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The Relationship of Polymorphism of LYSYL Oxidase Like-1 Gene and Pelvic Organ Prolapse in Balinese Women

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ABSTRACT

Background: Pelvic organ prolapse (POP) data from Sanglah General Hospital Bali revealed proportion of 91.2% Balinese women, and that proportion was not decreased even with modification of workload and contraception. One of the associated factors is the decrease of expression of elastin and proteins involved in elastin metabolism, such as lysyl oxidase-like 1 (LOXL-1). There is genetic factor in the pathogenesis of prolapse, and polymorphism of elastogenesis-related gene, such as LOXL1 gene, associated with elastinopathy.
Methods: We conducted an observational case control study in Balinese women population. Balinese women are women who have lineage of at least 3 generations (from grandparents) from Balinese people. Thirty patients with prolapse were taken as cases and 30 patients non prolapse as controls. DNA was isolated from venous blood, and then LOXL1 gene amplificated by polymerase chain reaction and then electrophoresis, and finally the DNA products were sequenced. Data was analyzed wih SPSS 21.0 for Windows. To determine whether the LOXL1 gene polymorphism is a risk factor for POP, a Chi-Square test was performed, and then variables were controlled by multivariate analysis using conditional logistic regression tests.
Results: From the distribution of polymorphism, there were 13 samples in the case group (43.34% of the case group), compared to 5 samples in the control group (16.67% of the control group), and for the distribution of non-polymorphism, there were 17 samples in the case group (56.66% of the case group), compared to 25 samples in the control group (83.33% of the control group). From the analysis of polymorphism of LOXL1 gene rs2304719 in Balinese women, we found OR 3.824, CI 95% 1.150-12.713, p = 0.024). The LOXL1 gene polymorphism remains a risk factor for prolapse occurrence after the control variables were controlled by multivariate analysis using conditional logistic regression tests. The LOXL1 gene polymorphism, after the four control variables were controlled, gave a risk for prolapse incidence of 3,791 times by multivariate analysis (adjusted OR = 3.791; 95% CI = 1.065-13.496; p = 0.04).
Conclusion: Polymorphism of LOXL1 gene rs2304719 is a risk factor for prolapse in Balinese women. Study for other gene polymorphisms is needed, before we can make a multigene panel testing for risk assessment. We hope in the future, multigene panel testing will be simpler, easier and cheaper, so that women at risk can be identified, and then can be informed and advised to avoid or reduce risk factors for prolapse.

Keywords: DNA sequencing, elastinogenesis, elastinopathy, fibroblast, genetic factor of prolapse, multigene panel testing, polymerase chain reaction, uterosacral ligament

INTRODUCTION

Pelvic organ prolapse (POP) is a woman's health problem, with complaints of discomfort, lumps that come out from the genitals, sexual dysfunction, urinary dysfunction, defecation disorder, vaginal discharge, back pain, and infections that can interfere with the quality of life. As the life expectancy of women increases, the incidence of POP also continues to increase. This health problem is not life threatening, but interferes with the social, economic and sexual function.

In Indonesia, hospital-based data from dr. Soetomo General Hospital Surabaya during a period of 5 years (2007-2011) found 371 women diagnosed with POP.[1] From data of the Obstetrics and Gynecology Department Sanglah General Hospital Denpasar, POP patients in 2015 were 91 cases, and most of them (91.20%) came from the Balinese ethnic group.[2]

Prolapse risk factors consist of intrinsic factors and extrinsic factors. Extrinsic factors include pregnancy and childbirth, work history and various conditions that cause an increase in intraabdominal pressure and history of hysterectomy. Intrinsic factors include genetic factors, connective tissue abnormalities, extracellular matrix changes, hypoestrogens, menopause, and the aging process.[2–4]

Various studies have shown that, in POP, there is an imbalance between the synthesis, maturation and degradation of elastin, one of the components of the extracellular matrix.[5] Decreased expression of elastin and proteins involved in elastin metabolism, such as lysyl oxidase-like 1 (LOXL-1) was reported to occur in the sacrouterine ligament, which is one of the ligaments supporting the pelvic floor.[6]

Based on the results of a study in Europe, the risk of POP increased 5-fold in women whose twin siblings had prolapse compared to the general population, and this family has a hereditary family history of POP, so it can be concluded that there is a genetic role in the pathogenesis of POP.[7]

