

Low Expression of p53 in Sacrouterine Ligament as A Risk Factor for Stage III-IV Uterine Prolapse

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ABSTRACT

Introduction: As life expectancy increases, so does the incidence of pelvic organ prolapse (POP). Low expression of p53 may lead to reduced production and deposition of elastin in the extracellular matrix thus it may increase the risk for POP. This study aims to determine the relationship between p53 expression and the incidence of grade III-IV POP.

Methods: This observational case-control study involved women with grade III-IV POP that underwent a total hysterectomy at Sanglah General Hospital Denpasar as the case group. The control group was non-prolapsed women who were matched to the case for the variables of age, parity, body mass index (BMI), and occupation to minimize bias. The p53 expression was grouped into low (<146.90) and high (≥ 146.90) expressions according to the optimal cut-off value obtained from the receiver operating characteristic curve. Bivariate analysis between p53 expression and risk of POP was performed using the Chi-square test.

Results: A total of 44 patients were included in this study with 22 patients with grade III-IV POP and 22 patients without POP. There were no significant differences in age, parity, BMI, and occupation between the two groups. Low p53 expression increased the risk of stage III-IV uterine prolapse by 20.25 times higher (OR = 20.25; 95% CI = 4.375-93.722; $p = 0.000$).

Conclusion: The low p53 expression in the sacrouterine ligament is a significant risk factor for grade III and IV POP.

Keywords: Elastin, Grade III-IV uterine prolapse, p53 Expression.

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I. INTRODUCTION

Pelvic organ prolapse (POP) is a condition in which one or more organs, such as the uterus, descends into the vaginal lumen resulting in protrusion of those organs in or through the vaginal canal. POP commonly occurs in older women. Barber and Maher reported that approximately 50% of women will eventually have POP in their lifetime. As the life expectancy increases, the incidence of POP was expected to be higher. This demographic shift is expected to be followed by an increase in the incidence of symptomatic POP from 46% to 200% or around 4.9 million to 9.2 million women in 2050 [1], [2].

The exact incidence of POP is still inconclusive. A study

conducted in the United States found that 40% of participants in the Woman Health Initiative (WHI) had various stages of uterine prolapse. Another study estimated that 1 in 8 adult women in the United States undergoes surgery for uterine prolapse by the age of 80 [3]. A higher POP incidence was reported in Sanglah Hospital Denpasar. The incidence of POP in Sanglah Hospital Denpasar increased from 82 cases in 2009 to 91 cases in 2015 and almost half of them underwent POP surgery [4].

Uterine prolapse occurs due to weakness in the muscle and ligament of the pelvic floor. Expression of p53 was suggested as one of the risk factors for POP. Some studies showed that low expression of p53 was found directly associated with low expression of estrogen receptor (ER) and progesterone receptors (PR) in the uterosacral ligament of women. These

findings indicate the possibility that p53 expression in the uterosacral ligament is related to the signaling process of female gonadal hormones [5], [6]. In contrast, Connell et al. reported that high activity of p53 was associated with a higher incidence of POP because women with POP had lower HOXA11 which was the suppressor of p53 expression [7], [8].

Although preliminary evidence and theory had shown that low p53 expression contributes to the risk of POP, this evidence was generally lacking and involved a small sample. In addition, the presence of contradictory studies regarding the expression of p53 also proved that additional evidence is still needed to establish the biomolecular pathophysiological theory of uterine prolapse, especially regarding p53 expression. Therefore, this study aims to determine the association between the p53 expression and the risk of grade III-IV uterine prolapse.

II. METHODS

This single-centered matched case-control study was conducted in the Reconstructive Urogynecology and Gynecology Polyclinic of Obstetrics and Gynecology Department of Sanglah General Hospital Denpasar from April until September 2021. This study had been approved by the Ethical Committee of Faculty Medicine Udayana University (1033/UN14.2.2.VII.14/LT/2022) and each patient involved in this study had signed written informed consent.

This study involved female patients aged 35-70 years old who underwent a total hysterectomy in Sanglah General Hospital. The case group consists of female patients who underwent a total hysterectomy due to a grade III-IV POP. Meanwhile, the control group consists of female patients who underwent total hysterectomy due to other diseases other than POP and were matched with the cases for age, parity, body mass index (BMI), and occupation. Female patients who had malignancy and endometriosis were excluded from this study because the expression of p53 is elevated in both conditions thereby may cause bias in the analysis. The sample size of this study was calculated using a power of 90% and α -error of 0.05 to detect a 20% difference. The minimum sample size required was 22 patients per group. All patients were selected using consecutive sampling until the number of required samples are met.

