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LOW TIMP1 EXPRESSION ON THE SACROUTERINA LIGAMENT AS A RISK FACTOR FOR DEGREES III-IV UTERINE PROLAPS

[1]I Nyoman Herlan Maharaga, [2,]* I Wayan Megadhana, [2]I Gede Megaputra, [2]Anak Agung Ngurah Anantasika, [2]I Made Darmayasa, [2]Ryan Saktika Mulyana

[1]PPDS-1 Obstetrics and Gynecology, Faculty of Medicine, Udayana University-Prof. Dr. IGNG Ngoerah, Denpasar, Bali, Indonesia
[2]Department/KSM of Obstetrics and Gynecology, Udayana University-Prof Hospital. Dr. IGNG Ngoerah, Denpasar, Denpasat, Bali, Indonesia.

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ABSTRACT

Objectives: To prove the low expression of TIMP1 in the sacrouterine ligament as a factor in the occurrence of grade III-IV uterine prolapse. **Methods:** This research is a study using an analytic observational design with case controls with a total sample of 44 cases and controls. The sample is the sacrouterine ligament 1.5 cm from the lateral edge of the cervix. The sample was then subjected to a paraffin block and further immunohistochemical preparations were made, to see the expression of TIMP1. **Results:** We found 14 (64%) women in this case group had a low TIMP1 expression, while only 3 (13%) women in the control group had a low expression of the gene. Low expression of TIMP1 in uterosacral ligament has 11 times risk of having advanced stage uterine prolapse (OR = 11.08.; 95% CI =2.48-49.46; p =0.001) compared with the high expression. The cut off value for TIMP-1 expression was 76.45. **Discussion:** The pathophysiology of PE is highly variable. Research by Xiao et al., stated that low expression of enzyme 1 alpha-hydroxylase increased the risk of severe PE by 2.83 times (OR, 2.83, 95% CI, 1.32-6.08). This study also proved that low placental alpha-hydroxylase expression was a risk factor for severe PE (OR 9.148; 05% CI 2.072-40.386, p=0.002). **Conclusions:** Low expression of TIMP-1 in the sacrouterine ligament is a risk factor for grade III and IV uterine prolapse. The cut off value for TIMP-1 expression was 76.45, with a sensitivity value of 86.4%, with a specificity of 63.6%; area under the curve (AUC) of 0.785; and a 95% confidence interval (CI) of 0.649-0.922.

Keywords: TIMP1, uterosacral ligament, uterine prolapse, pelvic organ prolapse.

INTRODUCTION

Uterine prolapse is a protrusion of the uterus into the vagina or out of the vagina caused by weakness of the pelvic floor muscles, especially the levator ani muscles, ligaments and fascia that support the uterus. Uterine prolapse can occur together with vaginal wall prolapse including bladder and rectum and cause disturbances that affect a woman's quality of life. Until now, this disorder is a common health problem in women and has not received much special attention in its management. Uterine prolapse can be a burden for patients, both psychologically, socially, and economically and quality of life. The burden is getting heavier if proper management is not carried out.

From various epidemiological studies, the incidence of uterine prolapse and pelvic organ prolapse is quite diverse. Aytan et al's study in 1320 subjects in Turkey found a prevalence of pelvic organ prolapse of 27.1%. 1 A study in Yogyakarta reported that pelvic organ prolapse occurred in 90% of subjects with vaginal delivery, of which 70% were uterine prolapse. 2A retrospective study at a hospital gynecology polyclinic in Denpasar reported a prevalence of pelvic organ prolapse of 11.38% within 2 years. The prevalence of pelvic organ prolapse has also been found to increase by 40% for every decade of a woman's age Related to pelvic organ prolapse, previous studies found that in patients with pelvic organ prolapse, TIMP1 expression in the connective tissue of the vaginal wall was lower than individuals without pelvic organ prolapse. Based on the background explanation above, researchers wanted to examine the role of TIMP 1 expression in the occurrence of grade III-IV uterine prolapse.

