ORIGINAL RESEARCH

The *Matrix Metalloproteinase-9* Gene Polymorphisms as Risk Factor for Pelvic Organ Prolapse in Balinese Women

Kadek Ary Widayana[1], I Gede Mega Putra[2], I Wayan Megadhana[3], Tjok Gde Agung Suwardewa[4], I Nyoman Bayu Mahendra[5], Anak Agung Gede Putra Wiradnyana[6], Ida Bagus Gede Fajar Manuaba[7]

Received on: 21 November 2022; *Accepted on:* 13 December 2022; *Published on*: 19 April 2023

Abstract

Aim: Pelvic organ prolapse (POP), although not life-threatening, is a serious condition that can affect a patient's quality of life. As life expectancy rises, the number of women with POP continues to rise. The weakness of the pelvic floor is always found underlying each case of POP. This weakness in the pelvic floor is associated with repeated damage to muscles and connective tissue due to the process during pregnancy and delivery. Balinese women have risk factors for developing POP related to the obligation to continue their offspring by conceiving and giving birth more than 4 times and working as heavy workers. The presence of genetic factors is also thought to play a role as a risk factor for POP in Balinese women. The purpose of this study was to prove the existence of polymorphism matrix metalloproteinase-9 (MMP-9) rs17576 as a factor that puts Balinese women at risk for POP.

Materials and methods: A paired case–control study was conducted on each of 30 Balinese women with POP and without prolapse, which was done matching based on parity and occupation variables at Sanglah Hospital, Denpasar and Integrated Biomedical Laboratory, Faculty of Medicine, Udayana University. The EDTA tube contains 3 milliliters of venous blood, from which the sample material is taken. DNA isolation, PCR, and sequencing examinations were performed to determine the presence of *MMP-9 rs17576* gene polymorphism.

Results: Balinese women's POP risk was quadrupled by the gene polymorphism of the *MMP-9 rs17576* genotype AG compared to without prolapse (OR = 4.00, 95% CI 1.13–14.18, $p = 0.04$).

Conclusion: In Balinese women, the presence of *MMP-9 rs17576* gene polymorphism raises the risk of POP.

Keywords: Matrix metalloproteinase-9, Pelvic organ prolapses, Polymorphism.

Journal of South Asian Federation of Obstetrics and Gynaecology (2023): 10.5005/jp-journals-10006-2190

Introduction

Pelvic organ prolapse is a serious condition in woman when there is prolapse in part or all of the pelvic organ, which is referred generally to the uterus, vaginal cavity, and surrounding organs like the rectum, intestine, and urinary bladder.[1] The number of women suffering from POP keeps increasing as the increase of life expectancy of woman in these last years.[2] In developing countries, POP prevalence is estimated around 19.7%, with variation of 3.4–56.4%.[3] In Indonesia, the incidence of POP is not well-reported. The case of POP in Sanglah General Hospital was higher during 2015 with 91 cases, 36 cases (39.56%) underwent surgery, and 83 cases of these patients were of Balinese ethnicity.[4]

Every case of POP is always based on weakness in the pelvic floor support, which is associated with repeated damage to muscles and connective tissue due to the process of repeated stretching and relaxation during pregnancy and childbirth.[5,6] This supporting function is influenced by the components of the connective tissue's extracellular matrix, which are mainly collagen types I and III. Biochemical changes in collagen can be influenced by matrix metalloproteinase (MMP) activity which plays an important role in the tissue remodeling process.[7,8]

Matrix metalloproteinase is an extracellular proteinase enzyme that regulates developmental processes and physiological functions. Matrix metalloproteinase activity is limited by tissue inhibitors of metalloproteinases (TIMP) as inhibitors. To keep the levels of collagen in the extracellular matrix in balance, the balance of MMP and TIMP activities is very important.[9–11] Research in 2014 discovered that patients with POP had higher levels of MMP-1, MMP-2, MMP-3, and MMP-9 expression and lower levels of TIMP-1 expression in the anterior vaginal wall than patients without POP.[12–14]

[1]Department of Obstetrics and Gynecology, Faculty of Medicine Udayana University, Bali, Indonesia

[2,3]Urogynecology and Reconstructive Division, Department of Obstetrics and Gynecology, Faculty of Medicine, Udayana University, Bali, Indonesia

[4,6]Maternal Fetal Medicine Division, Department of Obstetrics and Gynecology, Faculty of Medicine, Udayana University, Bali, Indonesia

