

## The Prognostic Role of (CD34) In The Angiogenesis of Endometrial Adenocarcinoma

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### ABSTRACT:

#### BACKGROUND:

Tumor angiogenesis is essential for tumor growth and metastases. Recently ,studies showed that microvessel density (MVD),a measure of tumor angiogenesis has found to have a prognostic significance for predicting metastases and survival in endometrial adenocarcinoma and identifying patients at high risk for recurrences .

The aim of this study is to understand the correlation between the MVD and various clinicopathological factors in endometrial adenocarcinoma in order to evaluate the role of angiogenesis in the assessment of the biological behavior of endometrial adenocarcinoma and to prove that MVD can be used as a valuable prognostic factor and can detect high risk patients for post-operative recurrence who may need adjuvant therapy after surgery .

#### PATIENTS AND METHODS:

Forty-one (41) cases were included in this study ; one case is normal endometrium in the proliferative phase , 5 cases with sever endometrial hyperplasia and 35 cases with endometrial adenocarcinoma taken from the Pathology Department at the Medical City and Private Pathology Laboratory between the years 2000-2004 .

Paraffin blocks cut in two sections ; one for Haematoxylin & Eosin stain and the other for CD34 immunostaining slides were studied well for tumor grade ,stage ,myometrial invasion and pelvic and paraaortic lymph nodes metastases .

The mean MVD was studied by scanning the tumor sections at a low power X40 and X100 then identifying areas of highest neovascularization (hot spots) .Five hot spot areas were selected from each section ,and the MVD were counted at X200 magnification (x10 ocular ,x20 objective) by taking the mean of those microvessels.

The correlation between mean MVD and various clinicopathological factors were studied including the age ,tumor grade ,stage ,myometrial invasion and pelvic and paraaortic lymph nodes metastases .

#### RESULTS :

CD34 is a reliable and sensitive endothelial cell marker used in the assessment of tumor angiogenesis The study showed that a significant statistical correlation was found between the mean MVD and patients age , tumor grade and myometrial invasion. While there were no statistical correlation significance between the mean MVD and tumor stage and pelvic & paraaortic lymph nodes .

#### CONCLUSION :

We conclude that MVD is important in the assessment of the biological behavior of endometrial adenocarcinoma , where it is an indicator for the emergence and growth of tumor and it is important to detect patients at high risk of recurrences and to decide the post-operative adjuvant therapy

**KEYWORDS :**Angiogenesis(MVD), CD34 ,endometrial adenocarcinoma.

### INTRODUCTION:

Uterine cancer, The most common malignant, neoplasm of the female genital tract and the fourth most common cancer in women.

It is more common in post-menopausal women who are white, affluent, obese and of low parity, the median age (55-65) years.<sup>(1,2,3,6,9,10,11)</sup>

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### Tumor angiogenesis

Is defined as the formation of neovessels from preexisting vascular structures, mainly capillary and venules under the influence of malignant tumor. Angiogenesis plays an important role in the regulation of the female menstrual cycle through the physiological proliferation of the endometrium and formation of corpus luteum in the second half of menstrual cycle.<sup>(4&5&12&13)</sup>

The function of angiogenic factors in the emergence of endometriosis and in female and male infertility is still under study.

In obstetrics angiogenesis is significant for implantation of impregnated blastocytes and growth of the placenta.

Tumor angiogenesis is a key step in tumor growth, invasion and metastasis.

Most tumors in humans persist for months to years without neovascularization until a subset of tumor cells acquires an angiogenic phenotype. The switch to angiogenic phenotype involves a change in the local equilibrium between negative and positive regulators of angiogenesis.<sup>(7&8&14&15&22&23)</sup>

#### **Mechanism and regulation of angiogenesis**

Angiogenesis, the sprouting of new capillaries from small venules occurs through local degradation of the basement membrane lining the venules followed by migration, alignment and proliferation of endothelial cells toward the angiogenic stimulus. The formation of new blood vessels is essential for tumor expansion & spread.

Tumor induce angiogenesis through a variety of growth factors/receptors that induce a functional triad of motility, proteolysis, and growth in a paracrine manner.<sup>(16,17)</sup>

#### **PATIENTS, MATERIALS AND METHOD:**

The retrospective study included 41 cases, who underwent total abdominal hysterectomy and bilateral salpingo- oophorectomy. Between 2000-2004, where taken from the pathology department at the Medical City Hospital and a private pathology laboratory.

