUH, we retrospectively collected 102 cases of severe preeclampsia, HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet count), or eclampsia meeting the criteria of the American College of Obstetricians and Gynecologists [23,24], and another 148 normal controls. They all delivered at the Department of Obstetrics and Gynecology of NCKUH, between January 2006 and December 2010. Patients with severe preeclampsia, HELLP syndrome, or eclampsia were collected from the medical records of NCKUH. They were called back and asked to sign an informed consent at that time. During the same time, blood samples were collected from the normal controls who had given birth in NCKUH after uncomplicated pregnancies. All study participants were matched for maternal age within 5 years, gestational age, parity, and fetal sex. None of the normal controls had clinical signs of preeclampsia or other medical or pregnancy complications. None of the study participants were in labor at the time of blood sampling. Both groups were ethnically Taiwanese and were enrolled by random selection in this study.

Preeclampsia was defined as the development of hypertension that occurred in a pregnant woman known to be normotensive previously after 20 weeks of gestation, accompanied by new-onset proteinuria [3,23,24]. Hypertension was defined as a systolic blood pressure $\geq$140 mmHg or a diastolic blood pressure $\geq$90 mmHg on at least two occasions and 4 to 6 hours apart after the 20th week of gestation in women known to be normotensive previously [23,24]. Proteinuria was defined as 300 mg or more protein excretion in 24-hour urine collection, or protein concentration of 300 mg/L or higher in urine ($\geq$1+ on dipstick) [3,23,24]. Severe preeclampsia was diagnosed if one or more of the following were present: blood pressure of 160/110 mmHg or higher, excretion of 5 g or more of protein in a 24-hour urine sample, the presence of multiorgan involvement such as oliguria, pulmonary edema, visual or cerebral disturbance, pain in the epigastric area or right upper quadrant, abnormal liver enzymes, and thrombocytopenia (platelet count $<100 \times 10^9$/L) [3,23,24]. To diagnose HELLP syndrome, the following criteria had to be met: hemolysis, defined by increased lactic dehydrogenase ($\geq$600 IU/L); elevated liver enzyme levels (aspartate aminotransferase and alanine aminotransferase $\geq$70 IU/L); and thrombocytopenia [3,23,24]. Eclampsia was defined as the onset of convulsion in a woman with preeclampsia [3,23,24]. Gestational age was based on the precise date of the last menstrual period and/or ultrasound measurement of the crown-rump length in the first trimester. Women who met preeclampsia criteria but not severe preeclampsia were excluded from the study. Other exclusion criteria were as follows: multiple pregnancies, premature rupture of membranes, chorioamnionitis, chronic hypertension, diabetes mellitus, autoimmune disorders or fetal abnormalities.

Genetic studies

The methods described by Hefler et al [21] were used to examine polymorphisms of the IL-1β gene (promoter region and Exon 5). The IL-1βgene is located on the long arm of Chromosome 2 at position 2q12-14 [22]. Two di-allelic polymorphisms located in the IL-1β gene were checked: the *Ava*I polymorphism at position −511 in the promoter region and the *Taq*I polymorphism at position +3953 within Exon 5 [22]. Blood samples were obtained from a peripheral vein and then DNA was extracted from blood using the Puregene DNA Purification Kit (Gentra Systems, Inc. Minneapolis, MN, USA) and stored at 4 °C until analyzed [21]. The *Ava*I polymorphism at position −511 in the promoter region of IL-1β gene was searched by using a polymerase chain reaction (PCR) strategy as published by di Giovine et al [25]. For testing the *Taq*I polymorphism at position +3953 within Exon 5 of the IL-1β gene, PCR amplification was followed by restriction fragment length polymorphism analysis as previously described [26,27]. The target DNA sequence at position −511 in the IL1-β gene promoter was amplified using

primer 5′-TGG CAT TGA TCT GGT TCA TC-3′ and reverse primer 5′-GTT TAG GAA TCT TCC CAC TT-3′ [21]. The target DNA sequence in the IL1-β gene Exon 5 was amplified using primer 5′-GTT GTC ATC AGA CTT TGA CC-3′ and reverse primer 5′-TTC AGT TCA TAT GGA CCA GA-3′ [21]. For new mutations or variations in the PCR products of the IL-1β gene promoter and the IL-1β gene Exon 5, single strand conformation polymorphism (SSCP) and denaturing gradient gel electrophoresis (DGGE) were used [28]. We also did direct sequencing for the representative samples or equivocal results detected by SSCP and DGGE as described by Chang and Kidd [29].

