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Original research Article

Micro-vessel density as a guide to angiogenesis in human endometrium

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ABSTRACT

Objectives: To evaluate the role of angiogenesis by the assessment of mean vessel density and to quantify angiogenesis as an important variable in different benign and premalignant endometrial lesions.
Materials and Methods: Endometrial biopsies and hysterectomy specimens were examined to evaluate and define any correlation between angiogenesis and different phases and premalignant diseased states of the endometrium. Microvessel counts were performed by examining the microvessels thoroughly in terms of count, morphology and density after staining the tissues by hematoxylin & eosin stain, reticulin stain and immunostain CD 34.
Results: A total of 381cases of benign and premalignant endometrial lesions were included in the study. In 135 cases of simple hyperplasias, Microvessel Density (MVD) ranged from 2.4 - 6.7 and Mean MVD was 4.71 on H&E stain and MVD ranged from 2.8 - 8.3 with Mean MVD of 5.39 on reticulin stain. In 105 cases of complex hyperplasias, MVD ranged from 2.3 - 7.0 and Mean MVD was 4.93 on H &E satin and MVD ranged from 3.1-7.7 with Mean MVD of 5.68 on reticulin stain. In 67 cases of atypical hyperplasias, inbudding and outpouching of the glandular epithelium together with multilayering was appreciated with MVD from 4.5 - 8.8 and Mean MVD of 6.31 on H &E stain and MVD of 5.5 - 9.4 with Mean MVD of 7.19 on reticulin stain. A statistically significant difference was noted in all categories except in between simple and complex hyperplasia where the difference was not statistically significant. The role of angiogenesis becomes more significant with increasing severity of lesion, both in terms of hyperplasia and atypia when compared to normal endometrium. Although angiogenesis can be detected on immunostain more easily, it can be illustrated well by H and E or Reticulin stain.
Conclusions : Role of angiogenesis becomes more significant with increasing severity of lesion, both in terms of hyperplasia and atypia when compared to normal endometrium. Although angiogenesis can be illustrated well by routine H and E and Reticulin stains, it can be detected on immunostains with greater degree of ease and accuracy.

1. Introduction

Angiogenesis is the formation of new capillaries from the preexisting vascular network and is essential for various biophysiological and pathological conditions.[1] Neoangiogenesis is a cyclical event throughout the menstrual cycle.[2] It keeps on changing as the endometrium changes from normal to abnormal states. It is needed for regenerative, hyperplastic conditions as well as tumor growth and metastases.[3]

Angiogenesis has an essential part to play in endometrial growth, and is manifested in normal endometrium in its proliferative and mid-secretory phase as well as in hyperplasia and carcinomas. In the menstrual phase, when the superficial layers of the endometrium and the coiled

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arteries have been shed off, regeneration takes place with the sprouting of new vessels in the early phase of the cycle which reaches a peak at the time of ovulation. This shows the mutual relation between new vessel formation or angiogenesis and endometrial growth.[2,3]

In the present study, we have studied the status of angiogenesis in normal as well as in hyperplastic endometrium, and an attempt has been made to observe any correlation between the two. This was accomplished by evaluating the counts of microvessels in tissue sections stained by hematoxylin and eosin, reticulin as well as immunostain wherever possible. The main aim of the study was to compare the angiogenesis status by MVD in normal endometrium, endometrial hyperplasia and atypia and its correlation with histological diagnosis.

2. Material and Methods

This study was conducted on 331 endometrial biopsies and 50 hysterectomy specimens, in the Departments of Obstetrics & Gynaecology and Pathology, J N Medical College, AMU, Aligarh. Tissues obtained were fixed in formalin and processed in automatic tissue processor. Sections of 3-5 microns thickness were cut from paraffin embedded blocks and subjected to Hematoxylin & Eosin stain, Reticulin stain and Immunostain CD 34.

Based on histological diagnosis the cases were classified as normal and hyperplastic endometrium. Microvessel counts were done in all the samples. Basement membrane was delineated properly by the reticulin stain so the blood vesssels were identified easily. Microvessels were examined for their morphology and density. Immunostaining for endothelial cells using CD34 antibody was performed by avidin-biotin-peroxidase complex method. Positive staining was identified as strong dark brown staining of endothelial cells. Microvessels were examined thoroughly in terms of count, morphology and density.

