Gineco.eu [14] 135-140 [2018]
DOI: 10.18643/gieu.2018.135

The role of estrogen receptor α, COL3A1, and fibulin-5 genes polymorphisms as risk factors for pelvic organ prolapse in Balinese women

I. Gede Mega Putra[1], Ida Bagus Amertha Putra Manuaba[2,3], Anak Agung Gde Marvy Khrisna Pranamartha[4], Agha Bhargah[5]

1. Urogynecology and Reconstructive Division of Obstetrics and Gynecology Department, Faculty of Medicine, Udayana University, Sanglah General Hospital, Denpasar, Bali-Indonesia

2. PhD Student, International Program in Medicine, College of Medicine, Taipei Medical University, Taiwan

3. Medical and Health Education Development, Faculty of Medicine, Udayana University, Bali, Indonesia

4. Master of Hospital Administration, Medical Faculty, Indonesia University

5. Faculty of Medicine, Udayana University, Bali-Indonesia

Correspondence:
Dr. I Gede Mega Putra
E-mail: megaputra@unud.ac.id

Abstract

Introduction. *Pelvic organ prolapse (POP) is a common problem in women, though not life-threatening, it gives negative impacts on the patient's quality of life. Balinese women are known to have clinical risk factors for POP including multiple pregnancies and childbirth (multiparity). The existence of genetic variation, estrogen receptor α and extracellular matrix genes polymorphisms, are thought to be genetic risk factors.* ***Methods.*** *A paired case-control study on Balinese women was conducted in Sanglah Hospital, Prima Medika Hospital Denpasar, and Integrated Biomedical Laboratory to prove that estrogen receptor α, col3a1, and fibulin-5 genes polymorphisms as risk factors for POP. About 30 women with POP as the case group and 30 women without POP as a control group, matched according to parity and work variables. Three milliliters of venous blood sample was drawn for deoxyribonucleic acid isolation, polymerase chain reaction, and sequencing to determine the presence of genetic variation in the form of estrogen receptor α, col3a1, and/or fibulin-5 genes polymorphism.* ***Results.*** *Estrogen receptor α gene polymorphism in Balinese women increased the risk of POP to 5.5 times higher (p= 0.022) and 3.25 times higher in Col3A1 gene polymorphism (p= 0.049). It was also found that fibulin-5 gene polymorphism was not represented a risk factor for POP (p = 0.774).* ***Conclusions.*** *It was concluded that estrogen receptor α and Col3A1 gene polymorphisms are represented risk factors for POP in Balinese women.*

Keywords: *estrogen receptor α, col3a1, and fibulin-5 genes polymorphisms, pelvic organ prolapse*

Introduction

Pelvic organ prolapse (POP) has been a common public health problem in women during the last two decades. Its incidence is increasing along with the increasing life expectancy of a woman. Even though it's not a non-life-threatening condition, its impact on woman's quality of life is detrimental[1].

From a hospital-based study, POP was found in approximately 43-76% of gynecologic patients, and 11% of the cases needed surgical intervention. Thirty percent of patients who underwent surgery suffered recurrence[1]. One study in England in 2001 reported that 2 out of 1000 adult women underwent prolapse repair surgery annually[2]. Some other studies, using POP quantification (POP-Q) as a standard diagnostic tool, reported POP prevalence as high as 23.5%-49.4%[3]. The prevalence of POP, in general, imitates the iceberg phenomenon. The incidences reported by researchers and clinicians are only comprised of severe cases which come to the clinic, due to the need for treatment.

POP prevalence data in Indonesia has not been established. Obstetrics and Gynecology Division of Medical Faculty of Udayana University/Sanglah General Hospital Denpasar in 2009, recorded 82 POP cases visit, of which 34 cases underwent surgery. A total of 37.32% of those with POP experienced urinary incontinence[4]. In 2015, the center recorded higher POP patient visit; total 91 cases, 36 cases underwent surgery. Most cases of POP who visited Urogynecologic Reconstruction Clinic of Sanglah Hospital were of Balinese Tribe (83 patients, 91.20%).