Statistical analysis

Genotype and allele frequencies were compared by χ^2 testing, using the statistical package SPSS (SPSS Inc, Chicago, Illinois, USA). Yates correction of continuity was used when an observed number was ≤5. A *p-value* < 0.05 was considered statistically significant.

Results

Genotype distributions and allele frequencies of the *Ava*I polymorphism at position −511 in the promoter region of the IL-1β gene among women with severe preeclampsia and normal controls are given in Tables 1 and 2. Genotype was determined in the 102 women with severe preeclampsia (including HELLP syndrome and eclampsia) and 148 normal controls. Overall, three genotypes (C/C, C/T, T/T) and two alleles (C, T) were seen. The frequency of homozygotes (T/T) was 35.3% (36 of 102) in women with severe preeclampsia group and 25.0% (37 of 148) in normal controls. The frequency of T alleles was 55.9% (114 of 204 alleles) in women with severe preeclampsia group and 48.6% (144 of 296 alleles) in normal controls. For the *Ava*I polymorphism at position −511, neither genotype distributions nor allele frequencies showed statistically significant differences among the women with severe preeclampsia and normal controls.

Genotype distributions and allele frequencies of the *Taq*I polymorphism at position +3953 within Exon 5 of the IL-1β gene among women with severe preeclampsia and normal controls are given in Tables 3 and 4. Genotyping data for this polymorphism were only available in the 95 women with severe preeclampsia (including HELLP syndrome and eclampsia) and 144 normal controls. For 11 women, the PCR was unsuccessful after repeated attempts when testing the *Taq*I polymorphism at position +3953. Overall, two genotypes (C/C, C/T) and two alleles (C, T) were seen. The homozygotes (T/T) could not be detected in both severe preeclampsia and control groups among Taiwanese women. The allele frequency of T alleles was 2.1% (4 of 190 alleles) in the women with severe preeclampsia and 1.7% (5 of 288 alleles) in normal controls. No statistically significant differences were observed in genotype distributions and allele frequencies of the *Taq*I polymorphism at position +3953 among women with severe preeclampsia and normal controls.

Table 1
Genotype distributions of the *Ava*I polymorphism at position −511 in the promoter region of IL-1β gene among women with severe preeclampsia and normal controls.

	Numbers of cases (*n*)	Genotype distributions			χ^2	*p*-value
	n	CC	CT	TT		
Normal controls	148	41 (27.7%)	70 (47.3%)	37 (25.0%)	3.1	0.21
Women with severe preeclampsia[a]	102	24 (23.5%)	42 (41.2%)	36 (35.3%)		

[a] Women with HELLP syndrome or eclampsia were also included.

Table 2
Allele frequencies of the *Ava*I polymorphism at position −511 in the promoter region of IL-1β gene among women with severe preeclampsia and normal controls.

	Numbers of alleles (2*n*)	Allele frequencies		χ^2	*p*-value
	2*n*	C	T		
Normal controls	296	152 (51.4%)	144 (48.6%)	2.53	0.11
Women with severe preeclampsia[a]	204	90 (44.1%)	114 (55.9%)		

[a] Women with HELLP syndrome or eclampsia were also included.

