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HIGH EXPRESSION OF TRANSFORMING GROWTH FACTOR β-1 (TGF β-1) AND MATRIX METALOPROTEINASE-9 (MMP-9) IN THE SACROUTERINE LIGAMENTUM AS A RISK FACTOR FOR THE OCCURRING OF GRADE III-IV UTERINE PROLAPS

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ABSTRACT

Background: The incidence of pelvic organ prolapse increases with age. A retrospective study in a gynecology clinic at a hospital in Denpasar reported a prevalence of pelvic organ prolapse of 11.38%, over a period of 2 years. One type of TGF-β that influences the remodeling process in the sacrouterine ligament is TGF-β1. TGF-β1 controls collagen synthesis and degradation in pelvic connective tissue by stimulating tissue inhibitor of metalloproteinase (TIMP) synthesis and inhibiting MMP activity. MMP-9 is secreted by a large number of cell types, including neutrophils, macrophages, and fibroblasts. MMP-9 is thought to be related to cell migration, invasion and tissue remodeling in the reproductive process. This research is to prove the expression of Transforming Growth Factor Beta 1 (TGF-β1) and high expression of Matrix Metalloproteinase-9 (MMP-9) as risk factors for grade III-IV uterine prolapse..

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Method: This type of research is research that uses an analytical observational design with case control. The case was a patient with grade III-IV uterine prolapse who had undergone a total vaginal hysterectomy. Controls were non-uterine prolapse patients who had undergone total hysterectomy for indications of benign abnormalities such as uterine myoma or bleeding disorders. Each group was then divided into high and low TGF-β1 and MMP-9 expression.
Results: In this study, high TGF-β1 expression was more frequently found in sacrouterine ligament samples from women who experienced uterine prolapse compared to women who did not experience uterine prolapse. The results showed that high TGF-β1 expression in the sacrouterine ligament significantly increased the risk factor for grade III-IV uterine prolapse up to 14.00-fold (OR 14.00; 95% CI 2.370 – 82.717; p=0.004). This study also showed that high MMP-9 expression in the sacrouterine ligament significantly increased the risk factor for grade III-IV uterine prolapse up to 8,867-fold (OR 8.867; 95% CI 2.246–34.998; p=0.002).
Conclusion: High expression of TGF-β1 and MMP-9 is a risk factor for grade III-IV uterine prolapse.

Keywords: Prolapse, Transforming Growth Factor, Matrix Metaproteinas

INTRODUCTION

Uterine prolapse is the herniation of the uterus from its normal anatomical location into the vaginal canal, through the hymen, or even out of the vaginal introitus caused by weakening of the supporting structures of the pelvic organs. The structures that play an important role in maintaining the uterus in its normal position are the sacrouterine and cardinal ligament complexes. Clinically, uterine prolapse is not life threatening, but it greatly affects the quality of life both physically, psychosocially and economically. The incidence of pelvic organ prolapse increases with age.(1)Data in the United States (US) shows that around 50% of elderly women experience pelvic organ prolapse to varying degrees (2). In developing countries like Nepal, more than 1 million women out of around 15 million women experience uterine prolapse or the equivalent of around 7% of Nepal's female population. (3). The incidence of pelvic organ prolapse is projected to increase by 46%, to 4.9 million, by 2050 (4). A study in Yogyakarta reported that pelvic organ prolapse occurred in 90% of subjects with vaginal birth, of which 70% were uterine prolapse. (5). A retrospective study in a gynecology clinic at a hospital in Denpasar reported a prevalence of pelvic organ prolapse of 11.38%,

over a period of 2 years. The prevalence of pelvic organ prolapse was also found to increase by 40% for every decade of a woman's age(6).

Regarding pelvic organ prolapse, previous research found that in patients with pelvic organ prolapse, the expression of TGF-β1 and MMP-9 in the sacrouterine ligament was higher than in individuals without pelvic organ prolapse(7). However, until now there has not been much research examining the expression of this gene in the sacrouterine ligament. Based on the background explanation above, researchers want to examine the role of TGF-β1 and MMP-9 expression in the occurrence of grade III-IV uterine prolapse..