Disturbance in elastogenesis causes POP. In studies on mice, there are genes responsible for elastogenesis. These genes include lysyl oxydase-like protein 1 (LOXL-1). The absence of LOXL-1 in mice causes various elastinopathies, such as emphysema, flaccid skin syndrome, vascular disorders and decreased vaginal elastin fibers.[3] Variations in DNA sequences between individuals are called polymorphism. Single nucleotide polymorphism (SNP) is a variation of DNA sequences that most often occurs in human genes.[8] The study of the LOXL-1 gene polymorphism was reported to be associated with the incidence of POP in Russian women.[3] However, studies in African-American and Caucasian women found no association between LOXL-1 polymorphisms and POP.[9] Ethnic factors may have an effect on the non-uniformity of the results of this polymorphism study, as stated by Maria Augusta in a review article in IUGA magazine.[8] Study on gene polymorphism as a risk factor for POP is still rare, making hopes to explain the genetic predisposition of POP still unsatisfactory.[2]

Genetic factors are thought to be a risk factor for POP in Balinese women, because the prevalence of POP is still high, although now the frequency of childbirth and workload has been limited by family planning and improved understanding of the Balinese traditional concept of Balinese women.

We hypothesized that Balinese women with polymorphism of LOXL1 gene have higher risk of pelvic organ prolapse than without polymorphism. This study was conducted to strengthen the suspicion about the role of genetics in the pathophysiology of POP. If we know the effect of polymorphism on the LOXL-1 gene, the risk of POP incidence in Balinese women can be explained, so we hope that the management or prevention of the occurrence and worsening of POP in Balinese women in the future will be better.

MATERIALS AND METHODS

The study design was observational case-control in Balinese women population. Balinese women were defined as women who have lineage of at least 3 generations (from grandparents) from Balinese people. Cases were patients diagnosed with POP. Controls were other gynecological patients without POP.

Samples were taken from the venous blood of POP patients and non-prolapse patients. The examination of polymorphism in the LOXL-1 gene was carried out at the Integrated Biomedical Laboratory, Faculty of Medicine, Udayana University, Denpasar, Bali. The target population were Balinese women aged 30 to 70 years with reachable population is all Balinese women aged 30-70 years underwent examinations at the Uroginecology Reconstruction Clinic and Gynecology Clinic, Sanglah Hospital Obgyn Polyclinic and Prima Medika Hospital Denpasar. Inclusion criteria were (1) Balinese women aged 30-70 years, (2) Pelvic organ prolapse and other gynecological patients undergoing examinations at the Reconstructive Uroginecology Clinic and Gynecology Clinic, Sanglah Hospital Obgyn Polyclinic and Prima Medika Hospital Denpasar, (3) Willing to participate in research after signing an informed consent. Exclusion criteria were (1) Patient with malignancy, (2) Pregnant.

Methods of data collection were (1) anamnesis to obtain characteristics such as identity, age, parity, complaints and work history, (2) General physical examination to determine the presence of comorbid conditions and to obtain a body mass index, (3) Urogynecological examination to determine the clinical degree of pelvic organ prolapse using the POPQ (Pelvic Organ Prolapse Quantification) methods.

Before this research was carried out, the research proposal was first sent to the research ethics commission of the research and development unit of the Medical Faculty of Udayana University Denpasar to obtain information on ethical feasibility, then permission was asked to start carrying out the research.

From the reachable population, urogynecological examination was performed, then inclusion and exclusion criteria were determined. In cases that met the inclusion criteria, informed consent was obtained and a non-prolapse patient control was sought according to the operational variable definition. Three milliliters of venous blood was taken to check for polymorphism. The venous blood was inserted into a tube containing EDTA and the DNA was examined in the Integrated Biomedical Laboratory of Medical Faculty of Udayana University. DNA isolation was carried out using GF-1 Blood Extraction. The lysyl oxidase like protein 1 gene with polymorphism LOXL-1 rs2304719 was amplified by polymerase chain reaction (PCR) using a forward primer: 5′-AGAGCAGTATTTGGAGTGTG-3 ′ and a reverse primer: 5′-CCCACTCTGAATGAATAAGC-3 ′, amplifying the 238 bp long fragments. PCR was performed using the PCR Kit from Promega. To see the success of PCR, electrophoresis was carried out using 1% agarose gel. To detect LOXL-1 rs2304719 polymorphisms, all PCR products were prepared for sequencing. Before sequencing, PCR products were purified using PCR DNA Fragments Extraction Kit from Geneaid. Finally, analysis of the results was processed using SPSS 21.0 for Windows.