A history taking and standard physical and gynecological examinations were performed on all patients involved in this study. Additional laboratory and imaging examinations were obtained as needed. POP was graded using POP Quantification (POP-Q) during examinations in the Reconstructive Urogynecology and Gynecology Polyclinic. A grade III uterine prolapse was defined as a decrease in the uterine cervix through the hymen by more than 1 cm, but not more than (TVL-2) cm. A grade IV uterine prolapse was defined as a decrease in the uterine cervix to (TVL-2) cm or a complete uterine eversion. All patients in the control group had no uterine prolapse based on the physical and gynecological examinations.

A sacrouterine ligament sample was obtained from each patient from both groups during the total hysterectomy. Sacrouterine ligament samples were taken by cutting the

ligament with a scalpel knife starting from the lateral edge of the uterine cervix for about 1.5 cm long. Then, the sacrouterine ligament samples were brought to the Anatomical Pathology Laboratory of Sanglah General Hospital to determine the expression of p53 in the sacrouterine ligament samples. The level of p53 expression was obtained using a digital analysis method in which the preparations were magnified 400 times and the stained area was calculated using the ImageJ program. The level of p53 expression was calculated using pixels of stained area divided by pixels of all area $\times 100\%$.

All data obtained in this study were analyzed using Statistical Product and Service Solutions (SPSS) version 21.0 for Windows. Numerical data were presented using mean and standard deviation (SD) for the normally distributed data and were presented using median and interquartile range (IQR) for the non-normally distributed. Categorical data are presented in frequency and percentage. The difference in numerical data between the two groups was assessed using the independent t-test (if the data was normally distributed) or the Mann-Whitney test (if the data were not normally distributed). The Chi-square or Fisher's exact test was used to determine the association between categorical data. The Chi-square test was also used to obtain the odds ratio (OR) to assess the risk of POP in patients with low expression of p53. The results of the analysis with a p-value <0.05 were considered statistically significant.

III. RESULTS

A total of 44 female patients were involved in this study which consist of 22 patients with grade III-IV uterine prolapse and 22 patients with no prolapse. The characteristics of patients involved in this study were showed in Table I. It was shown that the baseline characteristics of patients in both groups were comparable ($p > 0.05$). Therefore, the characteristics of age, parity, BMI, and occupation of the patients were not significantly associated with the incidence of POP in this study.

A receiver operating characteristic (ROC) curve analysis was done to obtain the optimal cut-off for p53 expression. A p53 expression cut-off value of 146.90 was found to have a sensitivity of 80% and specificity of 80% with an area under the curve (AUC) of 0.74. A strong p53 expression was shown as high-intensity staining on the immunohistochemistry examinations (Fig. 1A). Meanwhile, a weak p53 expression was shown as low-intensity staining on the immunohistochemistry examinations (Fig. 1B). The p53 expression from each patient was calculated using a digital analysis method and categorized into low and high expressions based on this cut-off.

TABLE I: THE CHARACTERISTICS OF PATIENTS IN CASE AND CONTROL GROUP

Variables	Groups		p-value
	Case (n=22)	Control (n=22)	
Age (year), mean \pm SD	61.04 \pm 7.7	55.95 \pm 9.	0.052 ^a
Parity (n), mean \pm SD	3 (2)	0	0.126 ^b
BMI (kg/m ²), mean \pm SD	29.31 \pm 4.24	2 (1)	0.068 ^a
Occupation		26.8 \pm 4.6	
Non-heavy lifting job, n (%)	17 (77.3)	21 (95.4)	0.233 ^c
Heavy lifting job, n (%)	5 (22.7)	1 (4.5)	

TABLE II: THE ASSOCIATION BETWEEN THE P53 EXPRESSION AND GRADE III-IV UTERINE PROLAPSE

		Groups		OR	95%CI	p-value
		Case	Control			
p53 Expression	Low (< 146.90)	18	4	20.250	4.375 – 93.722	<0.001
	High (≥ 146.90)	4	18			