The mechanism of uterine prolapse is still not very clear because it is multifactorial. The etiology of uterine prolapse is multifactorial. In general, uterine prolapse is caused by a decrease in the strength of the tissues supporting the pelvic organs, which is a complex interaction of the levator ani muscles, the vagina, and the connective tissue of the vaginal wall. In women without uterine prolapse, where the levator ani muscles have good tone and the vaginal canal is of adequate length/depth, the upper part of the vagina is almost in a horizontal position when standing. The position of the top of the vagina results in a "flap-valve" mechanism, where the top of the vagina presses on the levator ani muscle when there is an increase in intra-abdominal pressure. If the tone of the levator ani muscle decreases, the upper part of the vagina changes position from a horizontal to a semi-vertical position, resulting in widening of the genital hiatus (the distance from the external urethral meatus to the midline of the posterior hymen), which causes the pelvic organs to depend on connective tissue (ligament) as support. If the connective tissue also experiences abnormalities, for example due to decreased collagen levels or ligament tears, pelvic organ prolapse can occur (8).

One pathophysiology that is widely described is weakness of the sacrouterine ligament. Weakening of this ligament may be caused by an imbalance in the synthesis and degradation of connective tissue components such as elastin, smooth muscle and other matrices that make up the ligament. Pelvic floor soft tissue injuries, such as the levator ani muscle, pudendal nerve, and paravaginal fascia such as during childbirth, constipation, and a higher body mass index are

among the main risk factors for uterine prolapse. After injury, wound healing occurs which aims to restore tissue integrity. However, even though healing occurs, the damaged muscles, nerves, ligaments and vaginal walls cannot completely return to their normal condition as before the injury or what is often called remodeling (9). This remodeling process involves wound contraction and continuous synthesis and degradation of collagen. Degradation is mediated by various types of enzymes, especially MMPs, which are produced by fibroblasts. The synthesis and secretion of MMPs is tightly regulated by growth factors such as TGF-β, cytokines, and phagocytic stimulation to avoid degradation of essential proteins from the extracellular matrix, which can lead to impaired healing. (10)

One type of TGF-β that influences the remodeling process in the sacrouterine ligament is TGF-β1. TGF-β1 controls collagen synthesis and degradation in pelvic connective tissue by stimulating tissue inhibitor of metalloproteinase (TIMP) synthesis and inhibiting MMP activity(11). Research shows increased expression of TGF-β1 and pathological conditions including reduced fascia strength have been studied in inguinal hernias. In women with uterine prolapse, changes in TGF-β1 expression were found in both fibroblasts and pubovaginal fascia. Pathogenesis that can describe the relationship between TGF-β1 and uterine prolapse is the lack of balance of TGF-β1 levels which triggers a decrease in the function of the uterosacral ligaments, thus facilitating uterine prolapse (12).

MMP-9 is a subgroup of gelatinases and is known as gelatinase B because of its ability to degrade gelatin (8). MMP-9 is secreted by a large number of cell types, including neutrophils, macrophages, and fibroblasts (7).MMP-9 is thought to be related to cell migration, invasion and tissue remodeling in the reproductive process (Rahajeng, 2018). In uterine prolapse, MMP-9 may participate in mediating increased collagen and elastin degradation (12).

Based on the background above, TGF-β1 and MMP-9 may play a role in the pathogenesis of uterine prolapse. High expression of TGF- β1 and MMP-9 may be a risk for more severe uterine prolapse (grade III/IV). Knowledge about the role of these two markers in uterine prolapse is important so researchers are interested in conducting this research. The aim of this study was to prove high expression of Transforming Growth

Factor Beta 1 (TGF- β1) and Matrix Metalloproteinase-9 (MMP-9) as risk factors for grade III-IV uterine prolapse.

MATERIALS AND METHODS

This study used an analytical observational design with case control. The case was a patient with grade III-IV uterine prolapse who had undergone a total vaginal hysterectomy. Controls were non-uterine prolapse patients who had undergone total hysterectomy for indications of benign abnormalities such as uterine myoma or bleeding disorders. Each group was then divided into high and low TGF-β1 and MMP-9 expression.

