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ORIGINAL RESEARCH

Assessment of histological alterations in dysfunctional uterine hemorrhage

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ABSTRACT

Background: Up to 20% of women of reproductive age who attend an outpatient clinic do so because of menorrhagia, a common gynecological issue. Our goal was to examine the different histological alterations in dysfunctional uterine hemorrhage as well as blood vessel morphological changes, namely number morphology, and then compare the results with the clinical observations. **Methods:** The three-year research, conducted at the Department of Pathology in collaboration with the Department of Obstetrics and Gynecology, included around 220 patients. People in the reproductive age range who did not use an intrauterine device or an oral contraceptive medication and who had symptoms of irregular, heavy, intermittent, or prolonged menstrual bleeding coagulation problems were originally excluded from the research group. Per procedure, patient permission and ethics committee approval were obtained. **Results:** Proliferative pattern, secretory pattern/therapy related alterations, disorganized proliferative endometrial endometrial hyperplasia, and hyperplasia with atypia were the different patterns seen in this research. The proliferative pattern of the endometrium includes disorganized proliferation, endometrial hyperplasia, and hyperplastic endometrium with atypia, which are patterns that exhibit a significant alteration in the quantity and form of blood vessels. The vasculature did not significantly alter in any of the other patterns. **Conclusion:** It was concluded that abnormal vascular morphology in the various endometrial patterns mentioned above may be the pathogenic cause of dysfunctional uterine hemorrhage abnormal endometrial angiogenesis and poor vascular maturation are linked to AUB.

Keywords: Vascular alterations, endometrial hyperplasia, disordered proliferation,

INTRODUCTION

Disturbances in menstrual bleeding are a medical and social problem. Abnormal uterine bleeding (AUB) has a large economical effect since it greatly reduces quality of life. In women of reproductive age, menorrhagia is a common gynecological problem that accounts for up to 20% of outpatient clinic visits.[1] Apart from resulting in iron deficiency anemia and requiring a hysterectomy, the illness also significantly impairs life quality and creates social discomfort. Although menorrhagia is often associated with fibroid and polyps, around 50% of patients present with no obvious uterine illness. AUB is usually caused by a primary endometrial issue when fibroids, adenomyosis, or polyps are not found to be the underlying reason. Unusual bleeding may be caused by cellular processes and menstrual regulation mechanisms.[1,2] Dysfunctional uterine bleeding (DUB) should be diagnosed when the pelvic examination is normal and there is no other apparent extragenital reason for the patient's bleeding.[2, 3] Considering vascular modulation and function, AUB may develop from both abnormal vascularization in the endometrium due to modifications in the angiogenesis and vascular maturation processes.[4] The present study's objective was to use standard laboratory stains to investigate the various endometrial histopathologic patterns and the density of endometrial blood vessels in DUB patients in the era of antibodies and molecular testing.

EH is a histologic diagnostic that may be classified using either the widely used World Health Organization criteria or the more standardized endometrial intraepithelial neoplasia (EIN) criteria.[4]

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The probability of cancer progression depends on the extent of the lesion. Lesions associated with atypia are more likely to develop into cancer and to be detected concurrently with endometrial cancer. Most women with EH are either postmenopausal or perimenopausal. Important risk factors for endometriosis (EH) include obesity, tamoxifen usage, polycystic ovarian syndrome, estrogen-only hormone replacement, and prolonged anovulation. Clinical signs include aberrant uterine bleeding, postmenopausal bleeding, and atypical endometrial glands on Pap smears.

The uterine condition known as endometrial hyperplasia (EH) represents a variety of morphological endometrial abnormalities. Its main characteristic is an increase in the endometrial gland-to-stroma ratio as compared to a normal proliferative endometrium. EH is clinically relevant because it may proceed to endometrioid endometrial cancer (EC), and "atypical" types of EH are believed to represent premalignant lesions.[4,5]

The majority of EHs are thought to originate from a background of continuous oestrogen stimulation of the endometrium without progestin resistance, due to a number of possible causes.[5] Previous estimates indicate that 15% of all cases of postmenopausal hemorrhage are caused by EHs. Most women with EH will have abnormal uterine bleeding (AUB) when they first show clinically.[5] The main risk factors for EC development are similar to those for EH development. Obese perimenopausal and postmenopausal women, who may have irregular anovulatory cycles as a result of peripheral aromatization of androgens to oestrogens in adipose tissue, and premenopausal patients with polycystic ovarian syndrome (PCOS) are two patient categories who are especially vulnerable.

Therapeutic approaches, which sometimes rely on trial and error, do not address the aetiology of the problem. Understanding the pathogenesis of this illness is necessary to create new therapy alternatives.