Thirty-five cases were diagnosed as adenocarcinoma of the endometrium, five cases were diagnosed as severe endometrial hyperplasia and one case with normal endometrium (taken as a control group).

The patient's age, grading, depth of myometrial invasion, presence of pelvic and paraaortic LN metastases, and the stage of the disease were noted.

The specimens were already fixed in 10% formalin, and paraffin embedded. Two sections (each was 4 microns in thickness) were cut from the paraffin block. One section stained with Haematoxylin and Eosin (H&E) stain, and the other stained with CD34 antibody

#### **Quantitative Measurement of Angiogenesis**

The degree of angiogenesis can be evaluated by assessing MVD. Immunostained slides with CD34 antibody were examined at low-power magnification ( $\times 40$  and  $\times 100$  total magnification) to identify the areas of highest neovascularization (hot spots). The microvessel in a  $\times 200$  field ( $\times 20$  objective &  $\times 10$  ocular) in 5 areas (hot spots) were then counted. The average in the 5 areas per  $\times 200$

fields in each element of these tumors were calculated and thereafter were referred to as the microvessel density.<sup>(18&19&20&21)</sup>

#### **Statistical Analysis**

The average for vessel count, the mean for vessel density, and the standard deviation were determined.

Correlation between the mean MVD and various clinicopathological factors such as age, tumor grade, stage of disease, depth of myometrial invasion, and both pelvic and paraaortic lymph nodes metastases, were studied.

#### **RESULTS:**

A total of 41 cases were included in this study, 35 cases were endometrial adenocarcinoma, 5 cases were severe endometrial hyperplasia and one case was normal proliferative endometrium. Those 5 cases with severe endometrial hyperplasia and the normal proliferative endometrium are taken as a control group.

The mean age for the 35 cases with endometrial adenocarcinoma was 51.7 years (range, 20-80 years).

As in Table 1, 2.8% of the 35 cases were between (20 – 30) years, 8.5% between (31 – 40) years, 48.5% between (41-50) years, 34.2% between (51-60) years, 5.7% were more than 60 years.

And for the statistical analysis, the age was divided into < than 50 years, 12 cases (34.2%) &  $\geq$  50 years, 23 cases (65.7%).

The mean age for the 5 cases with severe endometrial hyperplasia was 39 years (range, 35-45 years).

One case with normal proliferative endometrium (age 42 years).

For the tumor grade, of the 35 cases of endometrial adenocarcinoma, 23 cases were grade I (65.7%), 8 cases were grade II (22.8%), 4 cases were grade III (11.4%).

According to FIGO staging system, we found 20 cases were stage I (57.1%), 6 cases were stage II (17.1%), 7 cases were stage III (20%), 2 cases were stage IV (5.7%).

We divided the cases into three groups according to myometrial invasion, the first group including 3 cases (8.5%) showed no myometrial invasion, second group including 12 cases (34.2%) with less than one half of the myometrial invasion, and the third group including 20 cases (57.1%) with more than one half of the myometrial invasion.

Pelvic lymph nodes metastases was seen in 4 cases only (11.4%), where 31 cases showed no pelvic lymph nodes metastases (88.5%). Paraaortic lymph nodes metastases seen in one case only (2.8%) & the rest 34 cases show no paraaortic lymph nodes involvement (97.1%).

**Table 1: The Characteristics (clinicopathological features ) of 35 Cases with Endometrial Adenocarcinoma studied**

Clinicopathological features	No. of cases	%
Age in years		
20 – 30	1	2.8
31 - 40	3	8.5
41 - 50	17	48.5
51 – 60	12	34.2
>60	2	5.7
Tumor grading		
I	23	65.7
II	8	22.8
III	4	11.4
Tumor staging		
I	20	57.1
II	6	17.1
III	7	20
IV	2	5.7
Myometrial ivasion		
No invasion of myometrium	3	8.5
<1/2 of myometrial invasion	12	34.2
>1/2 of myometrial invasion	20	57.1
Pelvic lymph nodes metastases		
-	31	88.5
+	4	11.4
<b>Paraortic lymph nodes metastases</b>		
-	34	97.1
+	1	2.8

The control group include: five cases present with severe endometrial hyperplasia, the age ranging from (35-45) years.

One case with normal endometrium in the proliferative phase (age =42)

#### **Correlation between MVD using CD34 antibody and various clinicopathological factors:**

##### **1.Endometrial hyperplasia and normal endometrium :**

The control group (5 cases with sever endometrial hyperplasia and one case with normal endometrium in the proliferative phase .