2.1. Counting procedure

In all cases, most discrete microvessels which appeared as lumina lined by endothelial cells were counted in high power using 40x objective lens and 10x eye piece (X400 magnification) in 10 fields and average microvessel density (MVD) was calculated for every case. In very small biopsies, maximum possible fields were evaluated. The range of MVDs was recorded in each category of endometrial lesion and further the mean of MVDs of total number of cases in each category was calculated. Simultaneously we also observed the morphology (size, shape & thickening of vessel wall) and distribution of blood vessels in each group. To avoid bias, counting was done by two different observers. Finally data from different groups were compared to assess, if the observations had any statistical significance by using student t-test (using the software SPSS version 15.0). The data was interpreted as significant if the value of $p < 0.05$ and not significant if $p > 0.05$.

3. Results

In the present study, a total of 381cases of endometrial biopsies and hysterectomy specimens were examined to evaluate and define any correlation between angiogenesis and different phases and diseased states of the endometrium. Amongst 381 cases, 74 cases constituted normal endometrium (07- proliferative and 67- secretory), 135 cases simple hyperplasia, 105 cases complex hyperplasia and 67 cases of atypical hyperplasia.

The bulk of patients in our study came from reproductive, perimenopausal and menopausal age group. The youngest patient was 19 years old and the oldest patient was 63 years. The major complaints of the patients were menstrual irregularities, lower abdominal pain, infertility and postmenopausal bleeding.

3.1. Normal Endometrium

The group comprised of 74 cases of normal proliferative and secretory endometrium. Cases with any associated / superimposed changes in a few endometrial fragments together with normal endometrium were not included in the group. On H&E stain - MVD ranged from 2.1- 6.6 and Mean MVD was 3.92. On Reticulin stain - MVD ranged from 2.2-7.2 and Mean MVD was 3.98. Further the cases were divided into proliferative and secretory endometrial groups and the observations recorded in Table 1.

The microvessels in stroma were both longitudinal and circular on cross section and most of them were small or medium sized in normal endometrium. They were sparse except in late secretory phase where the stroma was reduced and appeared to be in close proximity to the glands and to each other. Microvessels were found lying singly or in groups in the stroma. Higher values of MVD were obtained on reticulin stain as the basement membrane of vessels was better demarcated. In proliferative endometrium, MVD was higher in late proliferative phase in comparison to early phase. In secretory endometrium, counts were noted to be higher in mid and late secretory rather than early secretory phase. On immunostained sections, using CD 34 stain, a similar distribution of microvessels was observed as with the above mentioned stains but with slight increase in the total number of microvessels count as it was possible to identify even single endothelial cell stained. The difference in MVD counts of normal proliferative and secretory endometrium was statistically significant ($p = 0.012$).

3.2. Simple Hyperplasia

A total of 135 cases of simple hyperplasia were studied. In most of the cases, it was a generalized process except where

disordered proliferation was noted in some fragments in a few cases.On H&E stain - MVD ranged from - 2.4 - 6.7 and Mean MVD was 4.71. On Reticulin stain - MVD ranged from - 2.8 - 8.3 and Mean MVD was 5.39.

Most of the blood vessels were small or medium sized except a few with large calibre and occasional dilated microvessel. Thickened blood vessels were also present but they were not counted in the study. The MVD counts observed on reticulin stained sections were higher than those on H and E stain. (Figure 1) On immmunostained sections, the findings were more or less same except the overall increase in MVD counts as obtained with above mentioned stains. (Figure 2)

3.3. Complex hyperplasia

The total number of cases in this group was 105. In most of the cases complex hyperplasia was focal, mostly overlying the simple hyperplastic or proliferative or even secretory changes in the native endometrium. However there were a few cases which showed diffuse changes of complex hyperplasia in all the endometrial fragments. Cases showing associated cytological atypia were excluded from this category. On H&E stain - MVD ranged from 2.3 - 7.0 and Mean MVD was 4.93. On Reticulin stain- MVD ranged from 3.1-7.7 and Mean MVD was 5.68 (Table 2).

The microvessels were of different sizes and shapes ranging from very small to very large. Even though the stromal tissue was reduced in between the glands due to complex hyperplasia, tissue adjacent to basement membrane exhibited relatively increased number of blood vessels encircling these glands. Counts on reticulin stain were increased due to better appreciation of basement membrane of blood vessels around the glands and better appreciation of morphological details. Cases with superimposed progesterone effect showed decrease in number and size of microvessels while the counts were increased in cases associated with endometritis. On immunostained sections, the microvessels around the endometrial glands were easily identified and the total counts were higher than the vessels counted on routine stains on the same tissue sections.