The exact cause of POP is unknown, but it is always associated with weakness of the pelvic floor support structure. Pregnancy and childbirth are considered as major risk factors for POP, although it is not clear how the disruption of pelvic floor function happens during pregnancy and childbirth. In fact, POP can also occur in women who have never been pregnant and do not always happen in multiparous women[5]. Risk factors of POP are classified into extrinsic factors, such as pregnancy and childbirth, occupational history, diseases leading to a continuous increase in intra-abdominal pressure, and history of hysterectomy. Intrinsic factors include a genetic factor, aging process, extracellular matrix changes in pelvic support and menopause.

Understanding the pathogenesis of POP can provide an overview of prevention and treatment of this female abnormality. The pathogenesis of POP has

Received:
October 04, 2018
Revised:
October 25, 2018
Accepted:
November 27, 2018

been widely described, but there is little knowledge about the tissue histomorphology of pelvic floor. Several studies have found an association between POP and low collagen expression, mainly type I collagen. Increased expression of type III collagen in POP is considered as a result of remodeling process after tissue trauma. In the Sacro uterine ligament of POP patients also found a decrease in the ratio of type I and type III collagen. There was also a decline in the expression of elastin and proteins involved in elastin metabolisms, such as fibulin-5 and lysyl oxidase-like 1 (LOXL1). Decreased expression of estrogen receptor in the pelvic floor support tissue was also found to be associated with POP[6].

The polymorphism of the type I collagen gene in the Col1A1 gene reported to be unrelated to the incidence of POP in Russian women[7]. Similar result was also reported in a Brazilian study[8]. Studies in Korea, Taiwan, and the Netherlands have different results on collagen III gene polymorphism in the Col3A1 gene. The three studies reported an association between col3A1 gene polymorphism on POP[9]. Huey et al. reported that type III collagen gene polymorphism, the GA type genotype Col3A1 rs1800255 has a significant relationship with the incidence of POP in Taiwan women[10].

In addition to collagen gene polymorphism, POP is also found to be associated with polymorphisms of proteins involved in elastin synthesis, i.e. fibulin-5. Research by Khadzieva and contributors found a significant association between the polymorphisms of the fibers-5 gene pads (1818736 (C/A) with the incidence of POP[11]. Estrogen a receptor gene polymorphism in Taiwanese women was also found to be associated with POP incidence, i.e. ESR1 rs2228480 gene (exon 8 G/A) with an odds ratio of 2.05[12].

These different results may be influenced by the ethnic factors of the subjects; there has been no research reporting on estrogen receptor, collagen, elastin gene polymorphisms or other protein-related polymorphisms in association with the risk of POP in Indonesian and Balinese women. The absence of research into the association of these genes polymorphisms with the risk of POP in Indonesian and Balinese women has led to unsatisfactory progress on understanding the genetic pathology of POP.

If the polymorphisms of the estrogen receptor, Col3A1, and the fibulin-5 gene and their association with the risk of POP incidence in Balinese women can be explained, it is highly expected that the prevention strategy on incidence and disease progression in Bali can be improved in the future.

Methods

This observational case-control study was achieved at Reconstructive Urogynecology clinic, Obstetric and Gynecology clinic of Sanglah Hospital, Prima Medika Public Hospital Denpasar, and Integrated Biomedical Laboratory of Medical Faculty of Udayana University, Denpasar. The study was conducted on 60 patients, 30 women with POP as the case group and 30 women without POP as a control group, from July 2016 to January 2017. Samples were Balinese women aged 30-75 years old, who visited Sanglah General Hospital and Prima Medika Hospital Denpasar. Samples were diagnosed for POP or not by Obstetrics and Gynecologist specialized in Reconstructive Urogynecology. Controls were other non-prolapse gynecologic patients. To decreased selection bias or confounding bias between cases and controls, variable matching on parity and occupation was done, resulting in 1:1 ratio. Samples were selected consecutively from the reachable population. Patients who were pregnant and suffered malignancy excluded. POP in this study was POP grade II-IV according to POPQ standard, while non-prolapse was POP grade 0-1 according to POPQ standard. Polymorphisms studied were estrogen receptor α gene on rs2228480, Col3A1 gene on rs1800255, and fibulin-5 gene on rs2018736.