Discussion

Since preeclampsia implies a genetic susceptibility [4–9], it is of interest to investigate which specific genetic loci are involved. Determination of such genetic factors would assist in identifying women at risk. In the present study, we did not find an association between the polymorphisms of IL-1β gene and preeclampsia, especially severe preeclampsia. Our study is the first investigating for the association between the polymorphisms of IL-1β gene and preeclampsia in Taiwanese women. Because the ethnic difference might play an important role in disease-gene association studies [30], we cannot adopt the conclusion from other countries without our own studies in the Taiwanese population. From our results, we can rule out the role of IL-1β gene polymorphisms in the pathogenesis of preeclampsia in Taiwanese women. Our conclusions were similar to previous studies in Hispanic and Dutch populations described by Helfer et al and Lachmeijer et al, which

Table 3
Genotype distributions of the *Taq*I polymorphism at position +3953 within exon 5 of IL-1β gene among women with severe preeclampsia and normal controls.

	Number of cases (*n*)	Genotype distributions			χ^2	*p*-value
	n	CC	CT	TT		
Normal controls	144	139 (96.5%)	5 (3.5%)	0 (0.0%)	0.53	0.77
Women with severe preeclampsia[a]	95	91 (95.8%)	4 (4.2%)	0 (0.0%)		

[a] Women with HELLP syndrome or eclampsia were also included.

Table 4
Allele frequencies of the *Taq*I polymorphism at position +3953 within exon 5 of IL-1β gene among women with severe preeclampsia and normal controls.

	Number of alleles (2*n*)	Allele number (frequency)		χ^2	*p*-value
	2*n*	C	T		
Normal controls	288	283 (98.3%)	5 (1.7%)	0.09	0.77
Women with severe preeclampsia[a]	190	186 (97.9%)	4 (2.1%)		

[a] Women with HELLP syndrome or eclampsia were also included.

demonstrated that the two polymorphisms at promoter region and Exon 5 of the IL-1β gene were not associated with the risk for preeclampsia [21,22].

There are several possible hypotheses that could explain our results. One explanation is the definition of pregnancy complications. For example, the severity of preeclampsia might affect the results [31]. The diagnostic criteria for preeclampsia used in our study were the same as previous studies investigating for the role of IL-1β gene polymorphisms in the pathogenesis of preeclampsia [21,22]. In our present study, women who met preeclampsia criteria but not severe preeclampsia were excluded from the study. In other studies, women with mild preeclampsia were included by Helfer et al [21], while Lachmeijer et al recruited women with preeclampsia, severe preeclampsia, HELLP syndrome, and eclampsia in the study group [22]. However, all the results showed that polymorphisms of the IL-1β gene promoter and Exon 5 were not associated with the risk for preeclampsia.

Another explanation for the negative results is the difference in prevalence of IL-1β gene polymorphisms and preclampsia in different populations. When the prevalence of IL1-β gene polymorphisms is very low in the population, the relatively small sample size increases the likelihood of a type II error [31]. In our results, especially the genotype distributions and allele frequencies of the *Taq*I polymorphism at position +3953 within Exon 5 of the IL1-β gene among women with severe preeclampsia and normal controls, the homozygotes (T/T) could not be observed in both severe preeclampsia and control groups and the allele frequency of T alleles was only 2.1% in women with severe preeclampsia group and 1.7% in normal controls, even though genotyping data for this polymorphism were available in the 95 women with severe preeclampsia and 144 normal controls. The prevalence of IL-1β gene polymorphisms is much lower in Taiwanese women when compared with Hispanic and Dutch populations [21,22]. It would be much more challenging to recruit more study patients to test the relationship between the polymorphisms within the IL-1β gene and the risk for preeclampsia in the Taiwanese women.

Another reason for our negative results could be the complex interactions among genetic and environmental factors [32]. While preeclampsia is generally considered to be a multifactorial disease, environmental risk factors for preeclampsia pathophysiology, such as diet, obesity, stress, smoking, and other social elements during pregnancy may influence the development of preeclampsia and these environmental factors may be very different among races [33]. A limitation of our study is that we did not analyze the interaction and co-morbidity among environmental risk factors, polymorphisms of IL-1β gene and preeclampsia.