[5]Gynecology Oncology Division, Department of Obstetrics and Gynecology, Faculty of Medicine, Udayana University, Bali, Indonesia

[7]Fertility and Reproductive Endocrinology Division, Department of Obstetrics and Gynecology, Faculty of Medicine, Udayana University, Bali, Indonesia

Corresponding Author: Kadek Ary Widayana, Department of Obstetrics and Gynecology, Faculty of Medicine, Udayana University, Badung, Bali, Indonesia, Phone: +6282146572932, e-mail: ary_widayana@yahoo.com

How to cite this article: Widayana KA, Putra IGM, Megadhana IW, *et al*. The *Matrix Metalloproteinase-9* Gene Polymorphisms as Risk Factor for Pelvic Organ Prolapse in Balinese Women. J South Asian Feder Obst Gynae 2023;15(1):65–70.

Source of support: Nil

Conflict of interest: None

The presence of mutations or genetic variations in genes that express MMP-9 can increase the activity of MMP-9 so that it can increase the degradation process of type III collagen.[15] The MMP-9 gene's presence of polymorphisms is one of the factors that can affect the enzymatic activity of MMP-9.[16] In Taiwanese women, polymorphisms in the MMP-9 rs17576 gene were linked to a fivefold increased risk of developing POP.[17,18] Other studies found that there was no significant difference in MMP-9 gene polymorphism on the incidence of POP.[19,20] The varying results are most likely influenced by ethnic differences in each study.

Bali, as one of the regions in Indonesia, has a female population who are known as hard workers. Balinese women often take menial jobs like lifting heavy loads. Balinese women are also obliged to continue their family lineage by containing and giving birth more than four times in the past.[21,22] Therefore, Balinese women are at risk of experiencing POP in the future. Another factor that is also associated with the occurrence of POP in Balinese women is the role of genetic factors that have been proven previously.

Until now, there has been no study that has reported a correlation between the prevalence of POP in Balinese women and the MMP-9 rs17576 gene polymorphism. In this way, research on the MMP-9 rs17576 quality polymorphism as a gamble factor for the event of POP should be completed to decide the presence of hereditary varieties that assume a part in the pathogenesis of POP, especially in Balinese women.

Materials and Methods

This was an observational study paired case–control study conducted from June 2020 to December 2020. The case group consisted of patients with POP, meanwhile, the control group was defined as another gynecological patient without prolapse. The inclusion criteria were: (i) Balinese women aged 30–70 years old, (ii) patients with POP and another gynecological condition who were examined in the Urogynecology and Reconstruction polyclinic, Obstetrics and Gynecology polyclinic of Sanglah General Hospital (iii) agree to be involved in this study by signing the informed consent. Meanwhile, the exclusion criteria were patients with malignancy and pregnancy.

Study samples were taken from venous blood from the case group and control group with a proportion of 1:1. The samples underwent DNA isolation, polymerase chain reaction (PCR), electrophoresis, and then sequencing to examine the MM-9 rs17576 gene polymorphism. Descriptive analysis was done to compare the characteristics between the case group and the control group, with a *p*-value <0.05 as significant. A comparison was shown in the cross-distribution table.

Paired bivariate non-parametric analysis using the McNemar test was done to examine the risk factor of MMP-9 rs17576 gene polymorphism with the occurrence of POP. The result was shown in a 2 × 2 table paired between the case group and the control group, significant if the *p* value <0.05. Odd ratio (OR) was calculated using formula b/c from the paired 2 × 2 table, with CI 95% and significant if *p* value <0.05. Multivariate analysis was done using the multiple logistic regression test to examine if MMP-9 rs17576 gene polymorphism is a risk factor for POP that can be controlled by other factors like age, body mass index (BMI), menopause status, and hysterectomy history. The result was shown in table form as an adjusted Odds Ratio with a CI of 95% and significant if the *p*-value <0.05.

Results

Samples' Characteristics

The following Table 1 describes the characteristics of the sample in this study.

Examination of *Matrix Metalloproteinase (MMP-9)* rs17576 Gene Polymorphisms

Examination of the MMP-9 rs17576 gene polymorphism began with DNA isolation from venous blood samples. The DNA isolation results were then examined for amplification by PCR using a forward primer: 5′-CCATGGGTCAAAGAACAGGA-3′ along 285 bp with an annealing temperature of 58 °C. The success of the PCR examination was assessed by electrophoresis using 1% agarose gel, then viewed with a transilluminator using UV light. The results of the PCR examination that looked good with electrophoresis were sent for sequencing examination. The results of the sequencing examination were then analyzed for the presence of the MMP-9 rs17576 gene polymorphism using the reference Homo sapiens MMP-9, RefSeqGene on chromosome 20 from the GenBank National Center for Biotechnology Information (NCBI).

