A Study of angiogenesis in human endometrial denocarcinoma, and premalignant endometrial lesions: A comparative clinic pathological study.

Assistant professor Dr. Maha Shakir Hassan*
*M.B.Ch.B., F.I.C.M.S.(Path)

SUMMARY

Background: The development of new blood vessels is essential to embryonic growth and throughout life for physiological repair processes. Tumors cannot enlarge beyond 1-2 mm in diameter or thickness unless they are vascularized. Angiogenesis is an essential step in growth of primary tumor and for distant metastasis. MVD (Microvessel Density) is one of the important measurement to asses angiogenesis.

Aim: The aims of this study are to asses the micro vessel density (MVD) of endometrium using two markers which are (CD31 and CD34),to investigate the relationship between the degree of angiogenesis and clinicopathological variants, to determine its usefulness in histopathological practice, and to compare between the two markers.

Materials and methods: This retrospective study includes formalin fixed, paraffin embedded tissue sections from patients underwent different gynecological diagnostic and therapeutic procedures. These samples were taken from the hospitals in the period between August 2005 and January 2007. forty nine cases were studied; 10 were normal functional endometrium; 21 hyperplasia, and 18 were diagnosed as endometrial carcinoma. Five slides for each case was done, one slide was stained with Hematoxylin and eosin (H&E), and re-examined to confirm the diagnosis, and the other four were stained immunohistochemically for monoclonal antibodies CD31 and CD34 (two slides for each one).

Results: This study showed that MVD was correlated with benign and malignant endometrial lesions. The mean MVD for normal functional endometrium cases was as follows: 53.9 using CD34 stain, and 47.5 using CD31 stain for secretory endometrium, and 32.2 using CD34 and 30.1 using CD31 for the proliferative type. The mean MVD for hyperplastic endometrium was found to be: 63.2,45.8 (by CD34, and CD31 respectively) for cystic; 75.1 and 69.7(by CD34, and CD31 respectively) for adenomatous, and 78.4,73.2 (by CD34, and CD31 respectively) for atypical endometrial hyperplasia.
Regarding the malignant cases, the mean MVD was: 87.43, 70.22 for well differentiated adenocarcinoma; for moderately differentiated cases they were 102.6, 88.35; and 114.8, 102.7 for poorly differentiated cases (by CD34, and CD31 respectively). The results of mean MVD using CD34 is significantly higher than that obtained by using CD31 for both benign and malignant cases (the p values are 0.0412, and 0.0327 respectively). This study showed that MVD is significantly correlated with the benign and malignant endometrial lesions, but no significant statistical correlation between age and the MVD of the malignant cases.

Conclusion: MVD is an important parameter to asses angiogenesis in functional, hyperplasic and malignant endometrium, and it was found to be higher in malignant cases than benign ones. Significantly higher results obtain for CD34 in comparison with CD31for both benign and malignant cases.

*Thi-Qar.College of medicine.Thi-Qar.Iraq

INTRODUCTION

Premalignant endometrial lesions:

Endometrial hyperplasia: Denotes a set of mixed epithelial and endometrial proliferation that comprise a spectrum of glandular, architectural, and cytological abnormalities, ranging from disordered proliferative endometrium to proliferation so complex that may resemble well differentiated adenocarcinoma. It is thought to be the result of persistent, prolonged estrogen stimulation. There are many systems of classification of this lesion, in our study we use Vellios Tavassoli and Kraus, which classify endometrial hyperplasia into: cystic, adenomatous, and atypical hyperplasia[1,2,3].

Endometrial carcinoma:

Cancer of the corpus uterus is the eighth most common malignant neoplasm in women worldwide, it is the most common gynecologic malignancy in the developed countries[4].In Iraq, in a report issued by Iraqi cancer registry for the years between 1998-2002, it was included in the first 10 cancers occur in females except in 1999 where it was the ninth. Generally, endometrial carcinoma is thought to be a disease of postmenopausal women, however one fourth of the cases may occur in women who are premenopausal[4].

Risk factors:

It is currently believed that endometrial carcinoma can be divided into two distinct types on the basis of their pathogenesis, one, by far the more common, occurring as a result of excess estrogenic stimulation and developing against a background of endometrial hyperplasia and the other developing de novo[6,7].

Some risk factors thought to be associated with the increase risk of endometrial carcinoma are:

* Hormone replacement therapy for the prevention of the postmenopausal morbidity as a result of progressive decrease in natural estrogen secretion.
*Oral contraceptives *Tamoxifen therapy *obesity * diabetes mellitus * hypertention[8].

Pathologically: 80% of endometrial malignant epithelial tumors are conventional adenocarcinomas[9].
Grading system[10]:

- well differentiated (grade I) carcinoma: five percent or less of the tumor is non squamous or nonmorular solid growth pattern.
- moderately differentiated (grade II): six percent to 50% of the tumor is solid growth pattern.
- poorly differentiated (grade III): more than 50% is solid growth pattern.

Angiogenesis: It is a process by which preexisting vessels sent out capillaries sprouts to produce new vessels[9].

Angiogenesis and cancer: Tumors cannot enlarge beyond 1-2 mm in diameter or thickness unless they are vascularized. Beyond this size the tumor fails to enlarge without vascularization because hypoxia induce apoptosis by activation of p53[11].

Assesment of angiogenesis:

- MAGS: Microscopical grading system score, calculated by measuring vessels number, endothelial cell hyperplasia and cytology in tiactorally stained tissue sections[12].
- Hot spot:Blood vessels are immunohistochemically highlighted and the number of microvessels quantified in the most vascular areas of the tumor[13].
- Computerized image analysis. The automated quantitative image analysis allows the accurate study of a large number of specimens, with

reproducibility and sensitivity exceeding 99%[14,15].

Endothelial markers:

Since endothelium is highly heterogeneous, the choice of antibody influences the number of microvessels available for assessment[16].