In conclusion, preeclampsia has an inherited component likely to involve many genes [31]. In this series, we observed that polymorphisms of the IL-1β gene do not play a major role in the development of preeclampsia in the Taiwanese women. Multilocus or haplotype analysis, rather than a single locus study, might be a preferable strategy in future investigations to obtain much more accurate results between genetic susceptibility and preeclampsia.

Acknowledgments

We are grateful to Mr. Ming-Hui Chen for his assistance in this study. This study was supported in part by grants from the National Science Council, Executive Yuan, Taipei, Taiwan.

References

[1] Sibai BM. Diagnosis and management of gestational hypertension-preeclampsia. Obstet Gynecol 2003;102:181–92.
[2] Sibai BM. Hypertensive disorders of pregnancy: the United States perspective. Curr Opin Obstet Gynecol 2008;20:102–6.
[3] Sibai BM, Dekker G, Kupferminc M. Pre-eclampsia. Lancet 2005;365:785–99.
[4] Chesley LC, Annitto JE, Cosgrove RA. The familial factor in toxemia of pregnancy. Obstet Gynecol 1968;32:303–11.
[5] Chesley LC. Hypertension in pregnancy: definitions, familial factor, and remote prognosis. Kidney Internat 1980;18:234–40.
[6] Chesley LC, Cooper DW. Genetics of hypertension in pregnancy: possible single gene control of pre-eclampsia and eclampsia in descendants of eclamptic women. Br J Obstet Gynaecol 1986;93:898–908.
[7] Williams RR, Hunt SC, Hopkins PN, Hasstedt SJ, Wu LL, Lalouel JM. Tabulations and expectations regarding the genetics of human hypertension. Kidney Internat 1994;44:S57–64.
[8] Williams RR, Hunt SC, Hopkins PN, Wu LL, Lalouel JM. Evidence for single gene contributions to hypertension and lipid disturbances: definition, genetics, and clinical significance. Clin Genet 1994;46:80–7.
[9] Broughton Pipkin F. What is the place of genetics in the pathogenesis of pre-eclampsia? Biol Neonate 1999;76:325–30.
[10] Redman CW, Sacks GP, Sargent IL. Preeclampsia: an excessive maternal inflammatory response to pregnancy. Am J Obstet Gynecol 1999;180:499–506.
[11] Haggerty CL, Ferrell RE, Hubel CA, Markovic N, Harger G, Ness RB. Association between allelic variants in cytokine genes and preeclampsia. Am J Obstet Gynecol 2005;193:209–15.
[12] Benyo DF, Smarason A, Redman CW, Sims C, Conrad KP. Expression of inflammatory cytokines in placentas from women with preeclampsia. J Clin Endocrinol Metab 2001;86:2505–12.
[13] Sanchez SE, Zhang C, Williams MA, Ware-Jauregui S, Larrabure G, Bazul V, et al. Tumor necrosis factor-alpha soluble receptor p55 (sTNFp55) and risk of preeclampsia in Peruvian women. J Reprod Immunol 2000;47:49–63.
[14] Rinehart BK, Terrone DA, Lagoo-Deenadayalan S, Barber WH, Hale EA, Martin Jr JN, et al. Expression of the placental cytokines tumor necrosis factor alpha, interleukin 1beta, and interleukin 10 is increased in preeclampsia. Am J Obstet Gynecol 1999;181:915–20.
[15] Visser W, Beckmann I, Knook MA, Wallenburg HC. Soluble tumor necrosis factor receptor II and soluble cell adhesion molecule 1 as markers of tumor necrosis factor-alpha release in preeclampsia. Acta Obstet Gynecol Scand 2002;81:713–9.