Humans have the MMP-9 gene on chromosome 20q11.2-q13.1, which contain 14654 base pairs of DNA. This gene consists of 13 exons and 12 introns. The MMP-9 gene polymorphism rs17576 is an SNP present at exon 6 of the MMP-9 gene (Fig. 1). The MMP-9 rs17576 gene polymorphism results obtained from sequencing each POP and nonprolapsed group sample based on the SNP position of the MMP-9 GenBank NCBI gene reference found three types, namely homozygous AA, heterozygous AG, and homozygous GG (Fig. 2).

Table 1: Study samples' characteristics

Variable	*POP group (N = 30)*	*Nonprolapse group (N = 30)*	*p-value*
Age (years), mean ± SD	57.47 ± 3.98	56.63 ± 3.43	0.25[a]
Parity, median (IQR)	4 (1)	4 (1)	–
Parity <4	14 (46.70)	14 (46.70)	–
Parity ≥4	16 (53.30)	16 (53.30)	
BMI (kg/m^2), median (IQR)	22.54 (4.09)	23.14 (3.66)	0.62[b]
Underweight (<18.5 kg/m^2)	2 (6.70)	4 (13.30)	0.64[c]
Normal (18.5–24.9 kg/m^2)	21 (70.00)	21 (70.00)	
Overweight (25.0–26.9 kg/m^2)	3 (10.00)	3 (10.00)	
Obese (≥27.0 kg/m^2)	4 (13.30)	2 (6.70)	
Occupation			
Light work, *N* (%)	10 (33.30)	10 (33.30)	–
Heavy work, *N* (%)	20 (67.70)	20 (67.70)	
Menopausal status			
Have not, *N* (%)	4 (13.30)	6 (20.00)	0.69[c]
Have, *N* (%)	26 (86.7)	24 (80.00)	
History of hysterectomy			
No, *N* (%)	29 (96.70)	29 (96.70)	1.00[c]
Yes, *N* (%)	1 (3.30)	1 (3.30)	

BMI, body mass index; IQR, interquartile range; N, frequency; SD, standard deviation; [a]Paired *t*-test; [b]Wilcoxon test; [c]McNemar test; *p*-value <0.05 is significant