Samples for estrogen receptor α, Col3A1, and fibulin-5 genes polymorphism examination were obtained from 3 ml venous blood collected in EDTA containing tubes and was sent to Integrated Biomedical Laboratory of Udayana University for deoxyribonucleic acid isolation and polymerase chain reaction (PCR). Estrogen receptor α gene with ESR1 rs2228480 (exon 8 G/A) polymorphism was amplified by PCR using forward primer: 5' GCTCTACTTCATCGCATTCC-3' and reverse primer 5'CCACTAAGAACTGAGCAAGC-3'. The Amplification of 238 bp long fragment by PCR was done using PCR kit from Promega. Type III collagen with COL3A1 rs1800255 (exon 30 G>A) polymorphism was amplified by PCR, using forward primer: 5' TCCTCTTTCTCCAGGCATTC-3' and reverse primer 5'TTTGTTCACAGGGTGATGCTG-3'. Amplification of 270 bp long fragment was done with PCR using PCR kit from Promega. Fibulin-5 gene with polymorphism on rs2018736 (C/A) was amplified by PCR using forward primer: 5'CTGCCTTCCTCACTGGAGAC-3' and reverse primer 5'GTCACACCCACAAACAGTGC-3'. Amplification of its 221 bp long fragment was done with PCR using PCR Kit from Promega. Electrophoresis using agarose gel 1% was done to evaluate PCR success. Sequential technique was used to assess ESR1 rs2228480 (exon 8 G/A), Col3A1 rs1800255 (exon 30 G>A) and Fibulin-5 (FBLN5) genes polymorphisms.

Statistical analysis in this study using SPSS version 21.0 for windows, chi-square test was used to determine the association between gene polymorphism, and calculation of ORs and logistic regression to determine the magnitude of the risk arising from the polymorphism that occurred.

Results

In this case-control study, a comparison of study subject characteristics between case group (POP) and control group (non-prolapse) is presented in Table 1. Body mass index, age, menopausal status and history of hysterectomy variables between case and control groups were not significantly different.

Table 1 Characteristics distribution of study subjects

Risk Factors	Case Group (n= 30) Frequency n (%)	Control Group (n= 30) Frequency n (%)
Body mass index		
Lean	1(3.33)	0(0)
Normal	20(66.67)	22(73.33)
Obese	9(30.00)	8(26.67)
Age (mean ± SD)	57.67±9.764	56.37±9.633
Menopausal status		
Pre-menopause	6(20.00)	7(23.33)
Menopause	24(80.00)	23(76.67)
History of hysterectomy		
Yes	1(3.33)	1(3.33)
Never	29(96.67)	29(96.67)

Table 2 ER α, Col3A1, and fibulin-5 genes polymorphisms distribution

Genotype	a	b	c	d	*p*-value
ER α(GG) Gene	11	4	11	4	1.000
ER α(AA) Gene	0	2	4	24	0.572
ER α(GA) Gene	2	11	2	15	0.022
Col3A1 (GG) Gene	15	5	7	3	0.774
Col3A1 (GA) Gene	5	13	4	18	0.049
Fib-5 (CC) Gene	3	3	9	18	0.583
Fib-5 (CA) Gene	3	7	5	15	0.774
Fib-5 (AA) Gene	3	3	6	18	0.240

Complete results of estrogen receptor α, Col3A1, and fibulin-5 genes polymorphisms found in this study are displayed in Table 2.

Chi-square test was performed to determine the role of estrogen receptor α as a risk factor of POP. Estrogen receptor α gene polymorphism contributes as a risk factor for POP as much as 5.5 times (OR = 5.50; CI 95% = 1.22-11.99; p = 0.022), see the results in Table 3.

Table 4 shows that the estrogen receptor α gene polymorphism is a risk factor for POP after variables are controlled by multivariate analysis using conditional logistic regression. Estrogen receptor gene polymorphism, after the four variables are controlled, presents 8.33 times greater risk for POP with this multivariate analysis (adjusted OR = 8.33; 95% CI = 1.84 -37.72; p = 0.006).

Table 5 shows that Col3A1 gene polymorphism exposes to 3.25 times greater risk for POP (OR = 3.25; CI 95%=1.06-9.97; p=0.049).