This research was previously carried out at Sanglah Hospital, Denpasar from November 2020 to May 2021. Material taken from research samples is still stored in the Anatomy Pathology Laboratory at Sanglah Hospital in the form of pieces of the sacrouterine ligament in patients with grade III-IV uterine prolapse and non-uterine prolapse who have undergone total hysterectomy. . With the approval of previous researchers, this research was permitted to use material from the research sample but with different research variables. Research sampling used previous research samples regarding research on the expression of P53, TIMP1, and HOXA13 as risk factors for grade III-IV uterine prolapse. This research lasted for 6 months from July 2022 to December 2022. The number of samples in this study consisted of 22 case groups (patients with uterine prolapse) and 22 control groups (patients with non-uterine prolapse).

The technique used in carrying out this research is to carry out anamnesis, physical examination and urogynecological examination on an affordable population, then inclusion and exclusion criteria are determined on this affordable population. Each subject from the case group who met the inclusion criteria received informed consent and subjects from the control group (non-prolapse) were sought according to the operational definition of variables who had undergone total hysterectomy. Next, each subject from the case and control groups underwent sampling from the sacrouterine ligament which was still attached to the uterus, by cutting the ligament with a scalpel starting from the lateral edge of the uterine cervix for 1.5 cm. The samples that have been taken are then subjected to a paraffin block and then immunohistochemical preparations are made. Immunohistochemical examination was carried out to assess the expression of TGF-β1 and MMP-9. The examination method is carried out by examining the expression of the TGF-β1 and MMP-9 genes using the immunohistochemical method on the sacrouterine ligament, while

examining grade III-IV uterine prolapse using a gynecological examination using a speculum..

The final stage of this research is data analysis. Data was processed using the SPSS 21 for Windows program. To determine the odds ratio of high TGF-β1 and MMP-9 expression with the occurrence of grade III-IV uterine prolapse, chi-square was used.

RESULTS

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

In this case control study, the Shapiro Wilk test was carried out to determine the normality of data on the variables age, BMI and parity. In the Shapiro Wilk test, data is said to be normally distributed if the significance value (p) is> 0.05 and not normally distributed if the p value is <0.05. From the results of the Shapiro Wilk test, a p value of >0.05 was obtained for data on age in the case group, BMI in the control group, and parity in the control group, which shows that the data is normally distributed. However, a p value <0.05 was also found for age in the control group.

BMI in the case group, as well as parity in the case group, showed that the data on these variables were not normally distributed. Because there was data that was not normally distributed, a homogeneity test was then carried out using Lavene's test. In Lavene's test, data is said to be homogeneous if the p value is> 0.05 and not homogeneous if the p value is <0.05. Based on the results of the homogeneity test, a p value of>0.05 was obtained for the three variables, which shows that the data for these three variables are homogeneous.

Tabel 1 Distribution of Sample Characteristics in Case and Control Groups

Variabel	Groups		p
	Cases (n=22)	Control (n=22)	
Age (years), mean±SD	61,18±5,26	5	<0,001[a]
BMI (kg/m^2), mean±SD	29,32±4,25	50,09±6,36 2	0,76[a]
Parity (Child), median (IQR)	3 (2)	6,91±4,55 2 (2)	0,014[b]
Job			0,233[c]
Light n (%)	19 (86,4)	22 (100)	
Heavy, n (%)	3 (13,6)	0 (0)	

[a]T-*independent* test; [b]Mann-Whitney test, [c]*fisher's exact* test

The characteristics of the case and control groups in this study are presented in Table 5.1 which shows the mean, standard deviation (SD), median, interquartile range (IQR), as well as differences in data in the two groups based on the T-independent, Mann-Whitney, and Fisher's tests. exact. Data in the case and control groups are said to be significantly different if the p value is <0.05 and there is no significant difference if the p value is> 0.05. From the results of the T-independent test, a p value <0.001 was obtained for the age variable, which showed a significant difference between the case and control groups, while for the BMI variable, a p value = 0.76 was obtained, which did not show a significant difference between the two groups. From the results of the Mann-Whitney test on the parity variable, the value of p = 0.014 was obtained, which shows that there is a significant difference between the means of the two groups. From the results of the Fisher's exact test on the work variable, the p value = 0.233 was obtained, which shows that there is no significant difference between the two groups.