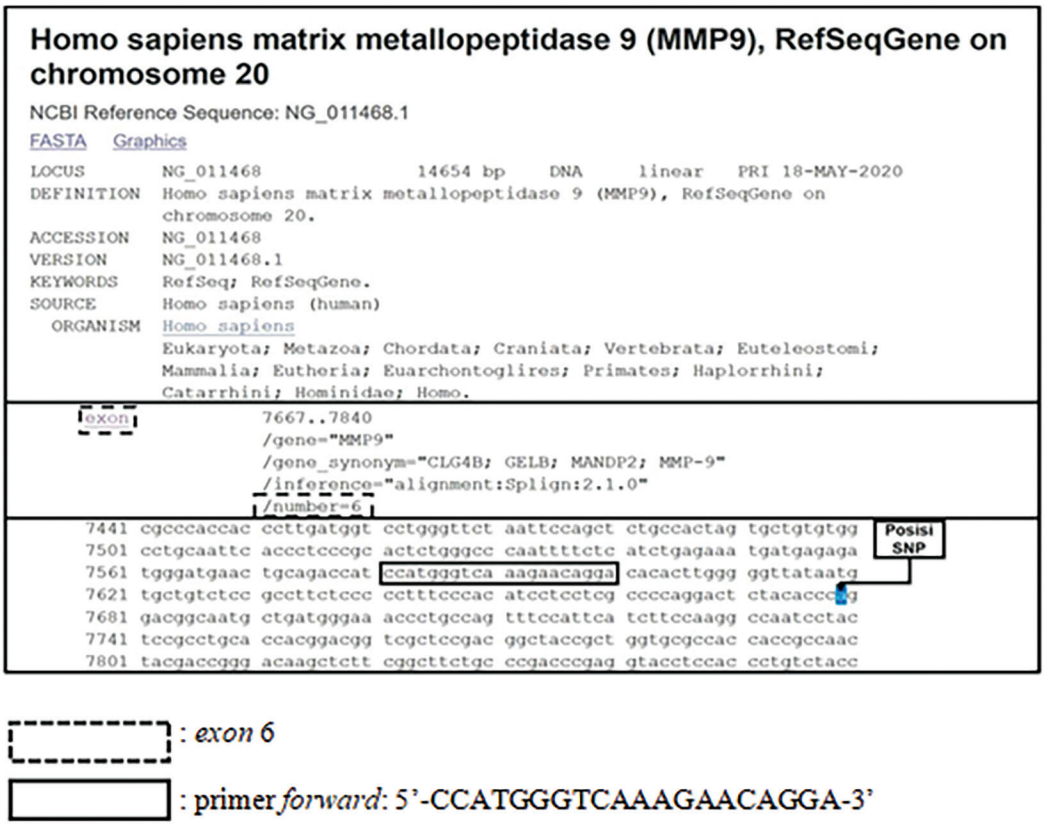


Fig. 1: SNP position of MMP-9 rs17576 gene on exon 6

The distribution of MMP-9 rs17576 gene polymorphisms in the POP and nonprolapsed groups were presented based on a paired 2×2 tabulation (Table 2). Analysis using the McNemar test on the three types of MMP-9 rs17576 gene polymorphisms, the *p* values were obtained, respectively, 0.29 for AA homozygotes, 0.04 for AG heterozygotes, and 0.36 for GG homozygotes. So, only the type of heterozygous AG was statistically significant.

The Matrix Metalloproteinase (MMP-9) rs17576 Gene Polymorphisms as Risk Factor of Pelvic Organ Prolapse

Analysis to determine the MMP-9 rs17576 polymorphisms in genes as POP risk factors were carried out by calculating OR using the b/c formula based on paired 2×2 tabulation on heterozygous AG. Calculations of b/c, 95% CI, and *p* values were obtained using the StatCalc algorithms and formulas application provided by OpenEpi.com for Windows. The calculation results are presented in Table 3. Based on this analysis, the heterozygous MMP-9 rs17576 gene polymorphism was found to quadruple the risk of POP compared to nonprolapse with 95% CI 1.13–14.18 (*p*-value = 0.04). Based on the results (Table 4), the MMP-9 rs17576 gene polymorphism was found to be a risk factor for the occurrence of POP with an adjusted OR value of 5.70 with 95% CI 1.44–22.57 (*p*-value = 0.01).