***Corresponding Author: I Wayan Megadhana,**
2Department/KSM of Obstetrics and Gynecology, Udayana University-Prof Hospital. Dr. IGNG Ngoerah, Denpasar, Denpasat, Bali, Indonesia.

The uterus is supported by several ligaments: the sacrouterine ligament, the cardinal ligament, the utero-ovarian ligament, the round ligament and the broad ligament. In cases of uterine prolapse, weakness of the pelvic floor tissue is always found. It is necessary to know in advance the three levels of pelvic floor tissue according to De Lancey. The first level of pelvic floor tissue is the endopelvic fascia, which includes the cardinal and sacrouterine ligament complexes and the pubocervical and rectovaginal fascia. The second level of pelvic floor tissue is the levator ani muscle or endopelvic diaphragm, and it is followed by the urogenital diaphragm at the third level. The mechanism for uterine prolapse is due to the weakness of the levator ani, causing the burden to hold the pelvic organs to be shifted to the sacrouterine ligament. Which in turn will result in the ligamentum sacrouterina will weaken as well. It is this weakness in the sacrouterine ligament that will cause grade III-IV uterine prolapse. One of the main structural proteins that make up the sacrouterine ligament are collagen type 1 and collagen type III. Collagen is also one of the structural proteins in the extracellular matrix, and plays a role in maintaining the structural integrity of the pelvic floor. The decrease in collagen levels is thought to occur due to an increase in the rate of collagen degradation and a decrease in collagen synthesis, which is caused by the MMP enzyme and the activity of the MMP enzyme can be suppressed by the TIMP enzyme. 5 Until now, there are four types of TIMP known to exist in humans, namely TIMP1, TIMP-2, TIMP-3, and TIMP-4. The four TIMP enzymes inhibit the activity of 23 types of MMPs found in the human body. The balance between MMP and TIMP levels plays an important role in controlling the rate of degradation of the extracellular matrix around cells. Of the four types of TIMP, TIMP1 is a specific inhibitor of MMP, has the widest spectrum of action against MMP compared to other TIMP types, and can combine with most types of MMP.

METHOD

The design of this study is a study that uses an analytic observational design with control cases that meet the inclusion and exclusion criteria. The sample will be divided into a control group and a case group, with 22 samples in each group. The case group was patients with grade III-IV uterine prolapse who had had a hysterectomy for indications of benign abnormalities such as uterine myomas or bleeding disorders. The research was conducted in the Delivery Room of the Emergency Room at Prof. Hospital. Dr. IGNG Ngoerah, Denpasar. Placental examination was carried out at the Integrated Biomedical Laboratory, Faculty of Medicine, Udayana University, from November 2020 to May 2021. The inclusion criteria in this study were grade III-IV uterine prolapse and non-prolapsed uterus that had undergone total hysterectomy, were willing to participate in the study and signed informed consent.

Exclusion criteria in this study were malignancy, endometriosis, history of previous HRT therapy. Data analysis in this study used SPSS software version 21 for Windows, the data normality was tested using the Shapiro Wilk test. Normality test results obtained data on age, BMI (Body Mass Index), parity and occupation in the case and control groups.

RESULTS

Research has been carried out on the case and control groups, each of which has 22 samples. The research results were obtained as follows:

Table 1. Distribution of Age, Parity, BMI, and Occupational Characteristics in the Case and Control Groups

Variable	Group		P value
	Case (n=22)	Control (n=22)	
Age (years), mean±SD	61.04±7.7	59.95±9.0	0.051a
Parity (children), median (IQR)	3(2)	2(1)	0.125b
BMI (kg/m2), mean±SD	29.31±4.24	26.8±4.6	0.068a
Work			0.233c
• Light, n (%)	17(77.3)	21(95.4)	
• Weight, n(%)	5(22.7)	1(4.5)	

Note: a=Independent T-test, b= Mann-Whitney Test, c=Fisher's Test

Based on the average comparison test, with the independent t test mean, median, with the Mann-Whitney and Fisher tests, it was found that age, parity, BMI, and occupation in the case group compared to the control group were not statistically significantly different. Thus, it can be concluded that the characteristics of the subjects, which included the mother's age, parity, BMI and occupation, were not risk factors for grades III-IV uterine prolapse (p <0.05).