B. Risk of Grade III-IV Uterine Prolapse Based on Transforming Growth Factor β-1 (TGF-β1) Expression

In this study, TGF-β1 expression was categorized into two, namely low and high based on the cut-off value from the results of Receiver Operating Characteristic (ROC) curve analysis. All TGF β-1 expression data was obtained through immunohistochemical examination which was calculated semi-quantitatively and then analyzed into a ROC curve. From the results of the ROC curve analysis, the cut-off value was 50.43 with a sensitivity value of 63.6%; specificity 36.4%; area under curve (AUC) of 0.678; and the 95% confidence interval (CI) is 0.518-0.837.

Furthermore, to prove the hypothesis in this study that high TGF-β1 expression in the sacrouterine ligament plays a role in the risk of grade III-IV uterine prolapse, a chi-square test was carried out. Based on the results of the chi-square test presented in Table 5.2, it is proven that high TGF-β1 expression is a risk factor for grade III-IV uterine prolapse with a risk of 14 times (95% CI=2.37-82.717; p=0.004) compared to expression Low TGF-β1. These results prove that H0 is rejected and Ha is accepted because the results are significant with a p-value of 0.004, meaning that high TGF-β1 in the sacrouterine ligament can provide a risk of uterine prolapse..

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Tabel 2. Hasil Analisis Ekspresi *Transforming Growth Factor β-1* (TGF-β1) sebagai Faktor Risiko Prolaps uterusDerajat III-IV

Risk Factor		Groups		Odd Ratio (OR)	CI 95%	p
		Case (n=22)	Control (n=22)			
TGF β-1 expression	Low (< 50.43)	7	14	14.00	2.370-82.717	0.004*
	High (> 50.43)	15	8			

*signifikan (p<0,05)

Microscopic Image of TGF-β1 Expression in the Sacrouterine Ligament

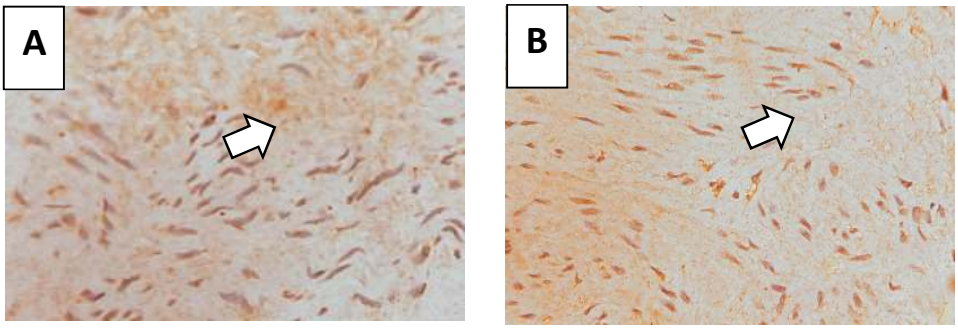


Image 1. Expression of TGF-β1 in Sacrouterine Ligament Tissue by Immunohistochemical Staining

Information:

A) Case Group. It can be seen that TGF-β1 expression (brown color) increases.

B) Control Group. The expression of TGF-β1 (brown color) appears to decrease.

C. Risk of Grade III-IV Uterine Prolapse Based on Matrix Metalloproteinase-9 (MMP-9)

In this MMP-9 research it was also categorized into two, namely low and high based on the cut-off value from the ROC curve results. So we got a cut-off value of 28.24 with a sensitivity value of 50%; specificity 50%; area under curve (AUC) of 0.514; and the 95% confidence interval (CI) is 0.338-0.691.

The results obtained from the ROC curve, namely 0.514, indicate that the results are considered acceptable because they are above the threshold of 0.5. All positive values above the threshold will be “True Positive” in predicting the diagnosis of a disease. Even though the results of the curve are still low, they have exceeded the threshold on the ROC curve.