[16] Teran E, Escudero C, Moya W, Flores M, Vallance P, Lopez-Jaramillo P. Elevated C-reactive protein and pro-inflammatory cytokines in Andean women with preeclampsia. Int J Gynaecol Obstet 2001;75:243–9.
[17] Velzing-Aarts FV, Muskiet FA, van der Dijs FP, Duits AJ. High serum interleukin-8 levels in afro-caribbean women with pre-eclampsia. Relations with tumor necrosis factor-alpha, duffy negative phenotype and von Willebrand factor. Am J Reprod Immunol 2002;48:319–22.
[18] Williams MA, Farrand A, Mittendorf R, Sorensen TK, Zingheim RW, O'Reilly GC, et al. Maternal second trimester serum tumor necrosis factor-alpha-soluble receptor p55 (sTNFp55) and subsequent risk of preeclampsia. Am J Epidemiol 1999;149:323–9.
[19] Mulla MJ, Myrtolli K, Potter J, Boeras C, Kavathas PB, Sfakianaki AK, et al. Uric acid induces trophoblast IL-1β production via the inflammasome: implications for the pathogenesis of preeclampsia. Am J Reprod Immunol 2011;65:542–8.
[20] Huang SJ, Chen CP, Schatz F, Rahman M, Abrahams VM, Lockwood CJ. Pre-eclampsia is associated with dendritic cell recruitment into the uterine decidua. J Pathol 2008;214:328–36.
[21] Hefler LA, Temper CB, Gregg AR. Polymorphisms within the interleukin-1beta gene cluster and preeclampsia. Obstet Gynecol 2001;97:664–8.
[22] Lachmeijer AM, Nosti-Escanilla MP, Bastiaans EB, Pals G, Sandkuijl LA, Kostense PJ, et al. Linkage and association studies of IL1B and IL1RN gene polymorphisms in preeclampsia. Hypertens Pregnancy 2002;21:23–38.
[23] American College of Obstetricians and Gynecologists. National high blood pressure education program working group report on high blood pressure in pregnancy. Am J Obstet Gynecol 1990;163:1689–712.
[24] American College of Obstetricians and Gynecologists. Report of the national high blood pressure education program working group on high blood pressure in pregnancy. Am J Obstet Gynecol 2000;183:S1–22.
[25] Di Giovine FS, Takhsh E, Blakemore AI, Duff GW. Single base polymorphism at −511 in the human interleukin-1 beta gene (IL1 beta). Hum Mol Genet 1992;1:450.
[26] Pociot F, Mølvig J, Wogensen L, Worsaae H, Nerup J. A TaqI polymorphism in the human interleukin-1 beta (IL-1 beta) gene correlates with IL-1 beta secretion in vitro. Eur J Clin Invest 1992;22:396–402.
[27] Bioque G, Crusius JB, Koutroubakis I, Bouma G, Kostense PJ, Meuwissen SG, et al. Allelic polymorphism in IL-1 beta and IL-1 receptor antagonist (IL-1Ra) genes in inflammatory bowel disease. Clin Exp Immunol 1995;102:379–83.
[28] Ruano G, Kidd KK. Modeling of heteroduplex formation during PCR from mixtures of DNA templates. PCR Methods Appl 1992;2:112–6.
[29] Chang FM, Kidd KK. Rapid molecular haplotyping of the first exon of the human dopamine D4 receptor gene by the heteroduplex analysis. Am J Med Genet 1997;74:91–4.
[30] Gard PR. Implications of the angiotensin converting enzyme gene insertion/deletion polymorphism in health and disease: a snapshot review. Int J Mol Epidemiol Genet 2010;1:145–57.
[31] Jääskeläinen E, Keski-Nisula L, Toivonen S, Romppanen EL, Helisalmi S, Punnonen K, et al. MTHFR C677T polymorphism is not associated with placental abruption or preeclampsia in Finnish women. Hypertens Pregnancy 2006;25:73–80.
[32] Roberts CB, Rom L, Moodley J, Pegoraro RJ. Hypertension-related gene polymorphisms in pre-eclampsia, eclampsia and gestational hypertension in Black South African women. J Hypertens 2004;22:945–8.
[33] Kim YJ, Park MH, Park HS, Lee KS, Ha EH, Pang MG. Associations of polymorphisms of the angiotensinogen M235 polymorphism and angiotensin-converting-enzyme intron 16 insertion/deletion polymorphism with preeclampsia in Korean women. Eur J Obstet Gynecol Reprod Biol 2004;116:48–53.