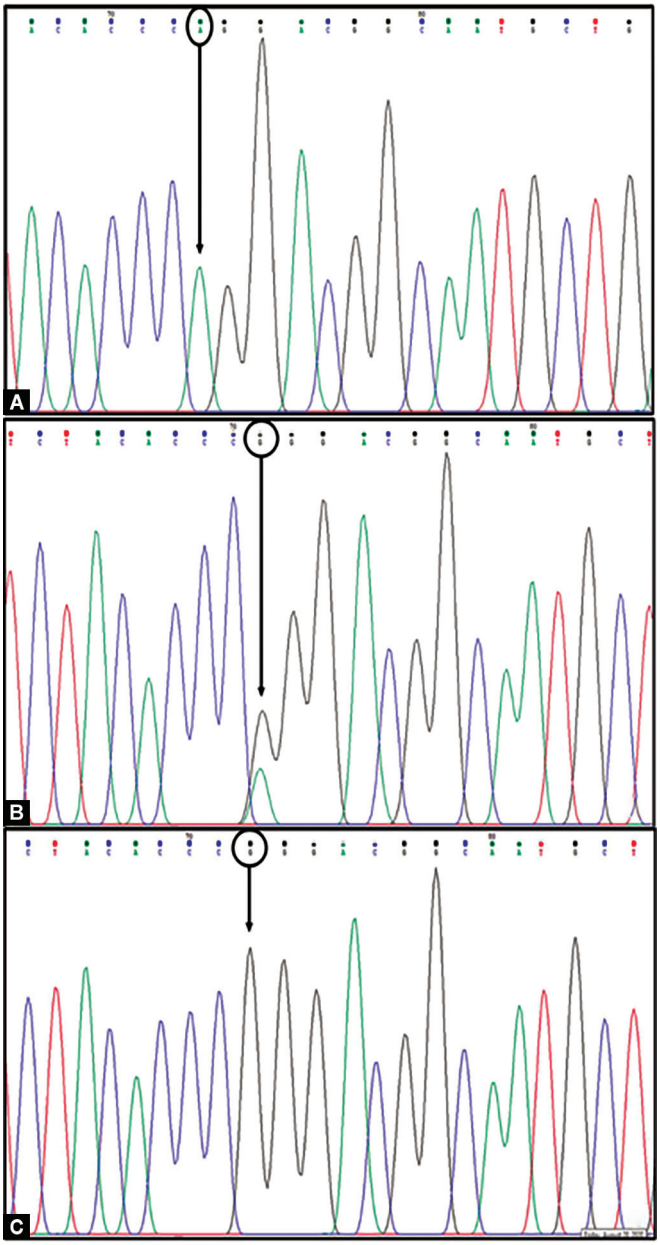
Discussion

Samples' Characteristics

The average age of the POP group sample was 57.47 years, which was not significantly different from 56.63 years for the nonprolapsed group. This study found that the average age of POP patients was nearly the same as that of several previous studies. Swift et al. discovered in 2005 that the prevalence of POP increased by approximately 40% every decade.[23] Postmenopausal women over the age of 50 face an approximately 5.2-fold increase in the risk of POP.[24] The risk of POP rises with age, peaking between the ages of 60 and 69. The aging process as a whole and the aging of all fibroblasts in the pelvic tissue probably have an impact on the pathogenesis of POP as people get older. Gene instability and mitochondrial dysfunction may be the causes of fibroblast aging.[25,26]

The median parity in the POP group was 4, with a parity ≥4 being 53.30%. Most of the POP patients in this study had a history of heavy work, namely 67.70%. Parity and prior work experience can affect the occurrence of POP due to expanded intra-abdominal pressure in the pelvic floor supporting tissues, especially the levator ani muscles. Women with multiparity and having given birth vaginally four times or more will increase the risk of POP to about 10 times compared to nulliparous women.[27] Heavy work during adolescence (more than 21 hours/week with strenuous activity) may present an increased danger of developing POP.[28–30]

In the POP group, the median BMI was 22.54 kg/m^2 with an IQR of 4.09 kg/m^2 and did not significantly differ from the group without prolapse. The results of BMI in POP patients in this study were different from several previous studies. Research carried out by Nygaard et al. found that women with BMI 25–30 kg/m^2 and BMI > 30 kg/m^2 had a 1.25- and 1.73-times risk of experiencing POP, respectively.[29] Similar to the research showed, the risk ratio with the same BMI category was 1.36 times and 1.47 times, respectively.[31]



Figs 2A to C: MMP-9 rs17576 gene polymorphism (A) Homozygote AA; (B) Heterozygote AG; and (C) Homozygote GG

Table 2: MMP-9 rs17576 gene polymorphism distribution

MMP-9	*Paired 2 × 2 tabulation*				
rs17576 gene polymorphism	*a*	*b*	*c*	*d*	*p-value*
AA	1	2	6	21	0.29[a]
AG	1	12	3	14	0.04[a]
GG	7	7	12	4	0.36[a]

[a] McNemar test; *p*-value < 0.05 is significant

Table 3: MMP-9 rs17576 gene polymorphism as a risk factor for POP

		Nonprolapse group MMP-9 rs17576 gene polymorphism					
			+	–	*RO*	*95% CI*	*p-value*
POP	MMP-9 rs17576 gene	+	1	12	4.00	1.13–14.18	0.04[a]
Group	Polymorphism	–	3	14			

[a]McNemar test, *p*-value < 0.05 is significant

Table 4: MMP-9 rs17576 gene polymorphism as risk factor of POP by controlling variables such as age, parity, BMI, occupation, menopausal status, and history of hysterectomy

Variable	*Adjusted OR*	*CI 95%*	*p-value*
MMP-9 rs17576 gene polymorphism	5.70	1.44–22.57	0.01[a]
Age	1.11	0.89–1.39	0.37[a]
Parity (<4/≥4)	1.11	0.34–3.66	0.86[a]
BMI (underweight normal overweight obese)	0.86	0.18–6.31	0.88[a]
Occupation (light heavy)	0.91	0.27–3.10	0.88[a]
Menopausal status (have not/have)	0.72	0.08–6.67	0.77[a]
History of hysterectomy (no/yes)	1.22	0.06–24.39	0.90[a]

[a]Multiple logistics regression test. *p*-value <0.05 is significant

These different results indicate that there are other factors besides BMI that cause POP in Balinese women.

The majority of samples in the POP group in this study had experienced menopause (86.70%) not significantly different from the nonprolapsed group (80.00%). Postmenopausal women over 50 years old face a 5.2-fold increase in their risk of POP, according to Indonesian research.[32] Menopause is characterized by a decrease in estrogen levels in the blood. Estrogen can boost muscle and connective tissue strength. By lowering MMP activity, estrogen can slow the degradation of collagen and elastin.[33] Therefore, postmenopausal women are more likely to develop POP.

