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By Yoga Paripurna

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The correlation between the vitamin D receptor and TIMP-1 in the placenta accreta spectrum

Yoga Paripurna, Hanom Husni Syam, Sonny Sasotya, Jusuf S Effendi, Setyorini Irianti, Siti Salima

Department of Obstetric and Gynecology, Faculty of Medicine, Padjadjaran University

Hasan Sadikin General Hospital, Bandung

Corresponding author:

Yoga Paripurna

E-mail: yogaparipurna0@gmail.com

ABSTRACT

Introduction: Placenta accreta is one of the high-risk pregnancy condition, which also stays as one of the pregnancy-related problem in Indonesia. Caused by the abnormal trophoblast invasion to the myometrium, there is a possibility that VDR and TIMP-1 expressions play an important role in the pathophysiology of abnormal trophoblast invasion. This study aimed to evaluate the expression of VDR and TIMP-1 in the placenta accreta spectrum and normal placenta, with further evaluation of the correlation between VDR and TIMP-1 expression in the placenta accreta spectrum.

Method: A cross-sectional study was conducted using samples obtained from Hasan Sadikin General Hospital, Bandung, Indonesia. Paraffin blocks of uteroplacental tissues were retreived and further evaluated through immunohistochemistry assays. Data analysis were performed using dichotomic and correlation analysis using the SPSS version 24.0 for Windows.

Results: A total of 40 samples were evaluated. VDR H-Score was significantly higher in the placenta accreta spectrum group compared to the normal placenta (median H-score: 2.50 vs 16.00; p=0.0001), with higher prevalence risk of H-Score <8 in the placenta

accreta spectrum group (PR=4.500; 95%CI=2.042-9.916; p=0.0001). TIMP-1 H-Score was significantly higher in the placenta accreta spectrum group compared to the normal placenta (median H-score: 4.00 vs 16.00; p=0.0001), with higher prevalence risk of H-Score <8 in the placenta accreta spectrum group (PR=3.115; 95%CI=1.706-5.689; p=0.0001). There was a very strong correlation of VDR and TIMP-1 expressions in the placenta accreta spectrum ((R=0.839; p=0.0001).

Conclusion: VDR and TIMP-1 expressions play an important role in the pathophysiology of placenta accreta spectrum.

Keywords: Placenta accreta, VDR, TIMP-1, Immunohistochemistry

INTRODUCTION

Placenta accreta spectrum is a high-risk pregnancy condition, with maternal morbidity reaching 60% after hysterectomy treatment in placenta accreta.[1] It is a condition where the placenta grows too deeply into the myometrium, with histopathological examination showing direct contact of the chorionic villi to the myometrium. It is caused by the absence or minimal appearance of the decidua basalis and the fibrinoid layer (Nitabuch's layer). Nitabuch's layer thinning usually occurs as a result of a cesarean section scar. The loss of this layer leads to a deeper infiltration of the villi and trophoblast.[2-4]

Nutrition is one of the most important factors for the placenta to achieve optimal function. In the first trimester of pregnancy, extravillous trophoblast invades the decidua to one third of the myometrium to obtain nutrition from the mother. In addition, macronutrients and micronutrients such as vitamin D, vitamin A, iron, folic acid and vitamin B12 also affect the process of placental development.[5]

In pregnancy, vitamin D has several functions during the implantation and placentation process. Vitamin D deficiency is often associated with pregnancy complications, characterized by impaired placental development.[6,7] The physiological active form of vitamin D, calcitriol

(1,25(OH)2D), is the main active ligand of Vitamin D Receptor (VDR) which plays a role in trophoblast invasion by suppressing MMP-9 expression and increased TIMP-1 expression. TIMP-1 is a biomarker for trophoblast invasion that is pathological of placenta accreta.[8-12] Decreased MMP-9 expression and increased TIMP-1 expression by the activation of VDR might play a role in decreasing the occurence of the placenta accreta. This research aims to specifically determine the difference of VDR and TIMP-1 expressions between the placenta accreta spectrum and normal placenta, also the correlation between VDR and TIMP-1 expression in the spectrum of placenta accreta.

MATERIALS AND METHOD

The research is a cross-sectional study using histopathological samples in Hasan Sadikin General Hospital, Bandung, Indonesia. Uteroplacental tissues from paraffin blocks were obtained from patients diagnosed either with normal placenta or having placenta accreta spectrum. Paraffin blocks collected were stored in refrigerators, which is then cut into thin slices using the 4 micron-thick microtome. Each paraffin block was cut twice for the measurement of both VDR and TIMP-1 immunohistochemical assay, presenting the expression of VDR and TIMP-1.