For those with sever endometrial hyperplasia , the mean MVD was 60.03 (range , 19-123) with SD 23.12.The case with normal endometrium in the proliferative phase , the mean MVD was 50.20 (range, 35-90) with SD 14.00.

The MVD in endometrial adenocarcinoma were significantly higher than those in the control group(sever endometrial hyperplasia and normal endometrium in the proliferative phase ). The P value were 0.0125 , 0.0132 respectively .

**2.Age:** ( TABLE 2 ) Of the 35 cases of endometrial adenocarcinoma , the highest figure seen between (41-50 years) were 17 cases , while the lowest figure seen between (20-30 years) was only one .

To facilitate statistical analysis the patients then classified into two groups : < 50 years , 12 cases ( 34.2%) and ≥ 50 years , 23 cases ( 65.7%) . For the first group the mean MVD was 73(range, 35-194) with SD equal to 17.8 and for the second group the mean MVD was 106( range, 31 – 250 ) with SD equal to 43.22 . A significant statistical correlation were found between MVD and the age of the patients where the P value was 0.046.

**Table 2 :Correlation Between MVD using CD 34 staining and the age of the patient with endometrial adenocarcinoma**

Age	Category	No. of patients	Range of MVD	Mean MVD	SD
	<50	12	35-194	73	17.8
	≥50	23	31-250	106	43.22
P value = 0.046**s					

S = significant

**3.Myometrial invasion(TABLE 3):**

Regarding the myometrial invasion was classified into , no myometrial invasion which is seen in 3 patients (8.5%, <1/2 myometrial invasion in 12 patients ( 34.2%) and >1/2 myometrial invasion in 20 patients (57.1% ) .

The mean MVD for the first group with no myometrial invasion was 47.50( range , 36-99) with SD 15.23 , , for the second group with <1/2 myometrial invasion the mean MVD was 66.40 ( range , 19-120) with SD 25.18, and for the last group where >1/2 of the myometrial invasion seen , the mean MVD was 80.01 ( range , 36-131 ) with SD 26.04 .The mean MVD found to be increased with increasing myometrial invasion , and the difference in the mean can be noticed in the different three groups as seen in table9, where the P value was 0.009 .

**Table 3: Correlation between MVD staining and myometrial invasion**

Myometrial invasion	Category	No .of patients (35)	Range of MVD	Mean MVD	SD
	No	3	36-99	47.50	15.23
	<1/2	12	19-120	66.40	25.18
	>1/2	20	36-131	80.01	26.04
P = 0.0096**s					

S =significant

**4. Tumor grade (TABLE 4) :**

In 23 patients tumor grade was grade I (65.7%), in 8 patients it was grade II (22.8%) , & in 4 patients it was grade III (11.4%).The mean MVD for grade I was 61.02( range,36-131) with SD 23.32, for grade II the mean MVD was 66.04(range ,19-120) with SD 25.92 , & for grade III mean MVD was 74.16(range, 44-127) with SD 28.55. It was found that the MVD ( microvessel density) increase with increasing histological grade where the P value was found to be 0.0156 which is statistically significant.

**Table 4: Correlation between MVD using CD34 staining & tumor grade**

Tumor grade	Category	No. of patients(35)	Range of MVD	Mean MVD	SD
	I	23	36-131	61.02	23.32
	II	8	19-120	66.04	25.92
	III	4	44-129	74.16	28.55
P = 0.0156**s					

S=significant

**5. Tumor stage ( TABLE 5)**

According to FIGO staging system ,endometrial adenocarcinoma classified into 4 stages.

Of the 35 cases of endometrial adenocarcinoma ,20 cases were stage I (57.1%),6 cases stage II(17.1%) ,7 cases stage III (20%),2 cases stage IV (5.7%)

The mean MVD for stage I was 62.00(range , 21-131)with SD equal to 23.83,for stage II mean MVD was 60.95(range ,37-120)with SD equal to26.08 for stage III mean MVD was 65.60(range,19-122) with SD 25.23,and for stage IV mean MVD was 89.54 (range, 94-102) with SD 32.00

**Table 5 :The correlation between MVD using CD34 staining antibodies and the tumor stage .**

stage	category	No .of patients	Range of MVD	Mean MVD	SD
	I	20	21-131	62.00	23.83
	II	6	37-120	60.95	26.08
	III	7	19-122	65.60	25.23
	IV	2	94-102	89.54	32.00
P=0.254 <sup>ns</sup>					

ns= not significant

**6. Pelvic and paraaortic lymph nodes metastases : (TABLE 6&7)** Pelvic lymph nodes metastases was seen in 4 cases (11.4%), while 31 cases show negative pelvic lymph nodes ( 88.5%).