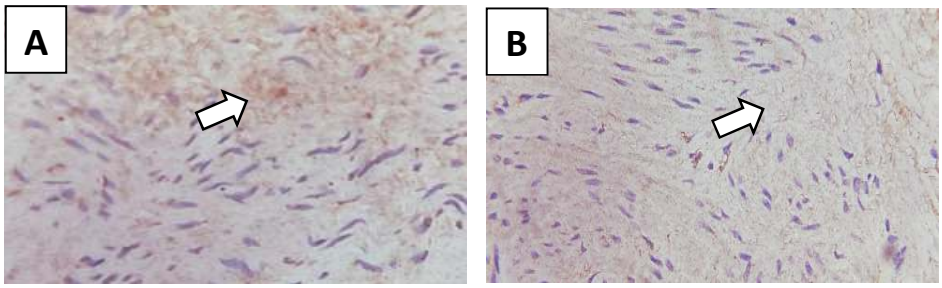
To prove the hypothesis in this study that high MMP-9 in the sacrouterine ligament plays a role in the risk of grade III-IV uterine prolapse, a chi-square test was carried out. Based on Table 5.4, it is proven that high TGF-β1 expression is a risk factor for grade III-IV uterine prolapse with a risk of 8,867 times (95% CI=2,246-34,998; p=0.002) compared to low MMP-9 expression. These results prove that H0 is rejected and Ha is accepted because the results are significant with a p-value of 0.002, meaning that high MMP-9 in the sacrouterine ligament can provide a risk of uterine prolapse..

Tabel 3. Results of Matrix Metalloproteinase-9 (MMP-9) Analysis as a Risk Factor for Grade III-IV Uterine Prolapse

Faktor Risiko		Kelompok		Odd Ratio (OR)	IK 95%	p
		Kasus (n=22)	Kontrol (n=22)			
MMP-9	Rendah (< 28.24)	11	11	8.867	2.246-34.998	0.002*
	Tinggi (>28.24)	11	11			

*signifikan (p<0,05)

Microscopic Image of Matrix Metalloproteinase-9 (MMP-9) in the Sacrouterine Ligament



Imagr 2 Expression of MMP-9 in Sacrouterine Ligament Tissue by Immunohistochemical Staining

Information:

A) Case Group. MMP-9 expression (brown color) appears to increase.

B) Control Group. MMP-9 expression (brown color) appears to decrease.

DISCUSSION

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

Uterine prolapse is a major health problem that affects the quality of life of many women (13). With increasing descent of the pelvic organs, symptoms of bladder, bowel and prolapse appear. Uterine prolapse is also a multifactorial disease, the pathophysiology of which is still not fully understood (14). Based on a large community-based retrospective cohort study, factors that increase the risk of uterine prolapse are older age, postmenopausal status, parity, increased intra-abdominal pressure (15). This is consistent with a large population-based cross-sectional study in Sweden which found that age and parity were the dominant risk factors, but a BMI greater than 26 kg/m2 and a strong family or personal history of hernias, suggest a weak connective tissue disorder. tissue, is an independent risk factor for uterine prolapse (16).

In this study, TGF-β1 expression was measured immunohistochemically to determine its role as a risk factor for grade III-IV uterine prolapse. In addition to examining TGF-β1 expression, research was carried out on several factors that could influence the incidence of uterine prolapse, such as age, BMI, parity, and work which was studied as a control variable and a case variable. In the control variable distribution data presented in Table 1, it can be seen that each research sample in the case and control groups has different characteristics.

The results of this study found that the mean age in the case group (women with uterine prolapse) was higher than the control group and there was a significant difference between the two groups ($p<0.001$). The mean age in the case group was 61.18 ± 5.26 years, while the control group was in the range of 50.09 ± 6.36 years. The results of this study show that cases of uterine prolapse will increase with age. The results of this study are in line with epidemiological surveys in the United States showing that examinations carried out on patients to detect uterine prolapse were found in women aged 50-79 years, 41-50% of these women suffered from uterine prolapse. Older age at first delivery is also an obstetric risk factor for uterine prolapse [(6)]. Uterine prolapse occurs due to weakening of the endopelvic fascia which functions as a support for the pelvic organs. One of the causes is age and menopause factors which result in a decrease in estrogen levels (hypoestrogen) so that muscle cells atrophy and weaken. [(7)]. Obesity is one factor that can increase intra-abdominal