During the progesterone-dominated luteal phase, decidualized human endometrial stromal cells (HESC) exhibit increased expression of tissue factor (TF), the primary coagulation initiator. Furthermore, plasminogen activator inhibitor-1 (PAI-1), a second hemostatic element of HESCs, is elevated by progesterone. Progestins, on the other hand, stabilize the vascular extracellular matrix and endometrial stromal by preventing the synthesis of HESC matrix metalloproteinase (MMP)-1, 3, and 9. During infertile cycles, progesterone withdrawal results in decreased expression of HESC TF and PAI, increased MMP activity, and the production of inflammatory cytokines. These factors all contribute to controlled menstrual hemorrhaging and related tissue sloughing. Because of unchecked angiogenesis, the endometrium is very vascular, non-hemostatic, and proteolytic. In contrast, endometrial bleeding associated with anovulation involves well-ordered metabolic processes.

Instead of poor hemostasis, uncontrolled angiogenesis that results in large, brittle endometrial vessels is the source of abnormal bleeding associated with long-term use of progestin-only contraceptives. Progestational endometrial blood flow inhibition causes this aberrant angiogenesis by fostering local hypoxia and reactive oxygen species generation, both of which increase the synthesis of angiogenic factors.[6,7] Vascular fragility results from this, which promotes bleeding. Aberrant angiogenesis also contributes to irregular bleeding associated with endometrial polyps and myomas.

Endometrial hyperplasia and disorganized proliferation are the outcomes of prolonged exposure to oestrogen without any resistance from progesterone, polycystic ovarian syndrome, tamoxifen, or hormone replacement treatment. The main conservative strategy for halting the growth of adenocarcinoma is the restoration of hyperplasia to normal endometrium. Because EH may advance or often occurs together with endometrial cancer, it is clinically relevant. At the moment, cyclic progestin or hysterectomy are the primary therapies for EH without atypia.[8,9] However, clinical studies of hormone therapy and definitive standard therapies for the management of EH are still awaited. Additionally, there are challenging therapy choices for endometriosis (EH) patients who want to preserve their fertility, which need nonsurgical care.

MATERIAL& METHODS

The three-year research, conducted at the Department of Pathology in collaboration with the Department of Obstetrics and Gynecology, included around 220 patients. People in the reproductive age range who did not use an intrauterine device or an oral contraceptive medication and who had symptoms of heavy, irregular, intermittent, or prolonged monthly bleeding made up the research group. Initially, coagulation issues were ruled out. Twenty individuals with prolapsed uteri and/or uterine/cervical fibroids—the sources of abnormal bleeding—made up the control group. Special stains, such as reticulin, were used if required to analyze specimens following hysterectomy or dilatation and curettage (D&C) surgeries. The endometrium was classified as having disorganized proliferation, endometrial hyperplasia without atypia, and endometrial hyperplasia with atypia based on architecture and atypia. Changes in progesterone and the endometrium's secretory and proliferative phases were not collected for study.

The total vascularity of the endometrium was evaluated by calculating the average number of blood vessels in 10 high power fields (HPF) and comparing the findings with a control. The number of blood vessels displaying vascular dilatation and congestion was also measured in 10 high-power fields (HPF). We counted arterioles and capillaries with thin walls. Dilatation-displaying vascular sinuses were excluded. The student's unpaired t-test was the statistical test

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that was used. P values < 0.05 and < 0.001 were regarded as significant (S) and highly significant (HS), whereas P values greater than 0.05 were regarded as non-significant (NS).

Examining the fundamental roles of vascular maturation and angiogenesis in AUB patients is our aim. We also believe that abnormal endometrial angiogenesis contributes significantly to the aetiology of AUB, although via distinct routes.[10,11]

OBSERVATIONS

A total of 220 DUB instances were looked at. The 40–50 year age group had the largest number of patients with dysfunctional uterine hemorrhage (109 cases, or 60.3%), with 51 cases (28.1%) occurring in the 30–40 year age group. The fewest patients seen were in the 21–30 age range. The most prevalent presenting type among the DUB was menorrhagia (39.8%), which was followed by polyhypermenorrhea (22.19%). Amenorrhea was seen in the smallest number of patients (2.11%).