♦ Those of low specificity antibodies and are present on many non-endothelial elements, directed against vimentin, lectin, alkaline phosphatase, and type IV collagen[17].

♦ Factor VIII-related antigen: is synthesized in endothelial cells of blood vessels, and it is found in megakaryocytes, platelets, and mast cells[13,18,19,20].

♦ CD31: This antigen identifies the hematopoietic progenitor antigen ER-MP 12, which is identical to the platelet endothelial cell adhesion molecule PECAM-1[21,22,23].

♦ CD34: It is a 110 kDa transmembrane cell surface glycoprotein, expressed developmentally on early lymphohemopoietic stem and progenitor cells[24,25,26].

In clinical setting, analysis of the intratumoral microvessel density (MVD) of solid tumor, highlighted with specific marker for endothelial cells, is CD34, which is found to have highest specific staining and least background staining compared with CD31 and factor VIII[9,27,28,29].

PATIENTS AND METHODS:

▪ This retrospective study includes samples of paients underwent total abdominal hysterectomy and bilateral salpingio-oopherectomy, subtotal abdominal hysterectomy, vaginal hysterectomy, dilatation and curettage (D&C) , and endometrial biopsy. These samples were taken from department of pathology in Al- Kadhemia teaching hospital, covering the period between August 2005- January 2007.The available information from the histopathological reports were only age, grade, and type of the operation. This study includes 49 samples taken from patients aged between 29- 70 years old, 10 were normal functional endometrium, 21 were hyperplastic endometrium and 18 were diagnosed as endometrial carcinoma.The normal functional endometrial cases: Secretory and proliferative endometrium, 5 cases for each.Hyperplastic endometrium: 6 cystic, 9 adenomatous, and 6 atypical hyperplasia.Malignant endometrial lesions: 7 well differentiated, 5 moderately differentiated, and 6 poorly differentiated.

▪ *MATERIALS*:

Immunohistochemical stain:

Reagents and solutions:

1- counter stain: Mayer's hematoxylin.
2- distilled water.
3- ethanol alcohol.
4- mounting medium (DPX).
5- Phosphate buffered saline (PBS).
6- Protein blocker.
7- Secondary detection kit.
8- Primary monoclonal antibody.
9- The specimen chosen were already formalie fixed and paraffin- embedded five sections for each representative part of the tissue were taken, one was stained with H&E and re-examined to confirm diagnoses, 2 were stained immunohistochemically for CD34, and the last two were stained immunohistochemically for CD31.
10- Xylene.

Equipments:

1- Aluminum foil.
2- Appendroff tubes.
3- Cotton –wool.
4- covered glass jars.
5- Disposable knives.
6- Filter papers.
7- Glass coverslides.
8- Hot air oven.
9- Humid chamber.
10- Incubator.

11- positively charged microscopic slides.
12- timer and alarm.

THE METHOD

1- paraffin embedded tissue block were sectioned into 5µm thickness, put on the positively charged slides, and allow to dry at room tempreture.
2- preparation of the concenetrated primary antibody.
3- Ethanol was prepared in 3 changes; absolute and two dilutions (95%, and 70%) with distal water.
4- PBS was prepared bydissolving each tablet in 100 ml DW.
5- Power blocker was prepared by adding 9µl DW for each 1µL power blocker.

Quantitative measures of angiogenesis:

All blood vessels were high lightened by staining endothelial cells for CD34 and CD31 with the use of the standard immunohistochemical technique, Tonsils were used as a positive control tissue for CD34, and CD31(figure 1). Negative control is obtained by omission of the primary antibody (figure2).Microvessel density was assessed by using Weidner's method. It is assessed without diagnoses ; areas of most intense neovascularization were examined by light microscopy. Tumors were frequently heterogeneous in their microvessel density, but the areas of highest neovascularization were found by scanning tumor sections at low power (40x and 100x). and identifying the areas on invasive carcinoma with the highest number of discrete microvessels staining for CD34, and CD31(brown).Individual microvessels were counted on 400x field. Any brown-stained endothelial cell or endothelial cell cluster that were clearly separated from adjacent microvessels, tumor cells and other connective tissue elements were considered a single, countable microvessel. Vessels lumens although usually present, were not necessary for a structure to be identified as a microvessel, and RBCs were not used to define vessel lumen.MVC(microvessel capacity) was expressed as the mean value of all the five fields examined. MVD is the MVC divided on the high power field area which is 0.1885mm^2. Larger vessels with thick muscular coat and their lumen larger than the size of 8 RBCs were excluded from the count[30].

Statistical analysis:

The statistical significance of the difference in mean between two groups was assessed by using student t-test, and more than two groups were assessed by using ANOVA test. P value less than the 0.05 level of significance was considered statistically significant.

Results.

Total number of 49 cases of endometrial specimens were studied, 10(20.4%) were functional endometrium, 21(42.85%) hyperplastic and 18 (36.7) were diagnosed as endometrial carcinoma (table1).

Age:

Concerning normal functional endometrial control group, as shown in table (1) 30% were at the age between 20-29 year, 10% between age 30-39, 40% between 40-49 year, and 20% fell between 50-59 years old.
The hyperplastic cases, as shown in table (1) 19.04% of the cases were at the third decade of life, 19.04% between 30-39, and 14.22% of the cases between 40-49 years; while the percentage of cases that fell between 50-59 was 23.57%. Only 4 cases were between 60-69 years of age and that represent 19.04 of the entire hyperplastic group.
Regarding the cases of endometrial carcinoma, as shown in the same table 38.88% was the percentage of cases aged between 40-49, 27.77% between 50-59, 22.22% between 60-69 years old; while cases above 70 years old were only 2 and the percentage was 11.11%.

The mean age of the hyperplastic cases was 46.2. The mean age of endometrial cases was 54.7%.

Histological subtypes:

As shown in table (2), the normal functional 10 cases(20.04% of total cases studied) comprises secretory and proliferative endometrium, 10 cases for each. The immunohistochemical staining using CD31, and CD34 endothelial markers show the results in figure (3,4, and5).