The mean MVD for the 4 cases with positive pelvic lymph nodes was 78.01(range , 41-102) with SD 20.13 , & for the 31 cases with negative pelvic lymph nodes the mean MVD was 65.04(range , 19 – 131) with SD 24.98 as seen in table 6 .

Regarding the paraaortic lymph nodes which was seen in one case only ( 2.8%) & the other 34 cases

were negative for the paraaortic lymph nodes(97.1%).

The mean MVD for the one case with positive paraaortic lymph nodes was 89.90( range , 39-102) with SD 24.93, while the mean MVD for the rest 34 cases with negative paraaortic lymph nodes was 65.20( range, 19-131) with SD 24.6 as in table 7.

There was no correlation between MVD & lymph nodes status, involving both pelvic and paraaortic lymph nodes , where the p value for the first group was 0.1287 & for the second group was 0.2544.

**Table 6 :correlation between MVD using CD 34 & pelvic lymph nodes metastases**

Pelvic lymph nodes metastases	category	No .of patients of 35	Range of MVD	Mean MVD	SD
	—	31	19-131	65.04	24.98
	+	4	41-102	78.01	20.13
P value = 0.1287* <sup>ns</sup>					

ns= not significant

**Table 7 :Correlation between MVD using CD34 staining and paraaortic lymph nodes metastases**

Paraortic lymph nodes metastases	Category	No .of patients of 35	Range of MVD	Mean MVD	SD
	-	34	19-131	65.20	24.6
	+	1	39-102	81.90	24.93
P = 0.2544* <sup>ns</sup>					

ns = not significant

### DISCUSSION:

Tumor angiogenesis is essential for tumor growth, invasion and metastases. The progressive growth of solid tumors is strictly dependant on their ability to stimulate formation of new blood vessels that will supply tumor cells with oxygen and nutrients (24&25).

Several clinical studies have shown that the degree of tumor angiogenesis is related to clinical outcome, suggesting that angiogenic properties correlate with tumor aggressiveness and metastatic potential. Recently, a close correlation has been observed between the density of microvessels (MVD) in tumor sections and patient survival in malignant tumors such as endometrial carcinoma, breast cancer, lung tumors, prostatic carcinoma, carcinoma of stomach, esophageal carcinoma, and colorectal carcinoma.

In this study we use CD34 antibody to stain the endothelial cells, which is highly sensitive and a reliable marker. A study by Siitonen et al 1995 using 3 endothelial markers (CD34, CD31 & anti factor VIII related Ag), evaluated the differences among the three markers in breast carcinoma and concluded that anti-CD34 showed a better staining performance than others (25). Another study by Zena Elyas 2003 using 2 endothelial markers (CD34, CD31) in gastric carcinoma also proved that CD34 is highly sensitive than CD31 in staining the endothelium. (26)

Because tumors are frequently heterogenous in their microvessel content, so areas of highest neovascularization (or "hot spots") are found by scanning the tumor sections at low magnification and easily identifying those areas of invasive carcinoma having the greatest numbers of microvessels per area. Then the MVD is measured by taking the mean of those 5 areas at 200x. (25).

The correlation between MVD using CD34 antibody staining and the age, tumor stage, tumor grade, myometrial invasion, and lymph nodes metastases were investigated.

This study has clarified several points regarding the significance of angiogenesis (by measuring the

MVD) in the assessment of the biological behavior of endometrial adenocarcinoma as appeared from the results.

Correlation between the mean MVD using CD34 antibody staining and various clinicopathological factors:

#### 1. Endometrial Hyperplasia:

In our study the mean MVD in endometrial adenocarcinoma was higher than those of severe endometrial hyperplasia.

A study by Abulafia et al 1995 using CD34 antibody evaluated angiogenesis in endometrial hyperplasia & stage I endometrial adenocarcinoma and reported that MVD of endometrial adenocarcinoma was higher than those of severe hyperplasia, and concluded that both severe endometrial hyperplasia & endometrial adenocarcinoma were angiogenic. (27)

**2. Age:** The mean age of patients with endometrial adenocarcinoma in our study was 51.7 years. A study done by Yassoub (6) in 1997, in Baghdad showed that the mean age of patients with endometrial adenocarcinoma was 55.6 years. In 1983

Ferezezy (29) showed that the mean age of patient with endometrial adenocarcinoma was 67 years. So we noticed that there is a regression in the age.