The immunohistochemical assay were further evaluated with a semi-quantitative approach, in which each data were assigned into a weak H-Score group and strong H-Score group. The intensity of membrane staining (negative, weak, moderate or strong) was determined for each fixed cell. The H-Score is calculated from the intensity multiplied by the expression distribution. Presented dichotomically, the cut off used was <8 for weak H-score and ≥8 for strong H-score. Statistical analysis was performed using SPSS version 24.0 for Windows, evaluated using the the unpaired T-test if the data were normally distributed, and Mann-Whitney or Exact Fischer test if not normally distributed. Correlation test using the Spearman test was also performed. P-value <0.05 was deemed statistically significant.

RESULTS

The expression of vitamin D receptor (VDR) in the spectrum group of placenta accreta and normal placenta can be seen in Figure 1 and Table 1. Significant difference of VDR H-Score was observed between the placenta accreta spectrum and the normal placenta (median H-score: 2.50 vs 16.00; p=0.0001).

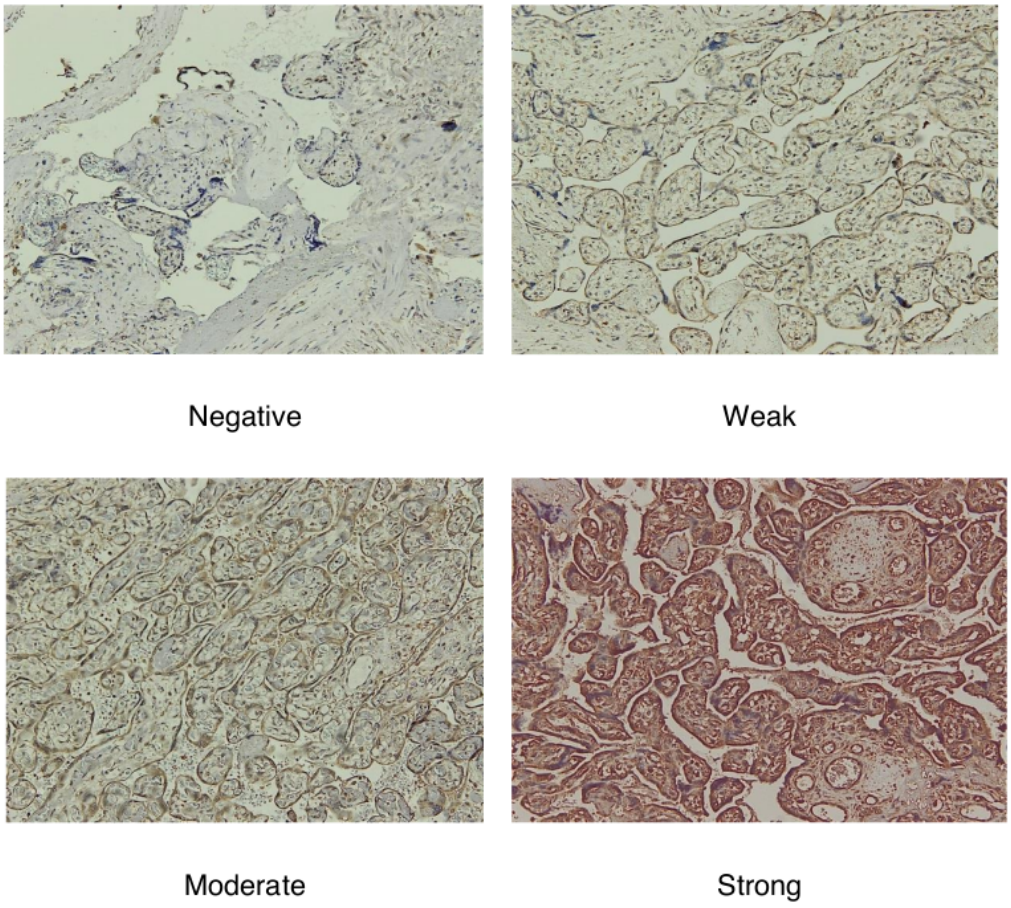

Figure 1. The immunohistochemical results of VDR Expression in Placenta Acreta Spectrum and Normal Placenta

Table 1. The Comparison of VDR H-Score in the Placenta Acreta Spectrum and Normal

Placenta

Variable	Group		P Value†
	Placenta Accreta Spectrum N=20	Normal Placenta N=20	
Total VDR H-Score			
Mean±Std	3.95±3.203	14.20±3.088	**0.0001***
Median	2.50	16.00	
Range (min-max)	1.00-9.00	6.00-16.00	