The 21 hyperplastic cases (42.85%) were subdivided into 6 cases(28.57%) cystic hyperplasia, 9 cases(42.85%) adenomatous hyperplasia, and 6 cases(28.57%) were atypical hyperplastic endometrium as shown in table(2).

Regarding the malignant cases, the studied cases were 18 table (2) (36.73 of the total selected cases), divided into: 7 (38.88% of malignant cases) were well differentiated, 5 (27.77%) were moderately differentiated, and 6 cases (33.33) were poorly differentiated endometrial adenocarcinoma table(2).

Correlation between MVD, clinicopathological, and histopathological features:

MVD and age:

For statistical analysis, the patients age (for the malignant cases only) was divided into two groups: The first group ≤ 55 years old which was 12 (66.66%) and the other > 55years old which include 6 cases (33.33%). As shown in table (3), the mean MVD for the first age group was 103.9 using CD31 and 110.5 using CD34 ; while the mean MVD for the age group >55 years old was 108.12 using CD31 and it was 111.7 using CD34, table(3). Student t-test showed the difference between the two means was statistically non-significant (p value=0.416, 0.321 for CD34 and CD31 respectively).

MVD with normal functional, hyperplastic and malignant endometrium

The MVD for normal functional endometrial cases was shown in table (4). For secretory endometrium the MVD was 53.9 using CD34 and it was 47.5 using CD31 .Th**e MVD** for proliferative endometrium was 32.2 using CD34 and it was 30.1 using CD31. The MVD for cystic hyperplasia was 63.2 using CD34, and it was 45.8 using CD31**.**

The MVD for endometrial hyperplasia was 75.1 using CD34, and it was 69.7 using CD31. The MVD for atypical hyperplasia was 78.4 by CD34 and it was 53.2 using CD31. Figure (6,7)

The MVD for well differentiated carcinoma was 87.43 using CD34 (table5) and it was 70.22 using CD31. The MVD for moderately differentiated carcinoma was 102.6 using CD34 and it was 88.35 using CD31 marker.The MVD for poorly differentiated carcinoma was 114.8 using CD34, and it was 102.7 using CD31 marker (figure,8,9, and10).

The mean MVD in endometrial carcinoma cases was 101.61 using CD34, and it was 87.09 using CD31; while in benign cases (including normal functional, and hyperplastic cases) was 60.56 using CD34, and it was 53.26 using CD31(table6).The results of mean MVD using CD34 is significantly higher than that obtained by using CD31 for both benign and malignant cases (the p values are 0.0212, and 0.0127 respectively).

This study showed that MVD is significantly higher for the malignant cases than the benign cases by both CD34 and CD31(p values are 0.00176, 0.0036 respectively).

DISCUSSION

*Immunohistochemical method:

Although the immunohistochemical staining has the same principles and steps, there are some differences according to the antibodies and specimens used.

In this work, we didn't get a good staining results when we used the same time advised in the literatures of the staining kits, the few minutes didn't give any color, we did many modifications such as prolongation of the incubation period of the primary antibody from 1-2 hours to overnight incubation in the refrigerator. The time of all the other staining steps was also not sufficient; the longer time of incubation of the biotinylated links strepavidin and the chromogen gave better results.

Non- specific background staining coasted us many runs, so we did many modifications to overcome this problem. As wax is one of the causes of the non-specific stains, prolongation of the period of deparaffinization, prolongation of the incubation period of the hydrogen peroxide.The hot whether resulted in drying of the tissue sections, which is one of the important causes of the background non-specific stains. We overcome this when we drain and blot one slide, put the second step reagent in the humid chamber then start with another slide.With each tissue section to asses the MVC, we noticed that there is heterogenicity in the MVC for the same slide of the same tissue. We tried to overcome this by assessing the MVC in all the hot spots found then we estimate the mean of the only higher 5 MVC detected.We calculate the microvessels by using 400x field then divided it on the area of that field which is 0.1885mm^2, so the MVD was obtained. In this study we used the panendothelial marker CD34 and CD31 to highlight the endothelial cells of the blood vessels.

* Age:

Our study revealed that the mean age of the patients with endometrial hyperplasia precedes that of endometrial carcinoma by about nine years this result agree with that done by others, and confirm the view that hyperplastic lesions of endometrium is potentially premalignant.The mean age of endometrial carcinoma was 45.7 which is near those studies done by others in Iraq, but these results disagree with that done in western countries, where the mean age of patients with adenocarcinoma is higher by about ten years. However a larger sample should be available to get clearer idea, this may be due to the exposure of Iraqi peoples to several insults during the years of wars[26,31,32].

*Correlation between angiogenesis and histological subtypes

In this study we asses angiogenesis using MVD, it is found that secretory endometrium is more vascular than proliferative one, this results is similar to that obtained by others[33]. The MVD (using CD34, CD31) was significantly higher in malignant cases than those obtained for benign cases (including the normal functional and hyperplastic groups). This results is consistent with that study done by Morgan KG[33] and Kaku T[19] who used factor viii –related Ag to stain vascular channels. This results confirm the theory that the angiogenesis is an essential step for tumor growth (Folkman J)[34].

Also we found that the MVD results using CD34 is significantly higher than that obtained using CD31 this result is consistent with obtained by Siitonen SM[35] who evaluate the differences among antibodies that act against vWF and cell surface markers CD34 and CD31 in breast cancer, they concluded that all three markers could be used but anti-vWF and anti-CD34 showed better staining results than anti –CD31.it is found that CD34 have the highest

specific staining and least background staining compared with CD31 and factor viii. In Iraq study done by AL-Sayegh ZA[36] using two markers to asses angiogenesis in gastric carcinoma, they also found the CD34 is more sensitive than CD31in staining endothelial cells.In this study the MVD was observed to be increasing when progressing from grade I to grade III, this in agreement with other studies done by Ozysal S[37] and Erden O[38];while disagree with Kaku T[19], Wagastsuma S[20], and others. The explanation of this may be related to the increase vascularity of the tumor with the increasing grade of the carcinoma.