3.4. Atypical hyperplasia

This group comprised of 67 cases showing cytological atypia. Inbudding and outpouching of the glandular epithelium together with multilayering and associated cytological and nuclear atypical features were the characteristics of this group. These changes were not only focal but generalized in some cases. Samples showing atypical features due to reactive inflammatory changes were not included in the group. On H&E stain - MVD ranged from 4.5 - 8.8 and Mean MVD was 6.31. On Reticulin stain - MVD ranged from 5.5 - 9.4 and Mean MVD was 7.19.

MVD counts were increased in these cases on both H&E and reticulin stain. Vessels ranging from small to large size and from circular to longitudinal in cross section were seen. Associated features of close approximation of glands and microvessels were also noted here. On immunostained sections, the above mentioned features were seen in addition to the increased MVD counts when compared to routine H&E stained sections. (Figure 3)

The values of MVD (Range and Mean) obtained in normal endometrium were correlated with values obtained in simple, complex and atypical hyperplasia to observe their statistical significance (using student t- test). The difference in MVD counts of normal endometrium and simple hyperplasia was statistically significant ($p = 0.012$). The difference in MVD counts of normal endometrium and complex hyperplasia was statistically significant ($p = 0.004$). The difference in MVD counts of normal endometrium and atypical hyperplasia was highly significant statistically ($p < 0.001$) (Table 2).

Further to evaluate statistical difference in counts obtained in different degrees of hyperplasia (simple v/s complex and atypical hyperplasia and complex v/s atypical hyperplasia) student t- test was applied and following values were observed: The difference in MVD counts of simple hyperplasia and complex hyperplasia was not statistically significant ($p = 0.413$). The difference in MVD counts of simple hyperplasia and atypical hyperplasia was statistically significant ($p = 0.003$). The difference in MVD counts of complex hyperplasia and atypical hyperplasia was statistically significant ($p=0.001$). Thus a statistically significant difference was noted in all categories except in between simple and complex hyperplasia where the difference was not statistically significant (Table 2).

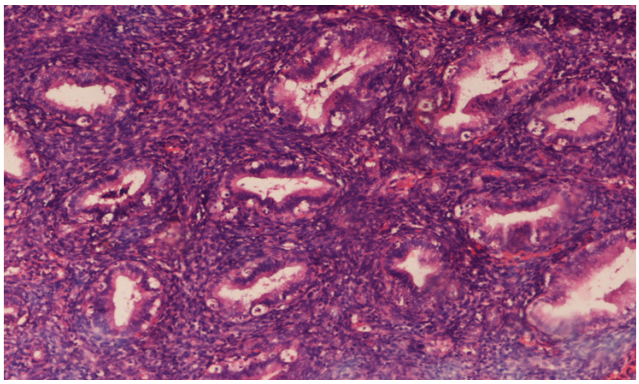

Fig. 1: Simple Hyperplasia: Small or medium sized blood vessels with large calibre and occasional dilated microvessel. H and E stain, 40X.

4. Discussion

Angiogenesis has different dimensions in different phases of the endometrium exposed to hormonal influences, and it

Table 1: MVD in Normal Proliferative and Secretory Endometrium:(n=74)

Type of endometrium	H&E stain		Reticulin stain		Immunostain CD 34	
	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD
Proliferative	2.1-2.7	2.4±0.01	2.5-3.0	2.73±0.02	2.8-3.5	2.99±0.04
Secretory	2.1-6.6	4.1±0.06	2.2-7.2	4.23±0.07	2.9-8.2	5.23±0.08

p= 0.012: Significant

Table 2: MVD in Different Types of Hyperplastic Lesions ofEndometrium (n=307)

	Types of Lesion	H & E stain		Reticulin stain		Immunostain CD34	
		Range	Mean±SD	Range	Mean±SD	Range	Mean±SD
1	Normal Endometrium	2.1-6.6	3.92±0.01	2.2-7.2	3.98±0.02	2.8-8.1	4.12±0.03
2	Simple hyperplasia	2.4-6.7	4.71±0.01	2.8-8.3	5.39±0.04	3.1-8.9	6.89±0.02
3	Complex hyperplasia	2.3-7.0	4.93±0.03	3.1-7.7	5.68±0.06	4.2-9.1	7.41±0.04
4	Atypical hyperplasia	4.5-8.8	6.31±0.04	5.5-9.4	7.19±0.07	6.5-10.4	8.19±0.03