Table 2. Results of TIMP1 Expression Analysis as a Risk Factor for Grade III-IV Uterine Prolapse

Risk Factors		*Group*		*Odd Ratio*	*CI 95%*	*P*
		Case (n=22)	*Control (n=22)*			
TIMP1 expression	*Low (<76.45)*	*14*	*3*	*11.08*	*2.48-49.46*	*0.001**
	Tall (≥76.45)	*8*	*9*			

[a]significant (p<0.05)

Table 2 above shows that in the case group there were 14 (63%) with low TIMP1 expression and in the control group there were 3 (13%) with low TIMP1 expression. Thus, low expression of the TIMP1 enzyme in the sacrouterine ligament significantly increased the risk of uterine prolapse by 11.08 times compared to high TIMP1 expression (OR = 11.08, 95% CI = 2.48-49.46, p = 0.001).

GFigure 1. Sacrouterine Ligament TIMP1 Expression in the Case Group

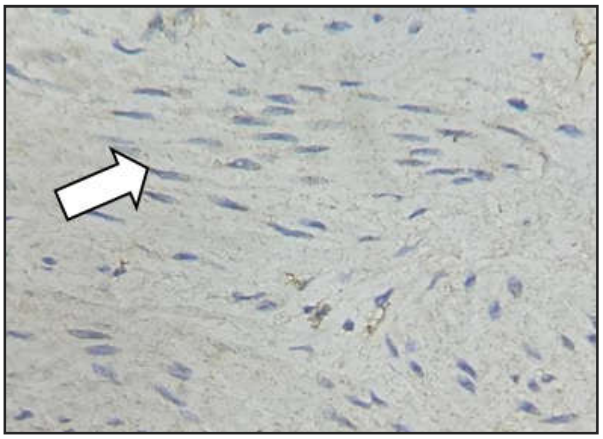
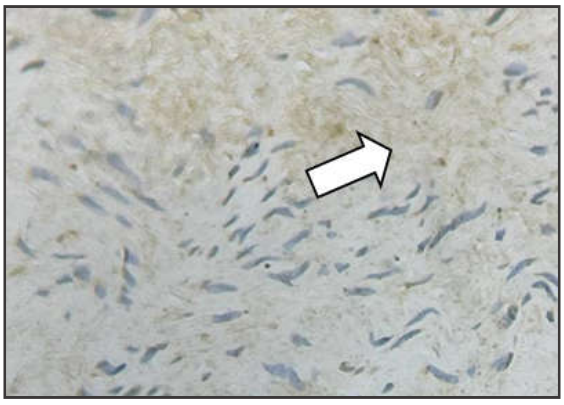


Figure 2. Sacrouterine Ligament TIMP1 Expression in the Control Group



TIMP1 expression can be seen in Immunohitochemical staining with brown cell nuclei (decreased intensity) in the case group and increased in the control group.

DISCUSSION

Characteristics of Age, BMI, Parity, and Occupation in Case and Control Groups

Uterine prolapse is a reproductive health problem that is quite common in women and can affect the patient's life, both from psychological, physical, social, and quality of life aspects. The main cause of this disorder is the decreased strength of the supporting tissues of the pelvic organs which is a complex interaction of the levator ani muscles, the vagina, and the connective tissue of the vaginal wall. 8 Apart from these causes, the incidence of uterine prolapse is also significantly influenced by several risk factors (multifactorial).), such as increasing age, race, family history, increasing BMI, increasing parity status, vaginal delivery, chronic constipation, menopause, and low socioeconomic status. In this study, immunohistochemical measurement of TIMP-1 expression was carried out to determine its role as a risk factor for grade III-IV uterine prolapse. In addition to examining TIMP-1 expression, several factors that could influence the incidence of uterine prolapse, such as age,

BMI, parity, and occupation were studied as control variables. In the control variable distribution data presented in Table 1, it can be seen that each study sample in the case and control groups has different characteristics.