Correlation between angiogenesis and age

Although the MVD is increasing with increasing age in malignant cases, which agree with some studies and disagree with others, the sample we used is small so that we can not give a final conclusion[20,39,40,41,42].

Table (1) :Number and percentage of cases according to the age.

Age in years	Normal(functional) No (%)	Hyperplastic No(%)	Malignant No(%)
20-29 years	3(30%)	4(19.04%)	
30-39 years	1 (10%)	4(19.04%)	
40-49 years	4(40%)	3(14.22%)	7(38.88%)
50-59 years	2(20%)	6(23.57%)	5(27.77%)
60-69 years		4(19.04%)	4(22.22%)
>70 years			2(11.11%)
Total No.	10 cases	21cases	18 cases

Table(2): Number and percentage of cases according to histological subtypes.

Histological subtypes	Normal No(%)	Hyperplastic No(%)	Malignant No(%)
Secretory endometrium	5(50%)		
Proliferative endometrium	5(50%)		
Cystic hyperplasia		6(28.57%)	
Adenomatous hyperplasia		9(42.85%)	
Atypical hyperplasia		6(28.57%)	
Well differentiated ca.			7(38.88%)
Modertely differentiated ca.			5(27.77%)
Poorly differentiated ca.			6(33.33%)

Table (3):Correlation between mean MVD and age.

Age group	Number	Mean MVD using CD31	Mean MVD using CD34
≤ 55years	12	103.9	110.5
>55years	6	108.12	111.7

Table(4): Mean MVD of normal functional, and hyperplastic cases.

Variable	Mean MVD using CD31	Mean MVD using CD34
Secretory	47.5	53.9
Proliferative	30.1	32.2
Cystic hyperplasia	45.8	63.2
Adenomatous hyperplasia	69.7	75.1
Atypical hyperplasia	73.2	78.4
Mean	53.26	60.56

Table(5): The mean MVD of malignant cases.

Variable	Mean MVD using CD31	Mean MVD using CD34
Well diff. ca.	70.22	87.43
Moderately diff. ca.	88.35	102.6
Poorly diff. ca.	102.7	114.8
Mean	87.09	1o1.61

Table (6): The mean MVD for benign and malignant cases using CD31, and CD34.

Variable	Mean MVD using CD31	Mean MVD using CD34
Benign cases	53.26	60.56
Malignant cases	87.09	101.61

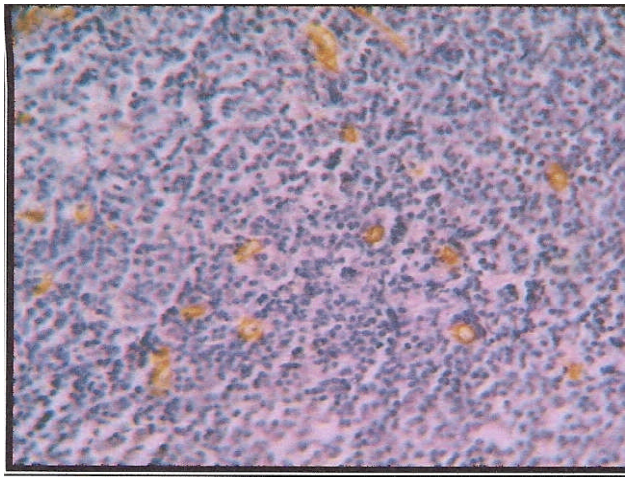

Figure 1: shows immunohistochemically CD31 stained tonsil tissue sample (positive control).

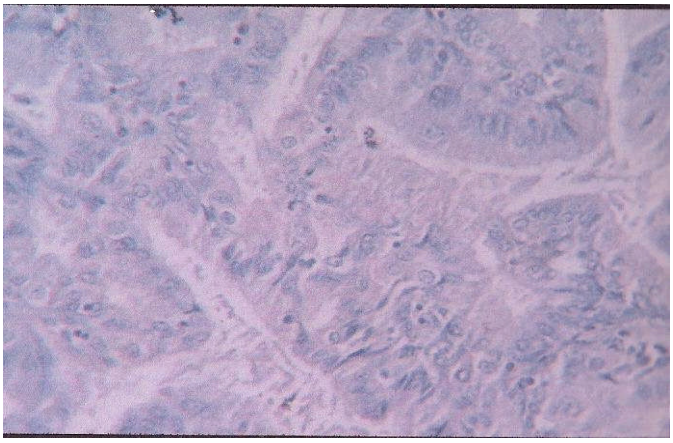

Figure 2: well differentiated endometrial carcinoma with omission of the primary antibody (negative control).

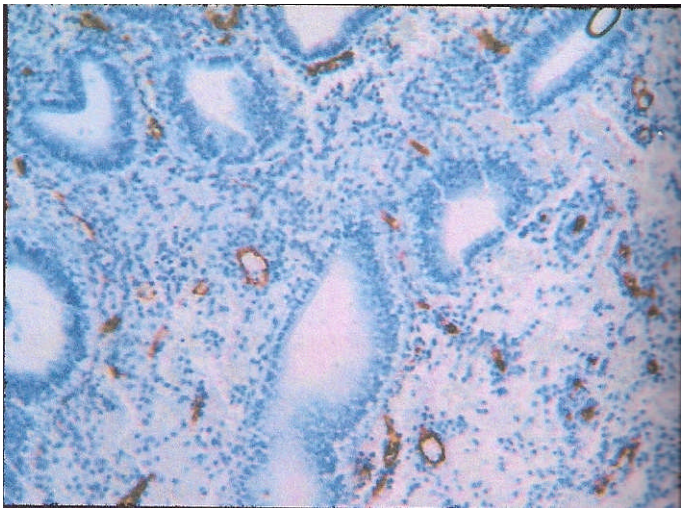

Figure3: secretory endometrium, immunohistochemically stained with CD31(200x).