As shown in Table 6, Col3A1 gene polymorphism is still to be a risk factor for POP and are statistically significant (p=0.014), variables were controlled by multivariate analysis using conditional logistic regression. ColA31 gene polymorphism, after variables were controlled, pose 4.26 times greater risk for POP in multivariate analysis (OR=4.26; 95% CI=1.34-13.60; p=0.014).

The polymorphism of the Fibulin-5 gene poses a POP risk of 1.4 times greater (OR=1.40), though this result was not statistically significant (CI 95%=0.44-4.41; p=0.774; Table 7).

Table 3 Estrogen receptor α gene polymorphism as POP risk factor

			Control Group		OR	CI 95%	*p*-value
			ER α Gene Polymorphism				
			(+)	(-)			
Case Group	ER α Gene Polymorphism	(+)	2	11	5.50	1.22-11.99	0.022
		(-)	2	15			

Table 4 The relationship between estrogen receptor α gene polymorphism and POP after controlling variables (BMI, age, menopause, and history of hysterectomy)

	Adjusted OR	CI 95%	*p*-value
ER α gene polymorphism	8.33	1.84-37.72	0.006
BMI (obese/normal/lean)	0.95	0.87-1.04	0.301
Age (year)	1.17	0.37-3.76	0.790
Menopause (yes/no)	0.80	0.09-6.75	0.837
History of hysterectomy (yes/never)	0.59	0.02-16.63	0.759

Table 5 Col3A1 gene polymorphism as POP risk factor

			Control Group		OR	CI 95%	*p*-value
			Co13A1 Gene Polymorphism				
			(+)	(-)			
Case Group	Co13A1 Gene Polymorphism	(+)	5	13	3.25	1.06-9.97	0.049
		(-)	4	8			

Table 6 The relationship between Col3A1 gene polymorphism and POP after variable control (BMI, age, menopause, and history of hysterectomy)

	Adjusted OR	CI 95%	*p*-value
Col3A1 gene polymorphism	4.26	1.34-13.60	0.014
BMI (obese/normal/lean)	1.14	0.36-3.60	0.823
Age (year)	0.98	0.89-1.07	0.601
Menopause (yes/no)	0.81	0.11-6.00	0.834
History of hysterectomy (yes/never)	0.42	0.02-8.26	0.565

The polymorphism of the fibulin-5 gene was not a significant risk factor for the occurrence of POP (p=0.051) after being adjusted for controlled variables by multivariate analysis using conditional logistic regression test. Polygenic polymorphism of fibulin-5, after controlled variables adjustment poses the risk for POP 1.48 times with this multivariate analysis (adjusted OR=1.48; 95% CI=0.47-4.63; p=0.051; Table 8).

The polymorphism of estrogen receptor gene α rs2228480 and Col3A1 rs1800255 gene were risk factors for POP in Balinese women after chi-square test was performed and after adjustment of controlled variables (BMI, age, menopause, and history of hysterectomy).

Table 7 Fibulin-5 gene polymorphism as POP risk factor

			Control Group		OR	CI 95%	p-value
			Fibulin-5 Gene Polymorphism				
			(+)	(-)			
Case Group	Fibulin-5 Gene Polymorphism	(+)	3	7	1.40	0.44-4.41	0.774
		(-)	3	15			

Table 8 The relationship between Fibulin-5 gene polymorphism and POP after variable control (BMI, age, menopause, and history of hysterectomy)

	Adjusted OR	CI 95%	*p*-value
Fibulin-5 gene polymorphism	1.48	0.47-4.63	0.501
BMI (obese/normal/lean)	1.06	0.36-3.15	0.920
Age (year)	0.98	0.98-1.06	0.586
Menopause (yes/no)	1.16	0.18-7.55	0.880
History of hysterectomy (yes/never)	0.97	0.05-17.69	0.985

Table 9 Comparison of OR for POP risk factors before and after controlled variables adjustment (BMI, age, menopause, and history of hysterectomy)

	Adjusted OR	Adjusted RO
ER α gene polymorphism	5.50	8.33
Col3A1 gene polymorphism	3.25	4.26
Fibulin-5 gene polymorphism	1.40	1.48

Other risk factors studied (fibulin-5 rs2018736 gene polymorphism) did not appear to be a risk factor for POP in Balinese women, using chi-square test and controlled variables adjustment. The OR of all three risk factors, before and after variables are controlled, can be seen in Table 9.