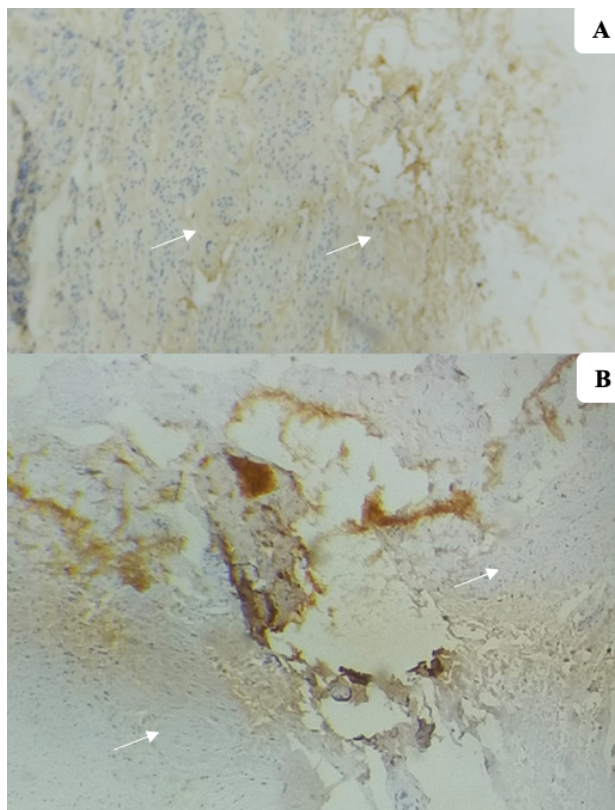


Fig. 1. The p53 Expression in the Sacrouterine Ligament Samples on Immunohistochemical Staining. A) A high p53 expression was indicated by a strong intensity staining found in the control group. B) A low p53 expression was indicated by a weak intensity staining found in the control group.

IV. DISCUSSION

Uterine prolapse is a common problem experienced by many women worldwide. Although it does not cause serious mortality or morbidity, uterine prolapse greatly affects the social, psychological, and physical life of women [9]. Weakened endopelvic fascia is the main cause of POP, but various factors also play a role in the weakness and damage to the fascia that eventually causes herniation of the organ and causes organ prolapse [10].

A previous study showed that age, parity, BMI, heavy lifting occupation, and history of vaginal births were the risk factors for POP [11]. Older age is associated with a higher risk of POP because the pelvic floor and pelvic muscle will become weaker with old age. Thus, older age is also associated with a higher degree of POP [9]. At the age of 80 years, it was reported that the risk for women in the United States to undergo at least one POP operation was 6.3% with the risk of undergoing reoperation reaching 30% [10]. Other studies in Indonesia also reported that almost all POP patients were aged older than 40 years old [5], [12]. The estrogen levels tend to decrease in older women (aged more than 40 years until before menopause) so that the production and degradation of extracellular matrix proteins that maintain

tissue stability and prevent collagen degradation will also decrease, causing weakness of the pelvic floor muscles which later manifests 5–10 years post-menopause. After menopause, the function of the ovaries and the estrogen levels will decrease even further. The decrease in estrogen causes a lower expression of type I collagen, which is elastic and resistant to pressure, and an increase in type III collagen, which plays a role in increasing tissue elasticity and stretch. Therefore, the strength of the pelvic organ and pelvic floor tissue is reduced. As a result, there is atrophy of the genital organs, weakness of the pelvic floor muscles, weakness of the sacrouterine and cardinal ligaments, and decreased ability of the endopelvic fascia to stretch resulting in POP [13], [14].

Vaginal birth and parity may significantly increase the risk of POP later in life. There is study found that most women with POP (80%) had more than two parities [12]. Similarly, Putra et al. also found a higher incidence of POP in multiparous women (68.5%) [13]. Also there is study that showed that the risk of POP increased 10-fold with one vaginal delivery [15]. Theoretically, a vaginal birth may cause causes trauma and strain of the endopelvic fascia, lacerations of the perineum and levator ani muscles, and mechanical and pudendal nerve damage. Trauma caused by pressure on the fetal head during vaginal delivery also causes pressure and tears in the pelvic floor muscles and fascia. Thus, a higher parity, mainly parity through vaginal birth, will increase the risk of POP. The risk of POP also increased even more with instrumental vaginal birth, especially when using forceps [13], [15], [16].

Regarding BMI, there were various controversial reports regarding the risk of POP in obese women. The WHI study stated that women with a BMI of 25-30 kg/m² had a risk of uterine prolapse increased by 31-39% and women with a BMI of more than 30 kg/m² had a risk of uterine prolapse increased by 40-75% [9]. There is study showed that greater BMI was a significant risk factor for POP [11]. However, another study analyzed the BMI as a continuous variable showed that higher BMI was a protective factor for POP [17]. Theoretically, there is chronic intraabdominal pressure increase in obese women which causes excessive load on the pelvic structures, including the pelvic floor muscles, fascia, and pudendal nerves. In addition, people who are obese generally have comorbidities such as diabetes which also contribute to poor tissue quality, and are more likely to experience symptoms of lower urinary tract syndrome (LUTS) such as urinary incontinence [16], [17]. A study also found that weight loss was not significantly associated with improvement in POP, thereby that the damage to the pelvic floor caused by weight gain was irreversible. However, weight loss was associated with improvement in prolapse symptoms [17].