pvalue: 1:2=0.012, 1:3=0.004, 1:4=0.001, 2:3=0.413 , 2:4=.0.003, 3:4=0.001

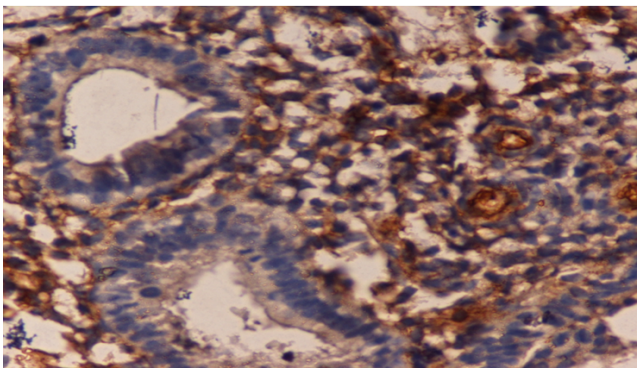

Fig. 2: Simple Hyperplasia: Tissue adjacent to basement membrane exhibited relatively increased number of blood vessels encircling these glands. IHC CD34, 40X.

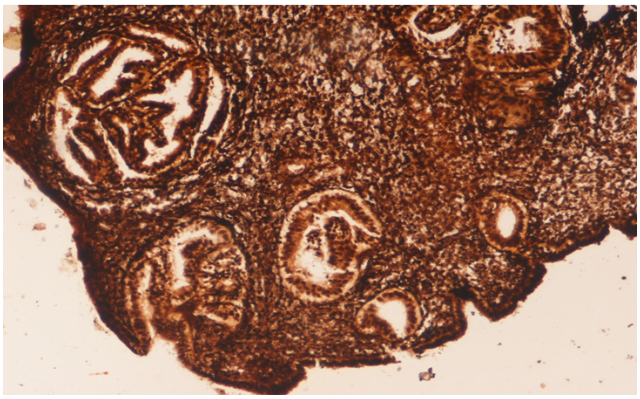

Fig. 3: Atypical hyperplasia: Inbudding and outpouching of the glandular epithelium together with multilayering and associated cytological and nuclear atypical features with raised MVD counts. Reticulin stain, 40X.

goes on changing as the endometrium changes from normal to abnormal states.[4]

The mean MVD in normal endometrium in our study on H&E was 3.15 which was in accordance with the study of Sahasrabudhe et al[5] and Makhija et al,[6] who found a mean MVD of 3.45 in normal endometrium. Makhija et al[6] did morphometric evalution of endometrial blood vessels in normal, dysfunctional states and in cases of unexplained infertility. But they did not find any significant difference between mean MVD of proliferative and secretory group whereas a significant difference was observed in our study (p = 0.0119).

Morgan et al[7] did a similar study to see angiogenesis in normal, hyperplastic and neoplastic endometrium by using Factor VIII related antigen and found increased counts in secretory as compared to proliferative endometrium which is in concordance with our study.Meduri et al[8] and Moller et al,[9] also found similar results with higher counts in secretory phase than proliferative phase.

Ozalp et al,[10] performed a study to assess MVD as a marker for angiogenesis in endometrial carcinoma (EC) and normal endometrium in the proliferative and secretory phase, and to determine its prognostic value on survival among cases with EC. Among control cases, endometrium from proliferative and secretory phases of the menstrual cycle was not statistically significant (48.5 +/- 3.6 vs 47.4 +/- 3.8, respectively) in their study. Similar results were obtained in studies by Shaw et al[11] and Rees et al,[12] who also did not find significant change in the average density of blood vessels during the various phases of the menstrual cycle.

Sahasrabudhe et al,[5] Fanger et al[13] and Nayha et al[14] have stated that the blood vessels were more concentrated in the basal layer in proliferative endometrium and were distributed more frequently in the functional layer by mid to late secretory phase, because the spiral arterioles are extremely sensitive to the changes in levels of ovarian

hormones. This could be the reason why different counts were obtained in normal proliferative and secretory phases of the menstrual cycle in the absence of any pathology as only the superficial layers of the endometrium were obtained in the biopsies alone.

Within the group of hyperplasias, the difference between simple and complex hyperplasia was not significant (p=0.413) but the difference between simple and atypical hyperplasia and complex and atypical hyperplasia was significant (p= 0.003 and p = 0.001 respectively) in our study, findings concordant with studies by Morgan et al,[7] who also noted increase in the counts with the severity of hyperplasia but with no statistical significance. Makhija et al[6] have also compared the angiogenesis status in normal and complex hyperplasia and found that the average blood vessels per HPF were significantly higher in complex hyperplasia (4.471 ± 0.095) as compared to normal (3.86 ± 0.7, $p < 0.01$) endometrium.