According to table 2, a correlation was found between mean MVD and the age in the present study. This result also proved by Kaku et al 1997 (16) using anti-factor VIII related antigen also called (anti Von willibrand's factor), while the studies done by Fujiwaki et al 2002 (28) and Wagatsuma et al 1998 (17) using anti-factor VIII related antigen showed no correlation between mean MVD and patient's age and this difference can be due to the use of larger number of patients by the last two studies.

**3. Myometrial invasion:** Our study showed that a significant correlation found between mean MVD and myometrial invasion where the mean MVD was higher when there is > 1/2 of myometrial invasion as in table 3.

This agree with studies done by Kaku et al 1997<sup>(16)</sup> using anti –factor VIII related antigen .Studies done by Fujiwaki et al 2002<sup>(28)</sup> & Wagatsuma et al 1998<sup>(17)</sup> using anti factor VIII related antigen show no correlation significance between mean MVD and myometrial invasion . In our study 20 cases out of 35 cases with endometrial adenocarcinoma show myometrial invasion in > 1/2 of the myometrium i.e more than half of the cases, while in the Fujiwaki et al and Wagatsuma et al the number of patients with > 1/2 myometrial invasion is less than half of the total number of patients with endometrial adenocarcinoma,this may give an idea about the difference in the result in these studies .

**4. Tumor grade :** A significant correlation was found between mean MVD using CD34 antibody and tumor grade in endometrial adenocarcinoma in our study as in

table4.Our results are similar to that of Kaku et al 1997 using anti- factor VIII related antigen , Abulafia et al 1995 using CD34 antibody and Wagatsuma et al 1998 using anti-factor VIII related antigen<sup>(17&27)</sup>, while Fujiwaki et al 2002<sup>(28)</sup> using anti-factor VIII related antigen found no significant association between mean MVD and tumor grade , in this study they classified the tumor grade into a low and high grade groups only, while in our study and the previous mentioned studies that show a significant correlation between mean MVD and tumor grade ,we classify the grade into grade I (well differentiated) , grade II ( moderately differentiated ), & grade III (poorly differentiated ), so this may explain the difference in the results between those studies and Fujiwaki et al study.

#### 5. Tumor stage :

In spite of the fact that the mean MVD was higher in patients with advanced disease ,a statistically significant correlation was not observed between mean MVD using CD34 antibody and tumor stage as seen in table 5 . This fact also proved by Fujiwaki et al 2002 & Kaku et al 1997 using anti-factor VIII related antigen<sup>(16 &28)</sup>

While Mazurek et al 1998<sup>(30)</sup> & Wagatsuma et al 1998<sup>(17)</sup> using anti-factor VIII related antigen proved that there is a statistical significant correlation between mean

MVD and tumor stage .The explanation for that is the last two studies study tumor stage more specifically i.e they take each stage and it's subtypes like, stage Ia , Ib,Ic , so these two studies are more detailed than the present study .

#### 6. pelvic and paraaortic lymph nodes :

Our study shows no significant correlation between mean MVD and lymph nodes metastases including both pelvic and paraaortic lymph nodes as seen in

table 6 &7.These results are in agreement with Kaku et al 1997<sup>(16)</sup> using anti – factor VIII related antigen .

While the study of Wagatsuma et al 1998<sup>(17)</sup> using anti-factor VIII related antigen showed a significant correlation between the mean MVD and lymph nodes metastases, in their study they deal with lymph nodes as one group , they did not divide them into pelvic and paraaortic lymph nodes Regarding distant metastases , only one case present with liver metastases and because this is not enough for statistical analysis so it was neglected .

Wagatsuma et al 1998 thought that the highly angiogenic tumor cells are the likely source of the growing metastatic foci .It is thus important to identify the area of most intensive neovascularization in determining tumor behavior<sup>(17)</sup>

Weidner N 1995 using CD34 antibody found that the increasing intratumoral MVD correlates with greater tumor aggressiveness such as a higher frequency of metastases , the study was done in early breast cancer<sup>(31)</sup>

In our study we did not mention the clinical outcome and the 5 years survival in relation to angiogenesis because of the difficulty to follow up the patients .