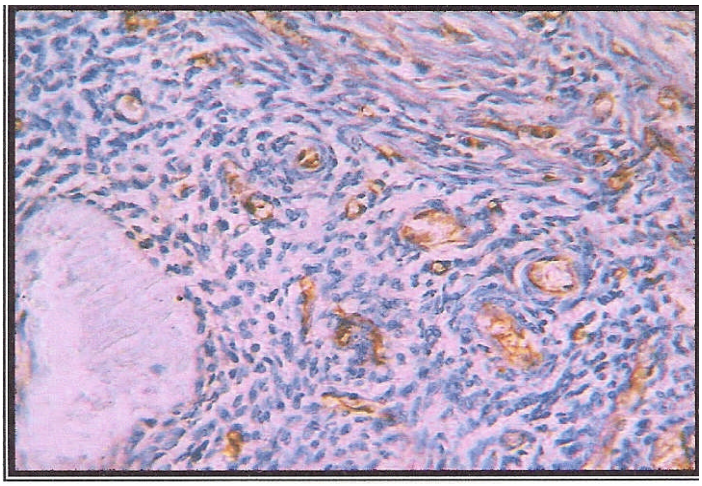

Figure 4: proliferative endometrium, immunohistochemically stained with CD34 (400X).

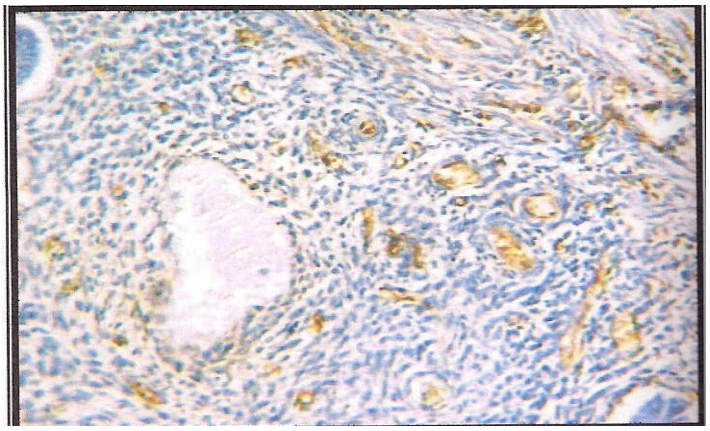

Figure 5: proliferative endometrium, immunohistochemically stained with CD31 (200X).

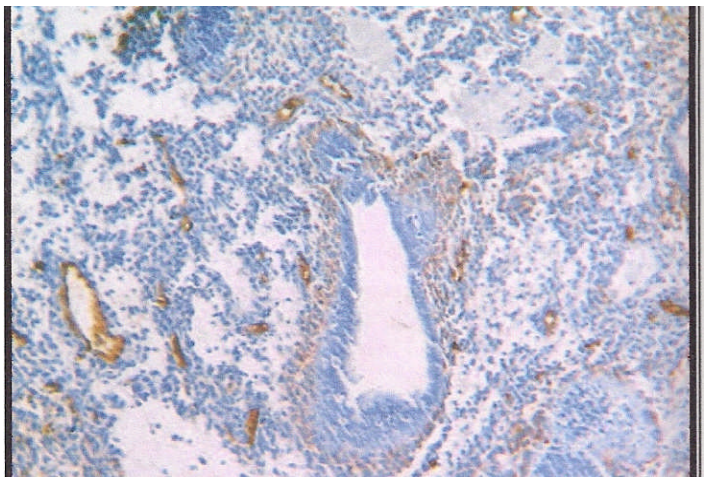

Figure 6: Cystic endometrial hyperplasia, immunohistochemically stained with CD31, (200X).

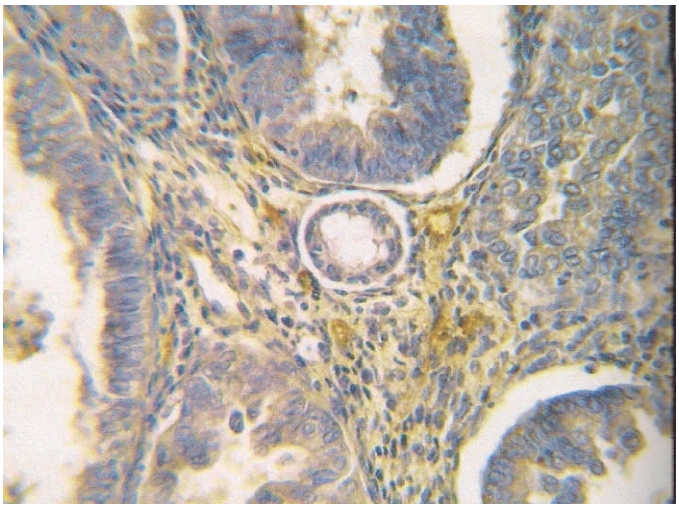

Figure 7: Atypical endometrial hyperplasia, immnohistochemically stained with CD31, (400X).

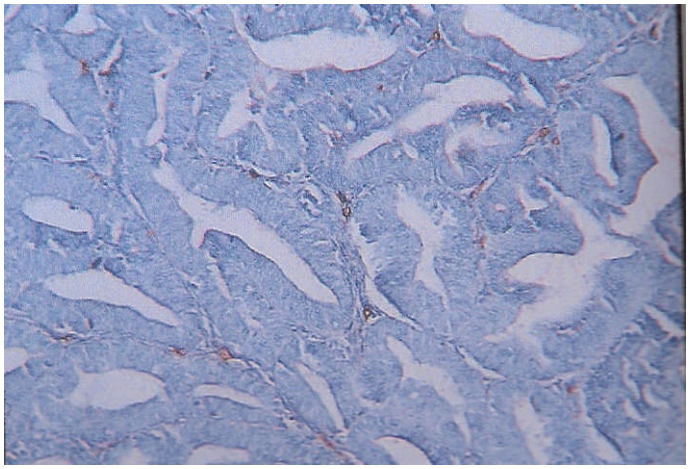

Figure 8: well differentiated endometrial adenocarcinoma, immunohistochemically stained with CD34, (200X).