Table 1: Different endometrial histology patterns in the research group

Endometrial pattern	Number of Cases	Percentage
Non secretory pattern	76.00	42.59%
1)Proliferative pattern	18.00	8.41%
2)Disordered proliferative endometrium	38.00	21.49%
3)Endometrial hyperplasia	22.00	12.71%
Secretory pattern	106.00	59.41%
1)Secretory pattern	35.00	19.91%
2)Hormonal changes	72.00	40.01%

Table 2: Results of Examination of the study group's microvascular density, dilatation, and congestion

Endometrial Patterns	No of cases	Mean number of blood vessels/10HPF	Mean number of dilated vessels/10HPF	Mean number of Congested Blood vessels/10HPF
1.Proliferativepattern	24	4.3±0.5	0.8± 0.3	0.8±0.08
2.Disorderedproliferative Endometrium	44	4.8±0.9	0.8±0.2	1.3±0.1
3.Endometrialhyperplasia	23	6±0.5	3.6±0.3	1.0±0.1
4.Endometrialhyperplasia withatypia	12	7.8±0.7	4.3±0.5	1.5±0.1
SECRETORYpattern		±	±	±
1)Secretorypattern	40	5.6±0.1	1.2±0.1	0.9±0.5
2)Hormonalchanges	77	13.54±1.07	7.94±0.47	4.8±0.87
Total	220			

The various endometrial histopathological patterns seen in DUB cases are shown in Table 1. Among the 220 case studies, the secretory pattern predominated over the proliferative pattern, which may have been influenced by hormonal treatment. 110 individuals exhibited secretory endometrial patterns, whereas only 80 patients had non-secretory patterns. Disordered proliferative cases accounted for 20.50 percent of the non-secretory endometrial pattern. Hormonal changes in the endometrium accounted for the largest proportion of cases in the other group (40.00%). (Figure 3)

Disordered proliferative endometrium (20.50%) was the most frequent cause of DUB in patients with nonsecretory endometrial pattern (Figure 2), followed by hyperplasia (Figure V), which accounted for 21.00% of cases.

The control group consisted of twenty patients. These individuals also had cervical lesions with regular cycles and no aberrant bleeding, serosal and intramural leiomyomas, and non-hormone generating ovarian tumors. According to their histological diagnosis, the endometrium was either in the secretory or proliferative phase.

Measurements were made of the microvascular density, dilated blood vessels, and congested blood vessels in the study group and control groups. Tables II and III provide the findings. As shown in Table IV, a significant computation was performed after the data were compared.

The number of blood vessels/10HPF (mean vascular density) was not substantially increased in endometrial hyperplasia with atypia, but it was in disorganized proliferative endometrium and endomerial hyperplasia without atypia. In the conrol group, it made no difference ($p>0.05$). Hormonal alterations caused a substantially significant ($p<0.001$) increase in the number of blood vessels in the endometrium when compared to the control group. While the mean vascular density did not substantially increase in 57.20% of the cases in the present study, it did significantly increase in 42.50% of the cases (hyperplastic endometrium, 21.00%; secretory and hormonal alteration endometrial, 39.00%).

Vascular dilatation, hyperplastic endometrium, and

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disorganized proliferation were seen in 71.70% of the patients in this study. Regarding the amount of vascular congestion, the majority of cases (70.70%) in the same group showed a statistically significant rise.

DISCUSSION

AUB may result from changes in the angiogenesis and vascular maturation processes as well as aberrant vascularization in the endometrium, taking into account vascular modulation and function. Endometrial hyperplasia (EH) is a uterine disorder that encompasses a range of morphological endometrial changes. Compared to a normal proliferative endometrium, it is primarily identified by an increase in the endometrial gland-to-stroma ratio. Proliferative pattern, secretory pattern/therapy related alterations, disordered proliferative endometrium, endometrial hyperplasia, and hyperplasia with atypia were among the several patterns seen in this research.[12-15] Disordered proliferation, endometrial hyperplasia, and hyperplastic endometrium with atypia are the three patterns that fall under the proliferative pattern of the endometrium and indicate a significant change in the quantity and form of blood vessels. The mean vascular density was substantially greater in 42.50% of the patients in this research (21 percent of cases had hyperplastic endometrium, and 39 percent had secretory and hormonal alteration endometrial). 71.70% of the patients in this study had vascular dilatation, which included disorganized proliferation and hyperplastic endometrium.

The vasculature did not significantly alter in any of the other patterns. Menorrhea in hyperplastic endometrium and cancer are caused by large, convoluted, thin-walled capillaries in the superficial endometrium that lack a properly formed wall. Prostaglandins (PGF2α and PGE2), prostacyclins (PGI2), nitric oxide, reduced endothelin-1 levels, and increased expression of VEGF-A and its receptors (VEGF-1 and VEGFR-2) are some examples of other local molecular processes that may cause menorrhagia in these circumstances. All of the aforementioned findings need more molecular investigation.