Abulafia et al[15] conducted a study to evaluate angiogenesis in endometrial hyperplasia and stage I endometrial carcinoma and to investigate the relationship between angiogenesis and tumor grade and depth of invasion. They noted a significant difference between the microvessel count of controls versus the group with complex endometrial hyperplasia (median 21, range 16-80, versus median 38, range 20-130; $p < 0.05$). They also reported that the microvessel counts in complex endometrial hyperplasia was significantly higher than simple hyperplasia (median 25, range 16 - 42; p <0 .05). Similar finding was noted by Nayha et al,[14] who have reported that microvessel density is associated with malignant transformation later.

Brawer et al[16] studied the histologic pattern of microcirculation using factor VIII immunostain and noticed that stromal tissue immediately adjacent to the epithelial glandular tissue exhibited a rich capillary network of microvessels, a finding similar to our observations.

Our study showed that neoangiogenesis is a cyclical event throughout the menstrual cycle and keeps changing with the cyclical endometrium whether physiological or pathological. The role of angiogenesis assumes greater significance with increasing severity of lesions ranging from simple hyperplasia to atypical hyperplasia. The stroma of secretory and hyperplastic is more vascular than proliferative endometrium, but the glands outstrip the stroma and can mask the angiogenic response in hyperplasias.

5. Conclusions

Role of angiogenesis becomes more significant with increasing severity of the lesion, normal endometrium to simple hyperplasia to atypical hyperplasia. Although angiogenesis can be illustrated well by routine H and E and Reticulin stains, it can be detected on immunostains with greater degree of ease and accuracy.

6. Source of Funding

None.

7. Conflict of Interest

None.

References

1. Carmeliet P, Moons L, Luttun A. Synergism between vascular endothelial growth factor and placental growth factor contributes to angiogenesis and plasma extravasation in pathological conditions. *Nat Med*. 2001;7:575–83.
2. Yamazaki Y, Morita T. Molecular and functional diversity of vascular endothelial growth factors. *Mol Divers* . 2006;10(4):515–27.
3. Holland CM, Day K, Evans A, Smith SK. Expression of the VEGF and angiopoietin genes in endometrial atypical hyperplasia and endometrial cancer. *Br J Cancer*. 2003;89(5):891–8.
4. Cursiefen C, Chen L, Borges LP. VEGF-A stimulates lymphangiogenesis and hemangiogenesis in inflammatory neovascularization via macrophage recruitment. *J Clin Invest*. 2004;113:1040–50.
5. Sahasrabudhe NS, Dalal SR, Jadhav MV. Endometrial blood vessels in health and disease. *Med J West India*. 2010;28:27–31.
6. Makhija D, Mathai AM, Naik R, Kumar S, Rai S, Pai MR, et al. Morphometric evaluation of endometrial blood vessels. *Ind J Pathol Microbiol*. 2011;51:346–50.
7. Morgan KG, Wilkinson N, Buckley CH. Angiogenesis in normal, hyperplastic, and neoplastic endometrium. *J Pathol*. 2006;179:317–20.
8. Meduri G, Bausero P, Perrot-Applanat M. Expression of vascular endothelial growth factor receptors in human endometrium: modulation during the menstrual cycle. *Biol Reprod*. 2012;62:439–47.
9. Möller C, Rasmussen B, Lindblom L, Olovsson M. Expression of the angiogenic growth factors VEGF, FGF-2, EGF and their receptors in normal human endometrium during the menstrual cycle. *Mol Hum Reprod*. 2011;7(1):65–72.
10. Ozalp S, Yalin OT, Acikalin M. Microvessel density (MVD) as a prognosticator in endometrial carcinoma. *Eur J Gynae Oncol*. 2003;24(3):305–8.
11. Shaw ST, Macaulay LK, Hohman WR. Vessel density in endometrium of women with and without intrauterine contraceptive devices: a morphometric evaluation. *Am J Obstet Gynecol*. 2013;135:202–6.
12. Rees MC, Dunhill MS, Anderson AB, Turnbull AC. Quantitative uterine histology during the menstrual cycle in relation to measured menstrual blood loss. *Br J Obstet Gynaecol*. 2011;91:662–6.
13. Fanger H, Barker BE. Capillaries and arterioles in normal endometrium. *Obstet Gynaecol*. 2012;17:543–50.
14. Nayha V, Viitanen T, Stenback F. Altered extent, pattern and characteristics of microvascular density are indicators of neoplastic progression in the endometrium. *Int J Cancer*. 2015;115:975–80.
15. Abulafia O, Triest WE, Sherer DM. Angiogenesis in endometrial hyperplasia and stage I endometrial carcinoma. *Obstet Gynecol*. 2005;86(4):479–85.
16. Brawer MK. Predictors of pathologic stage in prostatic carcinoma. The role of neovascularity. *Cancer*. 2014;73(3):678–87.

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