†= significant if <0.05; *=Mann Whitney test

Tissue metalloproteinase inhibitors (TIMP-1) in the placenta accreta spectrum and normal placenta can be seen in **Figure 2** and **Table 2**. Significant difference of TIMP-1 H-Score was also seen between the placenta accreta spectrum and the normal placenta (median H-score: 4.00 vs 16.00; p=0.0001)

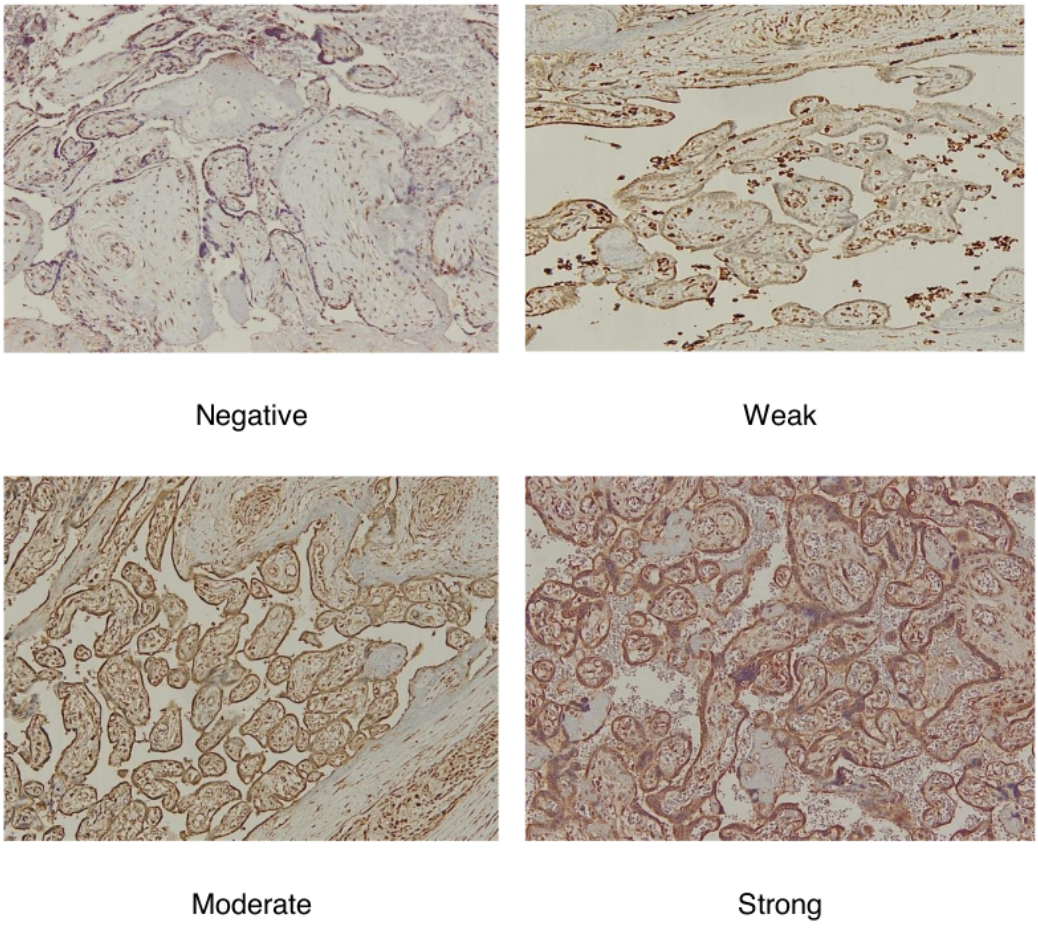

Figure 2. Immunohistochemical results of TIMP-1 Expression in Placenta Acreta Spectrum and Normal Placenta

Table 2. Comparison of TIMP-1 H-Score in the Placenta Acreta Spectrum and Normal Placenta

Variable	Group		P Value†
	Placenta Acreta Spectrum	Normal Placenta	

	N=20	N=20	
Total TIMP-1 H-Score			
Mean±Std	6.25±3.537	14.20±3.036	**0.0001***
Median	4.00	16.00	
Range (min-max)	1.00-12.00	4.00-16.00	

†= significant if <0.05; *=Mann Whitney test

The comparison of both VDR and TIMP-1 H-Score, along with the prevalence ratio (PR) of VDR and TIMP-1 H-Score between the placenta acreta spectrum and normal placenta were described in **Table 3**.