pressure which causes weakening of the pelvic muscles and fascia. Several studies show that obesity affects various types of lower urinary tract symptoms (LUTS) (18). Vergeldt's research (2015) shows that higher body mass is categorized as a variable that is a significant risk factor for primary factors (19). In the Women's Health Initiative (WHI) study, it was stated that women who were overweight (BMI 25-30 kg/m2) had an increased risk of uterine prolapse by 31-39%, while women who were obese (BMI> 30 kg /m2) the risk increases by 40-75% (Hardianti and Pramono, 2015).

A high body mass index is related to the progression of complaints of pelvic organ prolapse, placing additional load on the supporting muscles of the pelvis will cause weakness in the pelvic floor muscles. On the other hand, a low body mass index also has a higher risk of experiencing levator ani muscle injury due to anatomical defects in the pelvic floor, where levator ani muscle injuries often occur during vaginal delivery (17).

In this study, the mean BMI in the case group (29.32 ± 4.25 kg/m2) was higher than in the control group (26.91 ± 4.55 kg/m2). Based on the mean BMI value in the case group, it shows the obesity category according to the nutritional status criteria of the World Health Organization (WHO) and the Indonesian Ministry of Health. Even though different mean values were obtained, the difference in data between the two groups was not statistically significant (p=0.72). This is due to matching BMI values when sampling in the case and control groups. Obesity can increase the load on the muscles that support the pelvic floor, causing weakness in these muscles. These results are in line with systematic review studies reporting that large BMI is a significant non-obstetric risk factor for the risk of uterine prolapse, but is not a risk factor for uterine prolapse recurrence (12). From a meta-analysis study involving 22 studies, it was concluded that for the overweight category in women with uterine prolapse, the risk ratio was 1.36 (95% CI 1.2-1.53). Meanwhile, for the obesity category, the risk ratio is 1.47 (95% CI 1.35-1.59) (12).

Uterine prolapse disorders tend to occur in women with high parity. In several large studies and systematic reviews, parity is said to be an independent risk factor for the incidence of uterine prolapse. Both the number of parities and the method of vaginal delivery are known to be significant causes of uterine prolapse. This is caused by damage to the muscles supporting the pelvis and the patient's nerves. A study states that each vaginal delivery will increase the risk of prolapse 1.2 times (12).

In this study, the median parity value in the case group (3, IQR=2) was higher than

in the control group (2, IQR=2). In addition, a significant difference was found between the two groups (p=0.014). These results are in line with research conducted by Bachtiar (2018) showing that multiparity is a determining factor in the incidence of uterine prolapse with the results of the analysis showing that an average of 3-5 births is the risk limit (20). Several studies also agree that there is a relationship between parity and the incidence of uterine prolapse, such as research conducted by Azimatul Ilmiyah (2012) showing that the significance value between parity and prolapse is (p) = 0.000, ($p < \alpha$), meaning that there is a relationship between multiparity and uterine prolapse and for an OR value of 40.255. CI = 95% (5,314-34,919), meaning that multiparous menopausal mothers increase the risk of uterine prolapse 40.255 times compared to non-multiparous mothers (21).

The majority of the case group in this study did light work (86.4%) and no significant differences were found in the data distribution of the case and control groups (p= 0.233). Jobs that fall into the light category in this study are housewives; private employees; Teacher; as well as civil servants, while jobs in the heavy category are farmers. Similar results were also obtained in research by Putra et al. (2020) that the majority of uterine prolapse patients did light work (63.63%) and no significant difference was found between the work load in the case and control groups (p=0.226). Research by Devkota et al. (2020) reported that housewives were 2.29 times more likely to experience 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) . Housewives are susceptible to uterine prolapse due to sitting positions while working or resting at home. Women who work longer in a sitting or bending position are two to three times more likely to experience uterine prolapse than women who work more in a standing position (21)

B. High TGF-β1 Expression as a Risk Factor for Grade III-IV Uterine Prolapse

In this study, high TGF-β1 expression was more frequently found in sacrouterine ligament samples from a group of women who experienced uterine prolapse (n=15) compared to women who did not experience uterine prolapse (n=8). This study also shows that high expression of TGF-β1 in the sacrouterine ligament significantly increases the risk factor for grade III-IV uterine prolapse up to 14.00-fold (OR 14.00; 95% CI 2.370 – 82.717; p=0.004),

so that the hypothesis the null (Ho) is rejected and the alternative hypothesis (Ha) is accepted.