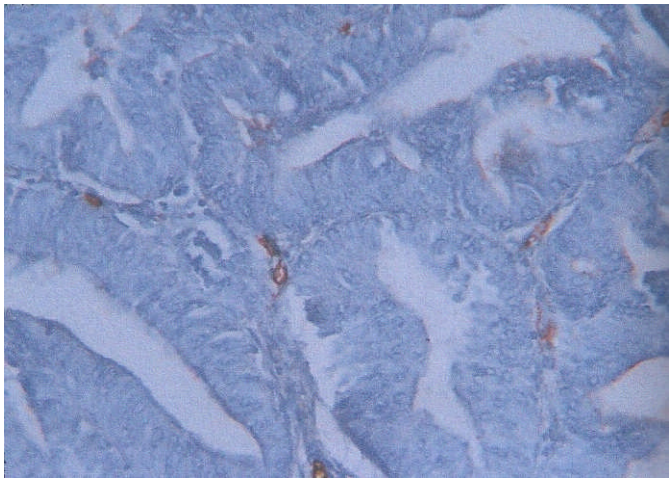

Figure 9: well differentiated endometrial adenocarcinoma, immunohistochemically stained with CD31, (400X).

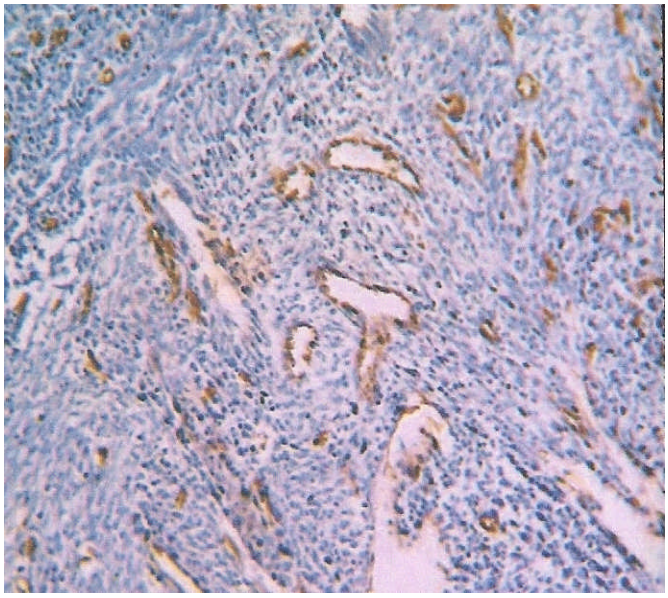

Figure 10: poorly differentiated endometrial adenocarcinoma, immunohistochemically stained with CD34, (200X).

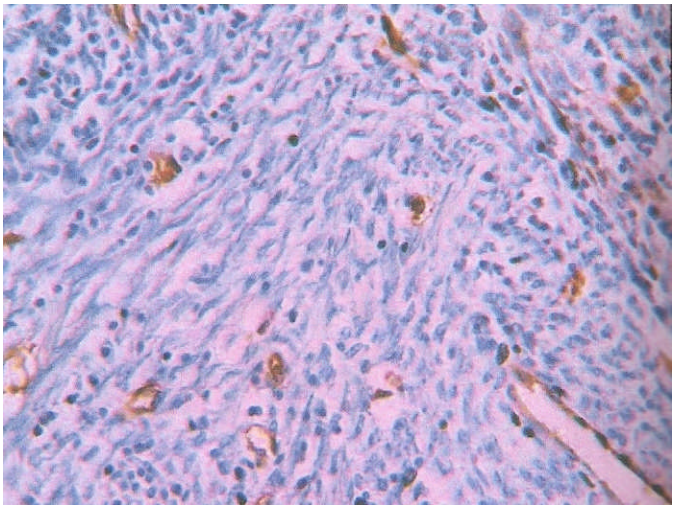

Figure 11: poorly differentiated endometrial adenocarcinoma, immunohistochemically stained with CD31, (400X).

References

1- Hendrickson MR and Kempson RL. The differential diagnosis of endometrial adenocarcinoma: Some view points concerning a common diagnostic problem. Pathology 1980; 12: 35-61.

2- Mutter GL, Wada H, Faquin WC and Enomoto I. K-ras mutations appear in the premalignant phase of both microsatellite stable and unstable endometrial carcinogensis. Mol Pathol. 1999 Oct; 52(5):257-62.

3- Kraus FT. High risk and premalignant lesions of the endometrium. Am J Surg Pathol 1985; 9(suppl): 3 1-40.

4- Parkin DM, Pisani P and Ferlay J. Estimates of the worldwide incidence of eighteen major cancers in 1985. Int J Cancerl993 Dec; 55(6):891-903.

5- Iraqi Cancer Registry (ICR) issued for the years between 1998 and 2002.

6- Gusberg SB and Milano C. Detection of endometrial carcinoma and its precursors. Cancer. 1981 Mar; 47(5 Suppl):1173-5.

7- Beckner ME, Mon T and Silverberg SG. Endometrial canrcinoma: Non-tumor factors in prognosis. Int J Gynecol Pathol. 1985; 4(2): 131-45.

8- Deligdisch L. Hormonal pathology of the endometrium. Med pathol 2000; 13(3): 285-94.

9- Rosai J. Special techniques in surgical pathology. Volume 1, chapter 3: pp.37-156.

10- Zaino RJ, Kurman RJ, Diana KL and Morrow CP. Pathologic models to predict outcome for women with endornetrial adenocarcinoma: The importance of the distinction between surgical stage and clinical stage--a Gynecologic Oncology Group study. Cancer. 1996 Mar; 77(6):l 115-21.