The history of the sample who had undergone hysterectomy in the POP and nonprolapse groups in this study was the same, namely 3.30%. Analysis showed an increased risk of POP by 1.27 times but not statistically significant. A cohort study found the cumulative incidence of POP following a hysterectomy of approximately 5.1% over 30 years.[34] Research by Robinson found that a history of hysterectomy increased the risk of developing POP by about 5.5 times.[35] Due to the removal of the pelvic organs' natural support, a hysterectomy may result in vaginal apex prolapse and/or genital prolapse.[36]

The Matrix Metalloproteinase (MMP-9) Gene Polymorphisms as Risk Factor for Pelvic Organ Prolapse

According to this study, Balinese women with the MMP-9 rs17576 gene polymorphism have a fourfold increased risk of POP. This risk consistently increased to more than 5.5 times (adjusted OR = 5.70) by controlling for age, parity, BMI, occupation, menopausal

status, and a history of hysterectomy, as well as all other risk factor variables. There are three types of MMP-9 rs17576 gene polymorphisms identified through DNA sequencing in this study, namely homozygous AA, heterozygous AG, and homozygous GG. Further analysis found only the MMP-9 rs17576 heterozygous AG gene polymorphism, which was significant as a factor that puts Balinese women at risk for POP.

An SNP in the MMP-9 gene's exon 6 is known as the rs17576 gene polymorphism (exon 6 A/G). Amino acid substitutions in the MMP-9 enzyme's catalytic domain are the result of single-nucleotide polymorphisms A/G in the rs17576 gene. The G-allele in the MMP-9 gene rs17576 encodes arginine as a substitute for the A-allele encoding glutamine.[37–39] The MMP-9 protein's structure is altered by this polymorphism by replacing an uncharged amino acid (arginine) with a positively charged one. The MMP-9 enzyme's catalytic region's affinity may be altered by this amino acid substitution, increasing substrate-binding and enzyme activity. This increased binding to substrates is accompanied by increased degradation of extracellular matrix components.[40,41]

Type III collagen is a substrate for MMP-9, so the increased activity of MMP-9 in connective tissue, where the majority of the fibers are type III collagen, can accelerate the breakdown of the tissue's extracellular matrix, which leads to a weakening of the tissue structure.[42] So, the MMP-9 rs17576 gene polymorphism can increase the activity of MMP-9, causing weakness of the pelvic floor support, which leads to POP. The results of this study can prove the role of genetic variation, namely the MMP-9 rs17576 gene polymorphism, as a potential cause of the occurrence of POP in Balinese women.

Conclusion

The study's findings suggested that Balinese women with the MMP-9 rs17576 gene polymorphism were more likely to develop POP.