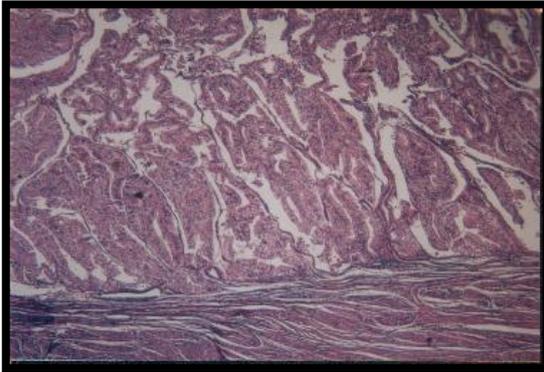
Selveston et al 1998<sup>(32)</sup> using CD34 antibody in endometrial adenocarcinoma reported a 5 years survival probability rates of 57% & 90% for patients with higher and low microvessel densities respectively , and in contrast Wagatsuma et al 1998<sup>(17)</sup> using anti-factor VIII related antigen reported a 0% survival rate in patients with high microvessel densities .

Obermair et al 1998<sup>(33)</sup> using anti-factor VIII related antigen show that high microvessel counts in endometrial carcinoma means poor prognostic factor & decrease in survival rate

Recently , several investigators have shown that the clinical outcome in patients with other tumors such as prostate , lung ,breast ,gastric carcinomas can be predicted by the degree of tumor angiogenesis<sup>(16)</sup>

Bermer et al 1996 using CD34 antibody<sup>(34)</sup> have demonstrated that MVD is an independent prognostic parameter for progression free survival in patients with stage IB & IIA cervical carcinoma. In women with advanced ovarian carcinoma microvessel density (MVD) were associated with disease free survival and with overall survival<sup>(16)</sup>

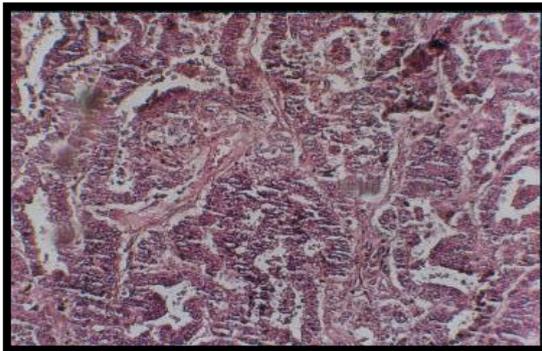
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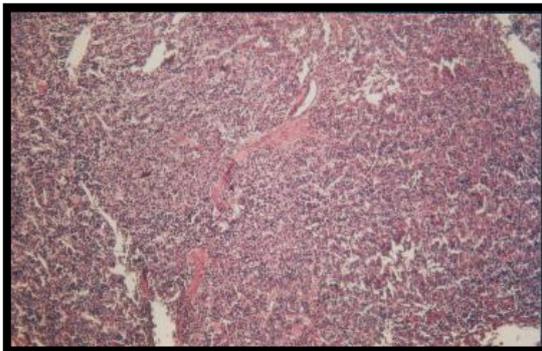
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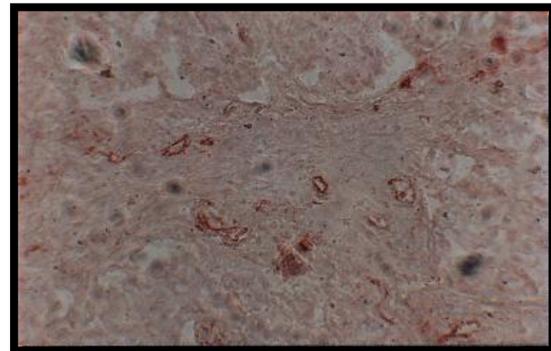
(4)



(5)



(6)



1. Well differentiated endometrial adenocarcinoma H.&E. stained section. (x100).
2. Well differentiated endometrial adenocarcinoma immuno stained with CD34 antibody. The microvessels are red stained.(x 200), mean MVD=61.02.
3. Moderately differentiated endometrial adenocarcinoma H.&E. stained section.(x200). (x200).
4. Moderately differentiated endometrial adenocarcinoma, immuno stained with CD34antibody. (x200) MVD=66.04.
5. poorly differentiated endometrial adenocarcinoma H.&E. stained.(x40).
6. poorly differentiated endometrial adenocarcinoma immunostained with CD34 antibody

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