After the data was collected, the analysis was carried out as follows: (1) Descriptive analysis was presented for all data obtained in this study, (2) To determine the Odds ratio of the LOXL-1 gene polymorphism with the occurrence of pelvic organ prolapse, a Chi-Square test was used, (3) and then variables were controlled by multivariate analysis using conditional logistic regression tests.

RESULT

The distribution of the characteristics of research subjects in the case group (POP) and the control group (non-prolapse) were

shown in Table 1. The mechanism of POP occurrence was multifactorial, including obesity as indicated by body mass index, age, menopausal status and history of hysterectomy. Descriptive analysis in this study aimed to assess the comparison between the case and control groups. It could be seen in Table 1, the variables of body mass index, age, menopausal status, and history of hysterectomy in the case and control groups were not too different.

Table 1 Characteristics Distribution of Study Subjects

Risk Factor	Cases (n=30)	Controls (n=30)
	Frequency n (%)	Frequency n (%)
Body Mass Index		
Lean	1(3,33)	0(0)
Normal	20(66,67)	22(73,33)
Fat	9(30,00)	8(26,27)
Age (Mean ± SD)	57,67±9,764	56,37±9,633
Menopausal status		
Premenopausal	6(20,00)	7(23,33)
Postmenopausal	24(80,00)	23(76,67)
Hysterectomy		
Yes	1(3,33)	1(3,33)
No	29(96,67)	29(96,67)

Data are n(%). SD, Standard deviation

The complete results of the LOXL1 receptor gene polymorphisms found in this study were presented in Table 2.

Table 2 Polymorphism Distribution of Gene LOXL1

Genotype		Case	Control
		Number (%)	Number (%)
Gene LOXL1	(TT)	2 (6,67%)	0 (0%)
Gene LOXL1	(CT)	11 (36,67%)	5 (16,67%)
Gene LOXL1	(CC)	17 (56,66%)	25 (83,33%)
		30 (100%)	30 (100%)

Data are n(%). LOXL1, lysyl oxidase like-1

To determine whether the LOXL1 gene polymorphism was a risk factor for POP, a Chi-Square test was performed. The results of the analysis were shown in Table 3. Table 3 showed that the LOXL1 gene polymorphism gave a risk of POP incidence of 3.8 times (RO = 3.824; 95% CI = 1,150-12,713; p = 0.024).

Table 3 Polymorphism gene LOXL1 as Risk Factor for POP

		Group		OR	CI 95%	p
		Case	Control			
Polymorphism Gene LOXL1	(+)	13	5	3,824	1,150-12,713	0,024
	(-)	17	25			

LOXL1, lysyl oxidase like-1; POP, pelvic organ prolapse; OR, odds ratio; CI, confidence interval

In Table 4, it was shown that the LOXL1 gene polymorphism remains a risk factor for POP occurrence after 4 control variables (Body mass index/BMI, age, menopausal status, history of hysterectomy) were controlled by multivariate analysis using conditional logistic regression tests. The LOXL1 gene polymorphism, after the control variables were controlled, gave a risk for POP incidence of 3,791 times by multivariate analysis (adjusted OR = 3,791; 95% CI = 1,065-13,496; p = 0.04).

Table 4 Relationship of Polymorphism Gene LOXL1 and POP after controlled with controlling variable (BMI, Age, Menopause, and Hysterectomy)

	Adjusted OR	CI 95%	p
Polymorphism Gene LOXL1	3,791	1,065-13,496	0,040
BMI (fat/normal/lean)	1,303	0,369-4,597	0,681
Age (years)	0,974	0,886-1,070	0,583
Menopause (yes/no)	1,088	0,142-8,326	0,936
Hysterectomy (yes/no)	0,832	0,036-19,462	0,909

LOXL1, lysyl oxidase like-1; POP, pelvic organ prolapse; BMI, body mass index; OR, odds ratio; CI, confidence interval

DISCUSSION

In previous studies, it was found that the LOXL1 gene polymorphism as a risk factor for POP were heterozygous CT genotype and TT homozygote. In this study, the genotypes that was analyzed and discussed as risk factors were heterozygous CT and TT homozygotes as in previous studies.[3,10]

In the LOXL1 gene polymorphism examination results (Table 2), from the distribution of polymorphisms, the case group found 13 samples with polymorphism (43.34% of the case group) compared to 5 samples in the control group (16.67% from the control group). Meanwhile, those without polymorphism found 17 samples in the case group (56.66% from the case group) compared to 25 samples in the control group (83.33% from the control group).