Uterine prolapse can occur in all age ranges, but this disorder is more common in women of older age. especially reproductive function Scott's theory states that increasing age causes a loss of tissue strength in supporting the pelvic floor. The weakness is caused by hypoestrogen conditions or decreased levels of estrogen. Estrogen decline begins to occur at the age of >40 years until just before the start of menopause. The hormone estrogen plays an important role in stimulating the skin to increase the synthesis of hydroxypoline and proline as tissue collagen. With decreased estrogen levels in the long term, In line with this theory, the results of this study found that the mean age in the case group (women with uterine prolapse) was higher than the control group. The mean age in the case group was 61.04 ± 7.7 years, while the control group was in the range of 55.95 ± 9.0 years. The results of this study were similar to Shrestha's study which stated that the incidence of grade I-IV uterine prolapse was higher at the age of >60 years (90.2%) compared to the age range of 41-60 years (83%). Meanwhile, Sayko's study found that the majority of grades I-IV uterine prolapse cases occurred at the age of >50 years with a percentage of 90.8%. More specifically, one study in Indonesia found that the average age of women who experienced grade III-IV uterine prolapse was 51.2 years.

Several theories state that obesity is a modifiable risk factor for uterine prolapse. Uterine prolapse can occur in obese women due to increased intra-abdominal pressure. This increase places an excessive burden on the pelvic floor muscles, causing weakness in the pelvic floor muscles. In the Women's Health Initiative (WHI) study, it was stated that women who are overweight (BMI 25-30 kg/m2) have an increased risk of uterine prolapse by 31-39%, while women who are obese (BMI> 30 kg /m2) the risk increases by 40-75%. Hardianti and Pramono's study (2015) also stated that the majority of grades I-IV uterine prolapse cases occurred in women with BMI≥25 kg/m2 (51.8%) compared to BMI<25 kg/m2 (48.2%). However, different results were reported by Sayko *et al.,* (2018), where the majority of grade III and IV uterine prolapse patients are not obese. Further research regarding the relationship of obesity with the incidence of pelvic organ prolapse (POP) still reports inconsistent results. Other studies reported a significant association between obesity and POP, including uterine prolapse with p=0.004. The results of this study were inconsistent with Hardianti and Pramono's (2015) study which reported an insignificant relationship between BMI and uterine prolapse (p=0.643).

Based on the number of parities, uterine prolapse disorders tend to occur in women with high parity. Multiparity or a history of more than one birth can lead to progressive labor injury to the endopelvic fascia and levator ani muscle lacerations.11 Vaginal delivery is also known to be a major risk factor for grade III-IV uterine prolapse with a risk of 8.4 times higher than women who do not have vaginal delivery. The risk will increase 10.9 times if you have more than four vaginal deliveries (Jelovsek *et al.,* 2007; O'Boyle *et al.,* 2002; Kim *et al.,* 2007). Apart from the method of delivery, long duration and difficult labor contribute significantly to damage to the pelvic floor. Dangerous actions performed by unskilled birth attendants, such as pressing on the fundus, using uterotonics (uterine contraction-stimulating drugs), and incorrect placenta delivery techniques can also cause damage to the pelvic floor, triggering uterine prolapse. (Tabaquero, 2017). In this study, the median parity value in the case group (3, IQR=2) was higher than the control group (2, IQR=2). The majority of uterine prolapse patients in the studyDevkota *et al.,* (2020) also reported experiencing labor or parity 3-4 times (51.1%). The results of this study are also similar to one study in Taiwan which reported that the median parity in POP patients was 3 with values ranging from 0-7. (Liao *et al.,* 2021). This suggests that overstretching during labour, tearing of the birth canal, and multiparity are major obstetric predisposing factors that trigger POP, including uterine prolapse.(Walker and Gunasekera, 2011). Occupational activities that cause increased intra-abdominal pressure, such as lifting heavy objects and activities requiring the Valsalva maneuver can directly contribute to POP, including uterine prolapse.(Gumanga *et al.,* 2014). However, this theory is not in line with the research resultsDevkota *et al.,* (2020) in Nepalese women, lifting or carrying heavy loads for a duration of ≥12 hours per week was not significantly associated with uterine prolapse (OR=0.18; 95% CI=0.05-0.71; p=0.33).