Discussion

In this study, the genotypes analyzed and discussed as risk factors were heterozygous genotypes, by the result that has been found in previous studies. From literature review conducted on similar studies, it was found that from the polymorphisms of the three genes, heterozygote genotypes (GA genotypes in ER α and Col3A1 genes polymorphisms) had been proved to be a POP risk factor(10,12). Heterozygous genotype of CA in the fibular-5 gene was found to be a risk factor for POP(11). A screening test for POP, by examining estrogen α rs2228480 and Col3A1 rs1800255 genes polymorphisms on Balinese women who have never suffered prolapse can be considered. Further research needs to be conducted to study the role of estrogen receptor α rs2228480, Col3A1 rs1800255 and fibulin-5 rs2018736 gene polymorphism as risk factors for prolapse in another ethnicity in Indonesia. Furthermore, more research needs to be conducted to study the role of fibulin-5 gene polymorphism as a risk factor of POP using other SNPs labels.

Conclusions

Estrogen α rs2228480 gene polymorphism and Col3A1 rs1800255 gene polymorphism (collagen III genes) were shown to be the risk factors for POP in Balinese women. Estrogen receptor α gene polymorphism represent the most dominant risk factor in the mechanism of genetic variations impact on POP incidence in Balinese women. However, Fibulin-5 rs2018736 gene polymorphism was not showed to be a risk factor for POP in Balinese women. ■

Funding: *This research doesn't receive any specific grant from the government or any private sectors.*

Conflict of interests: The authors declare no conflict of interests.

References

1. Jelovsek JE, Maher C, Baber MD. Pelvic Organ Prolapse. *Lancet.* 2007, 369, 27-38.
2. Anne MW, Linda B, Joseph S. An overview of pelvic organ prolapsed. Office Urogynecology. New York: Mc Graw Hill 2004, p. 189-96.
3. Bradley CS. Natural History of pelvic organ prolapse in postmenopausal women. *Obstet Gynecol.* 2007, 109(4), 848-54.
4. Megaputra G, Megadhana W, Suwiyoga K, Santoso B, Junizaf. Prevalence of Urinary Incontinence in Women with Pelvic Organ Prolapse at Sanglah Hospital Denpasar, Bali-Indonesia. *Bali Med J.* 2016, 5(1), 140-4.
5. Maria AT, Bortolini DEE. Genetics of pelvic organ prolapse: crossing the bridge between bench and bedside in urogynecology research. *Int Urogynecol J.* 2011, 22, 1211-9.
6. Ming PW. Regulation of Extracellular Matrix Remodeling Associated with Pelvic Organ Prolapse. *J Exp Clin Med.* 2010, 2(1), 11-6.
7. Skorupski P, Miotla P, Jankiewicz K, Rechberger T. Polymorphism of the gene encoding alpha-1 chain of collagen type I and risk of pelvic organ prolapse, a preliminary study. *Ginekologia Polska.* 2007, 78, 852-57.
8. Rodrigues AM, Girao MJBC, Silva IDCG, Sartori MGF, Martins K, Castro R. COL1A1 Sp1-binding site polymorphism as a risk factor for genital prolapse. *Int Urogynecol J.* 2008, 19, 1471-5.
9. Jeon MJ, Chung SM, Choi JR. The relationship between COL3A1 exon 31 polymorphism and pelvic organ prolapse. *The Journal of Urology.* 2009, 181, 1213-6.
10. Huey YC, Chung YW, Lin WY, Chen WC, Tsai FJ. Estrogen receptor alpha polymorphism is associated with pelvic organ prolapse risk. *Int Urogynecol J.* 2008, 19, 1159-63.
11. Khadzhieva MB, Kamoeva SV, Chumachenko AG. Fibulin-5 (FBLN5) gene polymorphism is associated with pelvic organ prolapse. *Maturitas.* 2014. 78, 287-92.
12. Huey YC, Chung YW, Lin WY, Chen WC, Tsai FJ. Estrogen receptor alpha polymorphism is associated with pelvic organ prolapse risk. *Int Urogynecol J.* 2008, 19, 1159-63.