References

1. Haylen BT, Maher CF, Barber MD, et al. An International Urogynecological Association (IUGA)/International Continence Society (ICS) joint report on the terminology for female pelvic organ prolapse (POP). Int Urogynecol J 2016;27(2):165–194. DOI: 10.1007/s00192-015-2932-1.
2. Barber MD. Pelvic organ prolapse. BMJ 2016;354:i3853. DOI: 10.1136/bmj.i3853.
3. Walker GJA, Gunasekera P. Pelvic organ prolapse and incontinence in developing countries: Review of prevalence and risk factors. Int Urogynecol J 2011;22(2):127–135. DOI: 10.1007/s00192-010-1215-0.
4. Mega Putra G, Bagus I, Manuaba A, et al. The role of estrogen receptor α, COL3A1, and fibulin-5 genes polymorphisms as risk factors for pelvic organ prolapse in Balinese women. Gineco.eu. 2018;14(4):135–140.
5. Word RA, Pathi S, Schaffer JI. Pathophysiology of pelvic organ prolapse. Obstet Gynecol Clin N Am 2009;36(3):521–539. DOI: 10.1016/j.ogc.2009.09.001.
6. Wibisono J, Hermawan G. Prolaps organ panggul. Medicinus 2019;7(1):27. DOI: 10.19166/med.v7i1.1444.
7. Wu MP. Regulation of extracellular matrix remodeling associated with pelvic organ prolapse. J Exp Clin Med 2010;2(1):11–16. DOI: 10.1016/S1878-3317(10)60003-4.
8. Leppert PC. Tissue remodeling in the female reproductive tract–a complex process becomes more complex: The role of Hox genes. Biol Reprod 2012;86(4):98. DOI: 10.1095/biolreprod.112.099283.
9. Vu TH, Werb Z. Matrix metalloproteinases: Effectors of development and normal physiology. Genes Dev 2000;14(17):2123–2133. DOI: 10.1101/gad.815400.
10. Verma RP, Hansch C. Matrix metalloproteinases (MMPs): Chemical–biological functions and (Q)SARs. Bioorg Med Chem 2007;15(6):2223–2268. DOI: 10.1016/j.bmc.2007.01.011.
11. Gong R, Xia Z. Collagen changes in pelvic support tissues in women with pelvic organ prolapse. Eur J Obstet Gynecol Reprod Biol 2019;234:185–189. DOI: 10.1016/j.ejogrb.2019.01.012.
12. Wang X, Li Y, Chen J, et al. Differential expression profiling of matrix metalloproteinases and tissue inhibitors of metalloproteinases in females with or without pelvic organ prolapse. Mol Med Rep 2014;10(4):2004–2008. DOI: 10.3892/mmr.2014.2467.
13. Swetha R, Gayen C, Kumar D, et al. Biomolecular basis of matrix metallo proteinase-9 activity. Future Med Chem 2018;10(9):1093–1112. DOI: 10.4155/fmc-2017-0236.
14. Budatha M, Roshanravan S, Zheng Q, et al. Extracellular matrix proteases contribute to progression of pelvic organ prolapse in mice and humans. J Clin Investig 2011;121(5):2048–2059. DOI: 10.1172/JCI45636.
15. Lim VF, Khoo JK, Wong V, et al. Recent studies of genetic dysfunction in pelvic organ prolapse: The role of collagen defects. Aust NZJ Obstet Gynaecol 2014;54(3):198–205. DOI: 10.1111/ajo.12169.
16. Fanjul-Fernández M, Folgueras AR, Cabrera S, et al. Matrix metalloproteinases: Evolution, gene regulation and functional analysis in mouse models. Biochim Biophys Acta 2010;1803(1):3–19. DOI: 10.1016/j.bbamcr.2009.07.004.
17. Chen H-Y, Lin W-Y, Chen Y-H, et al. Matrix metalloproteinase-9 polymorphism and risk of pelvic organ prolapse in Taiwanese women. Eur J Obstet Gynecol Reprod Biol 2010;149(2):222–224. DOI: 10.1016/j.ejogrb.2009.12.014.
18. Wu JM, Visco AG, Grass EA, et al. Matrix metalloproteinase-9 genetic polymorphisms and the risk for advanced pelvic organ prolapse. Obstet Gynecol 2012;120(3):587–593. DOI: 10.1097/AOG.0b013e318262234b.
19. Ghersel FR, Souto RP, Gonzales EWP, et al. Assessment of metalloproteinase matrix 9 (MMP9) gene polymorphisms risk factors for pelvic organ prolapse in the Brazilian population. Rev Bras Ginecol Obstet 2019;41(3):164–169:DOI: 10.1055/s-0039-1681112.
20. Ferrari MM, Rossi G, Biondi ML, et al. Type I collagen and matrix metalloproteinase 1, 3 and 9 gene polymorphisms in the predisposition to pelvic organ prolapse. Arch Gynecol Obstet 2012;285(6):1581–1586. DOI: 10.1007/s00404-011-2199-9.
21. Sudantra IK. Wanita Bali dan harta benda perkawinan: Suatu perspektif normatif 2002;2(2):79–87.
22. Windiyarti D. Pemberontakan perempuan Bali terhadap diskriminasi kelas dan gender: Kajian feminis novel tarian bumi karya Oka Rusmini. J Humaniora 2012;20(3):286–294. DOI: 10.22146/jh.945.
23. Swift S, Woodman P, O'Boyle A, et al. Pelvic Organ Support Study (POSST): The distribution, clinical definition, and epidemiologic condition of pelvic organ support defects. Am J Obstet Gynecol 2005;192(3):795–806. DOI: 10.1016/j.ajog.2004.10.602.
24. Vergeldt TFM, Weemhoff M, IntHout J, et al. Risk factors for pelvic organ prolapse and its recurrence: A systematic review. Int Urogynecol J 2015;26(11):1559–1573. DOI: 10.1007/s00192-015-2695-8.
25. Huang L, Zhao Z, Wen J, et al. Cellular senescence: A pathogenic mechanism of pelvic organ prolapse (Review). Mol Med Rep 2020;22(3):2155–2162. DOI: 10.3892/mmr.2020.11339.
26. Sharma G, Parulekar SV. Accuracy of clinical evaluation of patients with site-specific vaginal wall prolapse and its corelation to surgical diagnosis. J South Asian Fed Obstet Gynaecol 2015;10(3):175–181. DOI: 10.5005/jp-journals-10006-1584.
27. Lukacz ES, Lawrence JM, Contreras R, et al. Parity, mode delivery, and pelvic floor disorders. Obstet Gynecol 2006;107(6):1253–1260. DOI: 10.1097/01.AOG.0000218096.54169.34.
28. Nygaard IE, Shaw JM. Physical activity and the pelvic floor. Am J Obstet Gynecol 2016;214(2):164–171. DOI: 10.1016/j.ajog.2015.08.067.