To determine whether the LOXL1 gene polymorphism was a risk factor for POP, Chi-Square test was performed (Table 3), and odds ratio (OR) of 3.824 was obtained, with confidence interval (CI) of 95% 1.150-12.713, with *p value* of 0.024, which means that the LOXL1 gene polymorphism was a risk factor for POP with possibility of 3.824 times higher than those without polymorphism and this result was statistically significant, so the detection of LOXL1 gene polymorphism could be used as a screening candidate for POP.

In a study published at 2015 in the Russian Journal of Genetics, research was carried out on polymorphisms in genes involved in the synthesis of elastin fibers, one of which was the LOXL1 gene. In the initial study on mice, it found that mice without LOXL1 had decrease in the amount of elastin in the vagina, and only had POP after their first birth. This showed the importance of LOXL1 in the elastin fiber remodeling process during the postpartum period. Furthermore, the study found that the LOXL1 gene polymorphism rs2304719 increased the risk of developing POP with OR = 1.77, 95% CI: 1.01-3.09, p = 0.045. From these studies, the polymorphism of the LOXL1 rs2304719 gene was a risk factor for POP.[3]

In this study, we wanted to know whether the LOXL1 gene polymorphism was a risk factor for POP occurrence in Balinese women. The research samples were taken from Balinese women aged 30 to 70 years, with several other risk factors that had been controlled. The polymorphism examined was the LOXL1 gene polymorphism rs2304719, such as a study in Russia by Khadzhiev et al in 2015. The results of this study were presented in Table 3, namely, the LOXL1 gene polymorphism rs2304719 was a risk factor for POP in Balinese women by 3.824 times (OR = 3.824 ; CI 95% = 1,150-12,713; p = 0.024).

The LOXL1 gene has a molecular weight of 63 kDa, contains 7 exons and is located on chromosome 15q24 on the human chromosome.[11] Genetic examination with the aim of knowing the risk factors for POP in a woman is a very advanced scientific development, and it is also useful to provide information to these women so that women are better prepared and understand about POP. By knowing the single nucleotide polymorphisms (SNPs) that are responsible for disease, it can be a method to study genes associated with POP.[12]

Single nucleotide polymorphisms in the LOXL1 gene will result in defects in the elastin fibers in the postpartum uterus, where there is a decrease in the number of elastin cross-links, which in turn will cause POP.[3,13]

The result of this study indicated that the LOXL1 gene polymorphism was a risk factor for POP in Balinese women, with the hope that multigene panel testing could be carried out in the future, so that high risk women could be identified and they could, for example, be informed about pelvic muscle strengthening exercises, more focused and intense. They also could be advised to avoid or reduce other risk factors for POP such as multiparity, vaginal delivery, obesity, occupation or diseases that cause chronic increased intraabdominal pressure. Thus the likelihood of developing

POP in women who were at risk will be reduced. Although this had been done in many developed countries, in developing countries such as Indonesia, examination to determine the gene polymorphism is still difficult and relatively expensive. Study for other gene polymorphisms were needed, before we can make a multigene panel testing for risk assessment in the future. We hope in the future, multigene panel testing would be simpler, easier and cheaper, so that women at risk could be identified, and then could be informed and advised to avoid or reduce risk factors for prolapse. This was the first study to examine polymorphism of LOXL1 gene as a risk factor for POP in Balinese women. Limitations of this study included the small size of samples.

CONCLUSION

Polymorphism of LOXL1 gene rs2304719 was a risk factor for prolapse in Balinese women. Study for other gene polymorphisms were needed, before we could make a multigene panel testing for risk assessment. We hope in the future, multigene panel testing would be simpler, easier and cheaper, so that women at risk could be identified, and then could be informed and advised to avoid or reduce risk factors for prolapse.

RESEARCH ETHICS

This research has been approved by the Research Ethics Committee, Faculty of Medicine, Universitas Udayana/Sanglah General Hospital Denpasar with number 962/UN14.2.2.VII.14/LT/2020.

AUTHOR CONTRIBUTION

All authors have the same contribution in writing the report on the results of this study, from the stage of proposal preparation, data search, and data analysis, to the interpretation of research data and presentation of the final report.

Declaration by Authors

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