Table 3. The Comparison and PR 95% CI between Total H-Score VDR and Total H-Score TIMP-1 in the Placenta Acreta Spectrum and Normal Placenta

Variable	Group		PR (95% CI)	P Value†
	Placenta Acreta Spectrum N=20	Normal Placenta N=20		

VDR H-Score				
< 8.0	15(93.8%)	1(6.3%)	**4.500 (2.042-9.916)**	**0.0001***
≥ 8.0	5(20.8%)	19(79.2%)		
TIMP-1 H-Score				
< 8.0	12(92.3%)	1(7.7%)	**3.115 (1.706-5.689)**	**0.0001***
≥ 8.0	8(29.6%)	19(70.4%)		

†= significant if <0.05; *=Mann Whitney test

The prevalence ratio of VDR H-Score <8.0 was significantly higher in the placenta accreta spectrum when compared to the normal placenta group (PR=4.500; 95%CI=2.042-9.916; p=0.0001). Furthermore, the prevalence ratio of the TIMP-1 H-Score <8.0 was also significantly higher in the placenta accreta spectrum compared to normal placenta (PR=3.115; 95%CI=1.706-5.689; p=0.0001).

Table 4. Correlation Analysis of TIMP-1 Total H-Score with Total Vitamin D Receptor H-Score

Variabel	R^s	P-Value†

TIMP-1 Total H-Score Correlation with Vitamin D Receptor Total H-Score	**0.839**	**0.0001**

s=Spearman Correlation Test; †=significant if <0.05

The correlation between the Total H-Score VDR and Total H-Score TIMP-1 on the placenta accreta spectrum can be explained in table 4. The spearman correlation test between the TIMP-1 total H-Score and VDR total H-Score in the placenta accreta spectrum showed a very strong correlation with statistical significance (R=0.839; p=0.0001).

DISCUSSION

In our study, VDR expression was significantly lower in the placenta accreta group than the normal placenta. VDR has a major ligand, namely 1,25(OH)2D (calcitriol) which is the active form of vitamin D.[12] Previous research by Cross et al. Showed that vitamin D deficiency has an effect on decreasing VDR expression.[6] Low level of vitamin D causes inflammation, which indirectly decreased VDR expression.[6] The treatment using UVB light exposure can increase synthesis of vitamin D. Vitamin D supplementation has also been reported to increase VDR expression. Hence, increasing the serum vitamin D might play a role reducing the risk of low VDR expression.[8,13,14] During pregnancy, decreased levels of vitamin D and VDR are known to affect the formation of abnormal placenta with too deep the trophoblast which is a diagnosis of placenta accreta spectrum.[9] These findings support this study which states that the VDR in placenta accreta is lower than in normal placentas.

Our study also found that the expression of TIMP-1 was significantly lower in the placenta accreta spectrum group. TIMP-1 is an MMP-9 inhibitor, which plays a major factor in placental proteolysis. Increased levels of protease could cause an enhancement in MMP-9 inhibitor production, followed by excessive proteolysis. The production of MMP-9 needs to be balanced by TIMP-1 which is an inhibiting factor for the MMP-9. The balanced expression between MMP-9 and TIMP-1 has a major role in regulating the depth of trophoblast invasion into the uterus. Based on the results of previous studies, lower serum concentrations of the

biomarker TIMP-1 and higher MMP-9 in the placenta accreta spectrum resulted in deep placental invasion. TIMP-1 expression was lower in the spectrum of placenta accreta compared to normal placenta.[10,11,15]

Active form of vitamin D, 1,25(OH)2D, has the ability to modulate proliferation and differentiation, by suppressing MMP-9 production and increasing TIMP-1 expression. The ability of 1,25(OH)2D is supported by the presence of VDR as a receptor. Decreased VDR expression in the placenta may possibly disrupt the balance of MMP-9 and TIMP-1. This is due to a decrease in VDR expression, which may limit the effect of vitamin D. The balanced expression between MMP-9 and TIMP-1 has major implications for regulating the depth of trophoblast invasion into the uterus during pregnancy causing placenta accreta spectrum.[9,12,15] These theories are supported by the results of this study, showing a very strong correlation between VDR and TIMP-1 expression in the placenta accreta spectrum.

CONCLUSION

Our study showed that there is a significantly lower VDR and TIMP-1 expressions in the placenta accreta spectrum when compared to the normal placenta. Furthermore, VDR and TIMP-1 were very strongly correlated. The authors suggest that increasing the active form of vitamin D of the body might play a role in reducing the risk of placenta accreta spectrum. Further study evaluating the correlation between active increment of active vitamin D and the occurrence of placenta accreta are needed.

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