29. Nygaard IE, Shaw JM, Bardsley T, et al. Lifetime physical activity and pelvic organ prolapse in middle-aged women. Am J Obstet Gynecol 2014;210(5):477.e1–477.e12. DOI: 10.1016/j.ajog.2014.01.035.
30. Garg M, Sharma R, Banerjee BD, et al. Association of HOXA13 gene expression among premenopausal women with the severity of pelvic organ prolapse: A cross-sectional study. J South Asian Fed Obstet Gynaecol 2022;14(4):420–423. DOI: 10.5005/jp-journals-10006-2079.
31. Giri A, Hartmann KE, Hellwege JN, et al. Obesity and pelvic organ prolapse: A systematic review and meta-analysis of observational studies. Am J Obstet Gynecol 2017;217(1):11–26.e3. DOI: 10.1016/j.ajog.2017.01.039.
32. Rahmatyah R, Hardiyanto G, Susanti D. Correlation between pelvic organ prolapse symptoms and age of menopause. Majalah Obstet Ginekol 2017;25(1):30–32. DOI: 10.20473/mog.V25I12017.30-32.
33. Zhou L, Shangguan AJ. Estrogen and pelvic organ prolapse. J Mol Genet Med 2016;10(2). DOI: 10.4172/1747-0862.1000221.
34. Blandon RE, Bharucha AE, Melton LJ, et al. Incidence of pelvic floor repair after hysterectomy. Am J Obstet Gynecol 2007;197(6):664.e1–664.e7. DOI: 10.1016/j.ajog.2007.08.064.
35. Robinson D, Thiagamoorthy G, Cardozo L. Post-hysterectomy vaginal vault prolapse. Maturitas 2018;107:39–43. DOI: 10.1016/j.maturitas.2017.07.011.
36. Lee C-Y, Tseng C-J, Chang C-H, et al. Effect of modified laparoscopic hysterectomy on pelvic floor function: A retrospective observational study. Medicine 2019;98(8):e14616. DOI: 10.1097/MD.0000000000014616.
37. Vandooren J, Van den Steen PE, Opdenakker G. Biochemistry and molecular biology of gelatinase B or matrix metalloproteinase-9 (MMP-9): The next decade. Crit Rev Biochem Mol Biol 2013;48(3):222. DOI: 10.3109/10409238.2013.770819.
38. Wu H, Bai X, Chen D, et al. Association of genetic polymorphisms in matrix metalloproteinase-9 and coronary artery disease in the Chinese Han population: A case–control study. Genet Test Mol Biomarkers 2013;17(9):707–712. DOI: 10.1089/gtmb.2013.0109.
39. Huang H. Matrix metalloproteinase-9 (MMP-9) as a cancer biomarker and MMP-9 biosensors: Recent advances. Sensors 2018;18(10):3249. DOI: 10.3390/s18103249.
40. Kamal A, Elgengehy FT, Abd Elaziz MM, et al. Matrix metalloproteinase-9 rs17576 gene polymorphism and Behçet's disease: Is there an association? Immunol Invest 2017;46(5):460–468. DOI: 10.1080/08820139.2017.1296857.
41. Zou F, Zhang J, Xiang G, et al. Association of matrix metalloproteinase 9 (MMP-9) polymorphisms with asthma risk: A meta-analysis. Can Respir J 2019:9260495. DOI: 10.1155/2019/9260495.
42. Kerkhof MH, Hendriks L, Brölmann HAM. Changes in connective tissue in patients with pelvic organ prolapse—A review of the current literature. Int Urogynecol J Pelvic Floor Dysfunction 2009;20(4):461–474. DOI: 10.1007/s00192-008-0737-1.