The results of this study are in line with those conducted by Carlin et al (2020) who explained that TGF-ß1 was expressed significantly more often in cases of severe prolapse compared to moderate/mild cases (POP-Q stage IV versus POP-Q stages II and III; p = 0.001) in the study suggested that patients with severe prolapse (POP-Q Stage IV) showed significantly stronger immunoreactivity against TGF-ß1 specific antibodies compared with patients with POP-Q stage II and III prolapse (15). Another study by Ava Leegant et al (2015) showed that USL was obtained from women who underwent vaginal hysterectomy for stage two or greater uterine prolapse (cases, n=21) and from women without uterine prolapse who underwent vaginal hysterectomy for benign indications (controls, n=19) and the results showed that TGF-ß1 expression was positively correlated with MMP-9 expression (R=0.4, P=0.01). Furthermore, it was explained that there was a significant increase in fibrous tissue (P=0.008), and a decrease in smooth muscle (P =0.03) related to increasing patient age. This study focused on evaluating the expression of TGF-ß1 and matrix metalloproteinase-9 (MMP-9), key regulators of extracellular matrix composition, in the uterosacral ligament (USL) of women with uterine prolapse compared with controls (14). Increased expression of TGF-β1 and pathological conditions, including reduced fascia strength have been observed in inguinal hernias (Min et al, 2017). In women with uterine prolapse, changes in TGF-β1 expression were found in both fibroblasts and pubovaginal fascia (Qi *et al*, 2011; Li *et al*, 2013; Wen *et al*, 2006; Maijerink *et al*, 2013). This study provides evidence that TGF-β1 expression may be a risk factor for uterine prolapse and a candidate novel biomarker of uterine prolapse. Due to the prevalence of prolapse. The uterus is increasing rapidly along with the increase in the elderly population (7).

The role of TGF-ß1 in uterine prolapse is still controversial, a study conducted by Pascual (2007) stated that it did not detect any differences in TGF-ß1 levels in USL of women with and without uterine prolapse. In that

study also did not observe significant differences in TGF-ß1 expression between the uterine prolapse group and the control group. However, the TGF-ß1 pathway is known to be an important signaling mechanism for cellular responses to mechanical stress and thus may be related to the pathophysiology of uterine prolapse in postmenopausal women. (5).

Transforming growth factor-ß1 (TGF-ß1) is a fibrogenic cytokine that plays an important role in ECM remodeling and regulation of tissue integrity. TGF-ß1 activity can alter the balance between ECM synthesis and degradation and is implicated in the pathophysiology of many fibrotic disorders, including pulmonary fibrosis and cardiac fibrosis associated with heart failure. In addition, several studies showed that TGF-ß1 expression was altered in fibroblasts and pubic fascia of women with uterine prolapse. However, several reports indicate that TGF-ß1 is not associated with the prognosis of prolapseterus patients. Thus, there is no consensus regarding the role of TGF-ß1 in the pathogenesis of uterine prolapse, and further investigation is needed (15).

TGF-ß1 is currently considered an important regulator that is widely involved in the pathogenesis of fibrosis and degenerative fibrotic diseases, which can cause fibroblast differentiation, increase collagen synthesis and reduce degradation by inhibiting MMPs and upregulating TIMPs. TGF-ß1 reduces collagenase mRNA levels and increases TIMP mRNA levels. Sparse but growing evidence for TGF-ß1 modulation in pelvic connective tissue has been found. The TGF-ß1/Smad and MMP signaling pathways are involved in stress urinary incontinence caused by vaginal delivery in animal studies. However, to our knowledge, TGF-ß1 has rarely been studied in women with uterine prolapse. To identify the exact role of TGF-ß1 in the development of uterine prolapse, in follow-up in vitro experiments, several studies introduced a cellular disease model that mimics ECM remodeling in uterine prolapse, which was established in hUSLF subjected to CMS loading with the TGF-ß1 strain. (22).