The majority of the case group in this study did light work (77.3%) and found no significant difference in the data distribution of the case and control groups (p = 0.233). Jobs included in the light category in this study were housewives; private employees; Teacher; as well as civil servant employees, while jobs that fall into the heavy category are farmers. Similar results were also obtained in researchPutra *et al.,* (2020) that the majority of uterine prolapse patients did light work (63.63%) and found no significant difference between the workload in the case and control groups (p=0.226). Study Devkota *et al.,* (2020) reported that housewives were 2.29 times at risk of experiencing uterine prolapse (95% CI = 1.51-3.48; p <0.01) compared to women who worked in the agricultural sector or farmers (OR = 1.00). IRT are prone to uterine prolapse due to sitting position while working or resting at home. Women who work longer in a sitting or bent position are at two to three times higher risk of uterine prolapse than women who work more in a standing position.(Devkota *et al.,* 2020).

Low TIMP-1 Expression as a Risk Factor for Grade III-IV Uterine Prolapse

In this study, low expression of TIMP-1 was found more in the sacrouterine ligament samples of women who experienced uterine prolapse (n=14) compared to women who did not experience uterine prolapse (n=3). This study also showed that low TIMP-1 expression in the sacrouterine ligament significantly increased the risk factors for grade III-IV uterine prolapse up to 11 times (OR 11.08; 95% CI 2.48 – 49.46; p=0.001), so the null hypothesis (Ho) is rejected and the alternative hypothesis (Ha) is accepted. Results This study is in accordance with previous studies byChen *et al.,* (2002), Wang *et al.,* (2014), and Hu *et al.,* (2017). In a previous study by Chen (2002), TIMP-1 expression was reported to be significantly lower (p=0.03) in vaginal epithelial samples of women with uterine prolapse and urinary incontinence compared to the control group. Simultaneously, an increase in MMP-1 expression was also found (p=0.05) so that there was an increase in the MMP-1/TIMP-1 ratio (p=0.04) in women with uterine prolapse and urinary incontinence. (Chen *et al.,* 2002). In another study by Wang *et al.,* (2014), TIMP-1 expression was also reported to be significantly lower (p<0.05) in female patients with uterine prolapse (n=72) compared to controls. This study focused on the interaction of MMP and TIMP expression in patients with uterine prolapse, where it was also found that TIMP-1 expression was negatively correlated with MMP-3 (p=0.000, r=-0.944), and MMP-9 (p=0.003, r =-0.891), as well as a positive correlation between MMP-1 and TIMP-1 (r2=0.614). *(Wang et al.,* 2014). The study by Hu (2017) also found similar results, where the expression of TIMP-1 in female patients with uterine prolapse was significantly lower compared to the control group (p<0.05). This study also found a significant (p<0.05) decrease in the expression of collagen types I and III in the group of female patients with uterine prolapse compared to controls, consistent with previous theories regarding the role of collagen, MMP, and TIMP in uterine prolapse. (Hu *et al.,* 2017). The fascia and ligaments of the uterus are mostly composed of connective tissue