11- Kurman RJ, Kaminski PF and Norris HJ. The behavior of endornetrial hyperplasia. A long-term study of “untreated” hyperplasia in 170 patients. Cancer. 1985 Jul; 56(2):403-12.

12- Brem S, Cotran R and Folkman J. Tumor angiogenesis: A quantitative method for histological grading. J Nati Cancer Inst. 1972 Feb; 48(2):347-56. Breman ML and McHale MT. Uterus, chapter 57, pp.951-66. In Kaskell CM. Cancer treatment. Fifth edition, 2001.

13- Weidner N, Semple JP, Welch WR and Folkman J. El: tumor angiogenesis and metastasis-correlation in invasive breast carcinoma. N Engl J Med. 1991 Jan; 324(1): 1-8.

14- Laitakari J, Harrison D and Stenback F. Morphometric characteristics of cell proliferation and p53 expression in development of experimentally induced respiratoiy tumors. Analyt Quant Cytol Histol 2001; 23, 273-86.

15- Lohmann CM, Fields AC, Schiller A et al. Quantitation of angiogenesis by measuring CD3 1 and factor VIII visually and by image cytometry in renal cell carcinoma_prognostic significance. J Urol Pathol 1999; 11: 101-12.

16- Fox SB. Microscopic assessment of angiogenesis in tumors, chapter2. Human press 2001;pp.29-49.

17- Vesalainon S, Lipponen P, Taija M, Alhava E and Syrjanen K. Tumor vascularity and basement membrane structure as prognostic factors in Ti -2M0 prostatic adenocarcinoma. Anticancer Res. 1994 Mar-Apr; 1 4(2B):709- 14.

18- Rosai J. Rosai and Ackerman's surgical pathology. Appendix C: Staging of cancer: pp.28O1-29.Elsevier.Ninth edition, 2004c.

19- Kaku T, Karnura T, Kinukawa N, Kobayashi H, Sakal K, Tsuruchi N, Saito T, Kawauchi S, Tsuneyoshi M and Nakano H. Angiogenesis in endometrial carcinoma. Cancer. 1997 Aug; 80(4):741-7.

20- Wagatsuma 5, Konno R, Sato S and Yajima A. Tumor angiogenesis, hepatocyte growth factor and c-met expression iii endometrial carcinoma. Cancer. 1998 Feb; 82(3):520-30.

21- Ling V, Liixenberg D, Wang J, Nickbarg E, Leenen PJ, Neben S and Kobayashi M. Structural identification of the hematopoietic progenitor antigen ER-MP 12 as the vascular endothelial adhesion molecule PECAM- 1 (CD3 1). Eur J Immunol. 1997 Feb; 27(2):509-14.

22- Chu JS, Huang CS and Chang KJ. The prognostic significance of tumor angiogenesis in Taiwanese patients with invasive ductal breast carcinomas. Cancer Lett. 1998 Dec; 134(1):7-14.

23- Al-Obaidi AB. IFN-y versus IL-0 in situ expression and immunohistochemistry analysis of VCAM- I, PECAM- 1 and HCMV in recurrent spontaneous abortion. A thesis submitted to college of medicine — Al-Nahrain University in partial fulfillment of the requirements for the degree of Master of Science in medical microbiology. 2005.

24- Greaves MF, Brown J, Molgaard HV, Spurr NK, Robertson D, Delia D and Sutherland DR. Molecular features of CD34: a hematopoietic progenitor cell-associated molecule. Leukemia. 1992; 6 Suppl 1:31-6.

25- Katz FE, Tindle R, Sutherland DR and Greaves MF. Identification of a glycoprotien associated with hemopoitic progenitor cells. Leuk Res. 1985; 9(2):191-8.

26- Brown J, Greaves MF and Molgard HV. The gene encoding the stem cell antigen CD34 is conserved in mouse and expressed in hemopoietic progenitor cell lines, brain, and embryonic fibroblasts. IntImmunol. 1991 Feb; 3(2):175-84.

27- Schoell WM, Pieber D, Reich 0, Lahousen M, Janicek M, Guecer F and Ninrer R. Tumor angiogenesis as a prognostic factor in ovarian carcinoma. Cancer. 1997 Dcc; 80(12):2257-62.

28- Hansen S, Graban BA, Sorensen FB, Bak M, Vach W and Rose C. Vascular grading of angiogenesis: Prognostic significance in breast cancer. Br J Cancer. 2000 Jan; 82:339-47.

29- PoOn RT, Ng TO, Lau C, Yn WC, Yang ZF, Fan ST and Wong J. Tumor microvessel density as a predictor of recurrence after resection of hepatocellular carcinoma: A prospective study. J Clin Oncol. 2002 Apr; 20(7):l775-85.

30- Van Hoef MEHM, Knox WE, Dhesi 55, HoweJi A, Schor AM. Assessment of tumor vascularity as a prognostic factor in lymph node negative invasive breast cancer. Eur J Cancer 1993; 29A: 1141-5.

31- Daboagh SR. Assessment of AgNOR in normal, hyperplastic and endometrial adenocarcinoma. A thesis submitted to the scientific council of pathology in partial fulfillment of the requirement for the degree of followship of Iraqi commission for medical specialization in pathology. 2003.

32- Sherman Al and Brown S. The precursors of endometrial carcinoma. Am J Obstet Gynecol 1979; 135: 947-56.

33-Fox SB. Microscopic assessment of angiogenesis in tumors, chapter 2. In Murray JC. Angiogenesis protocols. Humana press 2001; pp.29-46.

34- Folkman J. Role of angiogenesis in tumor growth and metastasis. Semin Oncol. 2002 Dec; 29(6 Suppl16):15-8.

35- Slitonen SM, Haapasalo HK, Rantala IS, Helm HJ and Isola JJ. Comparision of different immunohistochemical methods in the assessment of angiogenesis: Lack of prognostic value in group of 77 selected node-negative breast carcinoma. Mod Pathol 1995; 8: 745-52.