C. High Matrix Metalloproteinase-9 (MMP-9) Expression as a Risk Factor for Grade III-IV Uterine Prolapse

In this study, high MMP-9 expression was found in sacrouterine ligament samples from groups of women who experienced uterine prolapse (n=11) and women who did not experience uterine prolapse (n=11). This study also showed that high MMP-9 expression in the sacrouterine ligament significantly increased the risk factor for grade III-IV uterine prolapse up to 8,867-fold (OR 8.867; 95% CI 2.246–34.998; p=0.002), so that the null hypothesis (Ho) is rejected and the alternative hypothesis (Ha) is accepted.

This study is in line with Ying et al (2022) who suggested that postmenopausal women with symptomatic uterine prolapse, and those without uterine prolapse, differ in terms of the expression of factors involved in synthesis and turnover. In particular, the levels of CD44 increased significantly lower, and MMP-2 and MMP-9 were higher in the USL uterine prolapse group than in the USL control group. In addition, CD44 levels were negatively correlated and MMP-2/9 levels were positively correlated with prolapse. These results support that CD44 may be involved in the pathogenesis of uterine prolapse and are consistent with the role of CD44, MMP-2, and MMP-9 in the mechanisms underlying the balance between ECM production and degradation (7).

The ECM is a connective tissue component that provides a framework for pelvic support; therefore, it may play an important role in the occurrence of uterine prolapse. The ECM is dynamically reshaped through quantitative and qualitative changes in cellular and non-cellular components, including enzymes such as collagenase and MMPs that regulate ECM turnover (23). MMPs are closely involved in ECM remodeling in normal and pathological conditions because they can cleave most ECM proteins (24). MMP-9 function is also involved in the degradation of ECM proteins and is involved in disease development (24). This is in line with research conducted by Ying (2022) which suggests that increasing MMP-2 and MMP-9 expression can accelerate ECM damage and accelerate the risk of uterine prolapse.

A literature discussing the relationship between increased expression of MMP-9 in pelvic supporting structures and uterine prolapse shows a statistically significant increase in MMP-9 expression in tissue from women with uterine prolapse. These findings suggest an important link between increased MMPs-9 expression and the risk of uterine prolapse. Since uterine prolapse is a disease of weakened supporting structures, which are mainly made of collagen, and MMPs are responsible for collagen

degradation, it seems reasonable that MMPs participate in the development of uterine prolapse and its progression. In fact, recent evidence suggests that changes in connective tissue composition are at least partially involved in the pathophysiology of uterine prolapse. It is also possible, however, that biomechanical changes associated with uterine prolapse result in altered MMP expression. Dviri's research (2011) showed higher expression of MMP-9 in the uterosacral ligaments of women with uterine prolapse indicating the possibility that uterine prolapse is associated with increased collagen degradation where the collagen degradation process supports the pelvic structure, causing weakening of the connective tissue and ultimately pelvic organ prolapse. It is also possible that biomechanical changes associated with uterine prolapse are responsible for the increased MMP expression (25)

CONCLUSIONS AND RECOMMENDATIONS

High expression of TGF-β1 and MMP-9 is a risk factor for grade III-IV uterine prolapse. This study suggests that future research should examine TGF-β1 as a possible new therapeutic target for uterine prolapse. Where TGF-β1 can not only be used as a biomarker to predict the severity of POP but also has a therapeutic effect on POP cell models and large-scale studies with various cut-off values of TGF-β1 and MMP-9 expression are needed to determine the cut-off values expression of TGF-β1 and MMP-9 which can be used to detect grade III-IV uterine prolapse

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DISCLOSURE STATEMENT

The authors declare that no relevant or material financial interests relate to the research described in this paper.

DATA AVAILABILITY STATEMENT

The data supporting this study's findings are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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