with collagen as the main structural protein. Collagen types I, II, and V are the main structural collagens of the vaginal epithelium and endopelvic fascia, with type I collagen playing a role in providing strength and type II collagen playing a role in tissue elasticity. (Chen and Yeh, 2011). The ability of the pelvic organs to maintain their anatomical position is closely related to the pelvic organ supporting tissue components at the molecular level. The mechanical stability of the genitourinary tract depends on intact, functional collagen fibers that serve to support the urinary bladder, urethra, and pelvic organs. (Zhang *et al.,* 2017). Meanwhile, collagen degradation depends on the activity of MMP enzymes, a group of proteolytic enzymes that play a role in the degradation process of almost all extracellular matrix protein components produced by fibroblast cells.(Chen *et al.,* 2010). MMP enzyme activity in degrading collagen in the supporting tissue of the pelvic organs is regulated by the TIMP enzyme *(Chen et al.,* 2002; Phillips *et al.,* 2006; Zhu *et al.,* 2021). TIMP is a specific MMP inhibitor that has the broadest spectrum of action against MMPs compared to other MMP inhibitors and can be combined with most other types of MMPs. Molecularly according to previous studies, TIMP-1 can bind MMP-1, MMP-3, and MMP-9, TIMP-2 can bind MMP-2, and TIMP-3 can bind MMP-1 and MMP-9 (Chen *et al.,* 2002; Wang *et al.,* 2014). By forming a complex with MMP (1:1 ratio), TIMP plays a role in inhibiting MMP activity and preventing MMP binding with its substrate, thereby limiting the rate of collagen degradation and maintaining the dynamic balance of collagen in connective tissue.(Hu *et al.,* 2017). Therefore, it can be concluded that low concentrations of TIMP-1 in the sacrouterine ligament can lead to increased concentrations of MMP. Ultimately, there is an increase in collagen degradation by MMP in the supporting tissues of the uterus, causing disruption of the mechanical and structural stability of the pelvic organs so that uterine prolapse occurs. (Phillips *et al.,* 2006; Chen and Yeh, 2011; Ruiz-Zapata *et al.,* 2014; Zhu *et al.,* 2021).

This research has several limitations. First, even though the number of samples used in this study was in accordance with the required minimum sample calculation. the number of samples used is still relatively small. Second, the inclusion criteria only included patients with grades III and IV uterine prolapse due to the limited number of patients with grades I and II uterine prolapse that could be found. Nonetheless, this research has made an important scientific contribution in molecular aspects related to uterine prolapse which can be used as a factor determining the prognosis, etiology, and treatment of the disease in the future. The use of other methods to quantify the protein level and enzymatic activity of MMPs and TIMPs and the effects of hormonal therapy could be used to increase the scope and validity of the findings of this study. In this study, it was found that low TIMP-1 levels were associated with uterine prolapse, so one of the practical methods of molecular therapy that has the potential to be developed in the future is to increase TIMP-1 expression. Increasing the expression of TIMP-1 can ultimately reduce collagen degradation so that the strength and elasticity of the supporting tissues of the pelvic organs can be maintained.

CONCLUSION

Low expression of TIMP-1 in the sacrouterine ligament is a risk factor for grade III and IV uterine prolapse. The cut off value for TIMP-1 expression was 76.45, with a sensitivity value of 86.4%, with a specificity of 63.6%; area under curve (AUC) of 0.785; and a 95% confidence interval (CI) of 0.649-0.922.

Conflict of Interest

The author declares that there is no conflict of interest regarding the publication of this research article.

Suggestion

Some suggestions related to the results of this study are as follows: This study can be used as a pilot study for further research related to differences in TIMP-1 expression in grades III and IV uterine prolapse, so that TIMP-1 expression can be used as a reference for predictors of progression or level severity of uterine prolapse in later life.

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