36- Fox SB. Microscopic assessment of angiogenesis in tumors, chapter3. Human press 2001;pp.29-49.

37- Ozuvsal *5,* Bilgin T, Ozan H, Kara HF, Ozturk H and Ercani. Angiogenesis endometrial carcinoma. Correlation with survival and clinicopathologic risk Factors. Gynecologic and Obstetric Investigation 2003; 55(3): 173-7.

38- Erdem 0, Erdem M, Dursun A, Akyol G and Erdem A. Angiogenesis, p53 and bcl-2 expression as prognostic indicator in endometrial cancer: Comparision with traditional clinicopathologic variables. Int J Gynecol Pathol 2003 Jul; 22(3): 254-60.

39- Salvesen HB, Iversen OE and Akslen LA. Prognostic significance of angiogenesis and Ki-67, p53 and p21 expression: A population-based endometrial carcinoma study. J Clin Oncol. 1999 May; 17(5):l382-90.
91. In Rosai and Ackerman's surgical pathology. Elsevier. Ninth edition, 2004a.
40-Fujiwaki R, Iida K, Kanasaki H, Ozaki T, Hata K and Miyazaki K. Cyclooxygenase-2 expression in endometrial cancer. Correlation with microvessel count and expression of vascular endothelial growth factor and thymidin phosphorylase. Human Pathol 2002 Feb; *33(2):* 213-8.

41- Bai X and Mi R. Expression of basic fibroblast growth factor and microvessel density in endornetrial carcinoma. Zhonghua Fu Chan Ke Za Zhi 2000 Jun; 35(6): 348-51.

42- AL-Qureishi LF. The prognostic role of CD34 in the angiogenesis of endometriall carcinoma. A thesis submitted to the scientific council of pathology as a partial fulfillment of the requirement for the degree of *Board inpathology. 2005*

دراسة تولد الاوعية الدموية في الأورام الخبيثة لبطانة الرحم وأمراض الرحم سابقة الخبث،دراسة نسيجية سريريه مقارنة

أ.م.د مها شاكر حسن*

*بكالوريوس طب وجراحة عامة، شهادة البورد العراقي في علم الأمراض (النسيج المرضي)

الخلاصة

الأهداف:أهداف هذه الدراسة هي لتقييم كثافة العروق الدمويه الصغيرة كعلامة لتولد الأوعية الدموية في حالات الرحم الطبيعية وفرط التنسج وأورام الرحم الخبيثة باستخدام المجهر الضوئي بعد تحديد المناطق الحاوية على اكبر تولد دموي وطريقة حساب جزء المساحة ، للتحري عن العلاقة بين درجة تولد الاوعية الدموية ومختلف العوامل السريرية المرضية لسرطان بطانة الرحم، وأيضا لتحديد فائدتها في الممارسة النسيجية المرضية.

الخلفية:إن تولد الأوعية الدموية هي خطوة مهمة للتكون الجنيني وخلال الحياة لعملية الترميم الفسيولوجي كما في اندمال الجروح، إعادة إحياء النسيج ما بعد العوز الدموي وتغيرات بطانة الرحم نتيجة الدورة الشهرية . لا تستطيع الأورام إن تنمو أكثر من ١-٢ ملم قطرا أو سمكا إلا إذا كان مجهز بالأوعية الدموية . من المحتمل إن منطقة ١-٢ملم تمثل أقصى مسافة التي عبرها يستطيع الأوكسجين والمواد الغذائية ان ينتشر من الأوعية الدموية . إن تولد الأوعية الدموية هي خطوة مهمة ليس فقط للنمو الأولي للسرطان لكن أيضا للانثبات . وكثافة العروق الصغيرة هو مقياس مهم لتقييم تولد الأوعية الدموية.

خطة الدراسة: هذه الدراسة الاستعادية تتضمن دراسة عينات نسيجية مثبتة بالفورمالين ومحفوظة بالشمع لمريضات خضعن الطرق التشخيصية والعلاجية للإمراض النسائية . هذه العينات أخذت من قسم الإمراض النسيجية من المستشفيات والمختبرات الخاصة للفترة بين آب ٢٠٠٥ وكانون الثاني ٢٠٠٧. ٤٩حالة درست وكانت ١٠ حالة طبيعية و٢١ حالة مفرطة التنسج و١٨حالة كانت سرطان بطانة الرحم . هذه العينات المختارة كانت أصلا مثبتا بالفورمالين ومحفوظا بالشمع.٥شرائح (بسمك ٥مايكرون لكل عينة) أخذت من الجزء المعبر من النسيج شريحة واحدة صبغت بصبغة الهيماتوكسلين والايوسين وأعيد فحصها للتأكد من التشخيص وشريحتين اخريتين صبغت بالصبغة الكيميائية النسيجية المناعية للمضاد المناعي الاحادي سي دي ٣٤وشريحتين صبغت بالصبغة الكيميائية النسيجية المناعية للمضاد المناعي الأحادي سي دي٣١.أن عدد العروق الدموية الصغيرة قيمت باستخدام المجهر الضوئي بعد تحقيق المناطق الحاوية على اكبر تولد دموي ، وجدت بواسطة مسح قطعة النسيج باستخدام القوه الصغيرة للمجهر الضوئي وتحديد المنطقة ذات أعلى رقم للأوعية الدموية المصبوغة بالون البني للمضاد الاحادي سي دي ٣٤،و سي دي ٣١. كل الأوعية الدموية حسبت باستخدام القوه الكبيرة للمجهر الضوئي

النتائج:
بينت هذه الدراسة إن كثافة العروق الدموية في سرطان بطانة الرحم هي اعلى بكثير من تلك المحسوبة في الحالات الطبيعية وأن النتائج التي تم الحصول عليها باستعمال ألمضاد الاحادي سي دي ٣٤ هي اعلي من النتائج التي تم الحصول عليها باستعمال المضاد الأحادي ٣١. وأنه لا توجد علاقة إحصائية بين العمر وكثافة الأوعية الدموية