Many previous studies found there was a significant association between heavy lifting jobs or heavy lifting in daily activities and the risk of uterine prolapse. Most uterine prolapse patients (78.6%) in India work as a manual laborers

[18]. Housewives in Surakarta also had a significant risk of POP due to heavy lifting in daily activities, such as carrying a child or heavy goods at home [12]. In theory, a heavy lifting job is considered to increase the risk of uterine prolapse because it increased the intra-abdominal pressure for a long time. However, some studies found the opposite results. There was no significant association between heavy lifting activities ≥ 12 hours/week and incidence of uterine prolapse. This difference in results may be due to variations in body position during heavy lifting where heavy load carried out in a sitting or bent position had a higher risk of causing an increase in intra-abdominal pressure compared to standing and squatting positions [19]. Another study also showed that the duration and weight of the load being lifted also determine the incidence of POP. Lifting a load of 26.89 ± 7.85 kg for 6.92 ± 7.76 years significantly increased the risk of uterine prolapse by 4 times (AOR 4.01, 95% CI 1.81-8.89) [20].

The result of this study showed that age, parity, BMI, and occupation were not the risk factors for POP incidence ($p > 0.05$) because this study used matching control for each patient in the case group. Therefore, the characteristics that should be confounding factors were successfully controlled by our study design.

Our study showed that low p53 expression in the sacrouterine ligament increased the risk of uterine prolapse up to 20-fold (OR 20.250, 95% CI 4.375-92.722, $p < 0.001$). This study was consistent with previous studies. Yamamoto et al. in 1998 found that p53 expression was significantly lower in the cardinal ligament culture cells of women with POP compared with women without POP ($p < 0.003$) [21]. Another study that compared the change in biological characteristics of fibroblast from the ligament of women with POP found that expression of p53 mRNA was 30-40% lower in a woman with POP than in non-prolapse woman [22]. Another study investigated the role of ER, PR, p53, and p21 in the pathogenesis of POP. Their result proved that women with POP had lower expression of ER, PR, p53, and p21 in the sacrouterine ligament ($p < 0.001$) [7].

Sacrouterine ligament had a vital role in supporting and stabilizing the uterus, cervix, and upper vagina. Damage to these ligaments significantly contributes to the development of uterine prolapse. The strength of the sacrouterine ligaments is highly dependent on the components of the cells and the extracellular matrix. The extracellular matrix of the sacrouterine ligament consists of fibrillar components, including collagen and elastin, and non-fibrillar components, including glycoproteins, proteoglycans, and hyaluronic. Collagen and elastin play an important role in the elasticity and resilience of the sacrouterine ligaments. The quantity and quality of collagen and elastin are regulated through the right balance between synthesis and degradation resulting in a dynamic elastin remodeling process [16], [23], [24].

The p53 tumor suppressor gene is a transcription factor that contributes to cell cycle regulation, cell repair, apoptosis, and cell aging in response to stress. The expression of p53 is tightly regulated to maintain the balance of cell proliferation. Increased p53 expression is commonly found during the late G1 phase (proliferation phase) to prevent the cells from entering the S phase (synthesis phase) resulting in the cell cycle stops. Thus, a low expression of p53 will cause cells to proliferate uncontrollably [8], [22], [24].

The p53 gene was also expressed by human fibroblast cells. The expression of p53 in fibroblast will prevent the proliferation of fibroblast and suppress fibronectin formation. On the other hand, low p53 expression will lead to an increase in fibroblast proliferation and fibronectin formation lead to an increase in type III collagen synthesis. A higher concentration of type III collagen in the sacrouterine ligaments makes the ligaments more rigid [25], [26]. Elastin synthesis also depends quantitatively and qualitatively on the stage of cell growth where this process is regulated by p53 expression. The highest accumulation of elastin was found when the fibroblast cells entered the quiet phase. The low expression of p53 will also cause a decrease in elastin production because fibroblast cells do not enter the quiet phase but continue to proliferate. Therefore, it can be concluded that the low expression of p53 in the sacrouterine ligament will affect the homeostasis of the extracellular matrix components of the ligament resulting in a more rigid sacrouterine ligament that is unable to support the pelvic organ [7], [24], [25].

V. CONCLUSION

Low p53 expression in the sacrouterine ligament was a significant risk factor for grade III-IV uterine prolapse. Large-scale multicentered studies may be needed in the future to confirm this association in different populations.

CONFLICT OF INTEREST

Authors declare that they do not have any conflict of interest.

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