Goteri et al. set out to examine if there were variations in the expression of VEGF, HIF-1alpha, and microvessel density (MVD) in women with and without adenomyosis, given that angiogenesis has been proposed to be significant. They came to the conclusion that adenomyosis development could be linked to VEGF-mediated angiogenesis. The creation of additional vasculature, the increase of VEGF production, and the augmentation of HIF-1a expression may all be related to the abnormal positioning of the ectopic foci. Nevertheless, neither the heightened MVD in the adenomyotic foci nor the elevated production of VEGF and HIF-1alpha seem to be related. On the other hand, hyperplasia, disordered proliferation, and cancer may all be impacted by the same cause in the endometrial vascular proliferation.[16]

According to Makhija et al., the endometrium's secretory phase shows notable dilatation and congestion.[17] In the past, decreased endometrial vasoconstriction and poor vascular plug development were the main causes of ovulatory dysfunctional uterine hemorrhage, which led to excessive bleeding.[18, 19] The aberrant bleeding seen in individuals with endometrial hormonal changes may be explained by this finding. Hormonal therapy is also often used as the first treatment for irregular uterine bleeding.

Unopposed estrogen stimulation causes cancer and endometrial enlargement. According to Livingstone and Fraser, menorrhagia in hyperplastic endometrium and cancer are caused by the vast, convoluted, thin-walled capillaries in the superficial endometrium that lack a fully formed wall.

However, low levels of endothelin-1 produced by the vascular endothelium, low levels of nitric oxide and PGF2α (both secreted by the vascular endothelium), and local prostaglandin (PGE2 and PGI2) effects may also cause menorrhagia. According to research by Livingstone and Fraser[20] and Shaw, increased tissue plasminogen activator is another important factor contributing to menorrhagia. This, in turn, results in increased fibrinolytic activity.[21]

Mints et al.'s research [22] shown that overexpression of VEGF-A and its receptors in capillaries, including VEGFR-1 and VEGFR-2, improves vascular permeability, induces fenestration in capillaries and venules, and promotes the development of vascular endothelial cells. Menorrhagia in these people is caused by these angiogenic factors. Thus, a variety of biochemical and molecular factors also contribute significantly to menorrhagia, in addition to the vascular morphological changes in the different endometrial patterns that were previously discussed.

Endoglein and VRGF were used to measure the microvascular density and proliferation in the Erdem et al., CD 34 investigation. Microvessel density (MVD) was measured using endoglin and anti-CD 34 in most vascular areas. VEGF expression was significantly greater in EC and EH but did not vary between the two groups. The CD 34 staining revealed no differences in MVD across the groups. However, endoglin showed that EC had much higher mean MVD numbers than EH. VEGF expression did not correlate with other angiogenesis-related measures, even though it was much higher in EC. It was determined that MVD using endoglin seems to more accurately depict neoplastic angiogenesis than CD 34.[23]

Zhang et al. performed amazing work on endometrial samples for vascularity evaluation employing an antibody to CD34 for immunohistochemical labeling of MMP-2 and -9, VEGF, and endometrial MVD.Zhang et al.'s research supported our findings by demonstrating that women with anovulatory DUB who had endometrial hyperplasia had significantly higher frequencies of VEGF expression in

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endometrial glands and MMP-2 and -9 expression in endometrial stroma compared to the control group. Additionally, the mean score of endometrial MVD was considerably greater in women with anovulatory DUB who had endometrial hyperplasia than in the control group. In women with anovulatory DUB, VEGF expression in the endometrial glands was statistically associated with MMP-2 and -9 expressions in the uterine stroma and endometrial MVD.[23]

Patients with AUB had substantially higher levels of vascular endothelial growth factor A and its receptors (1 and 2), Tie-1, and the ratio of angiopoietin-1 to angiopoietin-2. Several studies found that individuals with AUB expressed specific pro- and antiangiogenic factors, which suggests poor vessel integrity and aberrant vascular development. Overall, endometrial microvessel density (MVD) was comparable in patients with AUB. Particularly after short-term hormone therapy, patients with AUB exhibited elevated MVD and proangiogenic marker expression.[24] Both short- and long-term exposures altered the way vessels developed, while longer-term exposures progressively erased this impact.

CONCLUSION

Therefore, it was concluded that the pathogenic reason of dysfunctional uterine hemorrhage may be aberrant vascular morphology in the numerous endometrial patterns stated above. AUB is associated with inadequate vascular maturation and abnormal endometrial angiogenesis. The body of studies demonstrating that high levels of proangiogenic factors and low levels of antiangiogenic factors hinder the development of vasculature, making it more brittle and porous, is supported by our study and literature review. The various investigations revealed that the vascular morphology has changed in terms of mean vascular density, dilatation, and congestion in the proliferative and secretory patterns.

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