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To Evaluate Cd34 Protein Immunoreactivity in the Endometrial Stroma of Normal Endometrium and the Changes in Immunoreactivity in Abnormal Uterine Bleeding: An Observational Study

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KEYWORDS

Abnormal uterine bleeding; Endometrium; CD34; Immunohistochemistry; Endometrial stroma; Angiogenesis

ABSTRACT:

Background: Abnormal uterine bleeding (AUB) is a common gynaecological problem, particularly in the late reproductive and perimenopausal age group. Altered endometrial angiogenesis and stromal vascular remodelling are believed to play an important role in its pathogenesis. CD34 is a reliable immunohistochemical marker of endothelial cells and stromal vascularity.

Aim: To evaluate CD34 protein immunoreactivity in the endometrial stroma of normal endometrium and to assess changes in its expression in cases of abnormal uterine bleeding.

Materials and Methods: This observational study was conducted in the Department of Pathology, Integral Institute of Medical Sciences and Research, Lucknow, over a period of 18 months. A total of 65 endometrial biopsy and hysterectomy specimens were included. Routine histopathological examination was performed using hematoxylin and eosin–stained sections. Immunohistochemical staining for CD34 was carried out on formalin-fixed paraffin-embedded sections. CD34 expression was assessed semi-quantitatively and categorised as negative or positive. Statistical analysis was performed using appropriate descriptive and comparative tests.

Results: The majority of cases were in the 31–50-year age group. Of the 65 cases, 55 were diagnosed as AUB and 10 as normal endometrium. All normal endometrial samples showed positive CD34 expression, whereas 50.9% of AUB cases were CD34 negative, with a statistically significant difference between the two groups ($p < 0.001$). Functional causes of AUB showed significantly higher CD34 negativity compared to structural causes ($p = 0.002$). Variable CD34 expression was observed across different histopathological diagnoses.

Conclusion: CD34 immunoreactivity is significantly altered in abnormal uterine bleeding compared to normal endometrium, suggesting a role of disturbed stromal vascularity in the pathogenesis of AUB. CD34 immunohistochemistry may serve as a useful adjunct in the evaluation of endometrial changes associated with abnormal uterine bleeding.

INTRODUCTION

Abnormal uterine bleeding (AUB) is one of the most common gynaecological complaints encountered in women of reproductive and perimenopausal age and represents a significant cause of morbidity, impaired quality of life, and healthcare utilisation [1]. It encompasses bleeding that is excessive in amount, duration, or frequency and occurs in the absence of pregnancy. The etiopathogenesis of AUB is multifactorial and includes functional hormonal disturbances as well as structural uterine pathology [2].

The International Federation of Gynaecology and Obstetrics (FIGO) has proposed the PALM–COEIN classification system to standardise the causes of AUB

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into structural (Polyp, Adenomyosis, Leiomyoma, Malignancy and hyperplasia) and non-structural (Coagulopathy, Ovulatory dysfunction, Endometrial, Iatrogenic, and Not otherwise classified) categories [3]. Despite this classification, the underlying mechanisms responsible for abnormal bleeding at the endometrial level remain incompletely understood.

The endometrium is a hormonally responsive tissue that undergoes cyclical changes involving proliferation, differentiation, breakdown, and regeneration. These changes are closely linked to endometrial stromal vascular remodelling and angiogenesis [4]. Alterations in stromal vascularity and endothelial integrity have been implicated in the pathogenesis of AUB, particularly in functional causes where no obvious structural abnormality is identified [5].

CD34 is a transmembrane glycoprotein expressed on hematopoietic progenitor cells and vascular endothelial cells and is widely used as an immunohistochemical marker for assessing microvessel density and stromal vascular architecture [6]. In the endometrium, CD34 highlights stromal capillaries and vascular endothelial cells, providing insight into angiogenic activity and stromal remodeling during different phases of the menstrual cycle [7].

Previous studies have demonstrated that normal endometrium exhibits cyclical variation in CD34 expression, with higher vascular density during the proliferative phase compared to the secretory phase [8]. In contrast, altered CD34 expression has been reported in various pathological conditions such as endometrial hyperplasia, adenomyosis, polyps, and malignancy, suggesting a role of abnormal angiogenesis in disease progression and abnormal bleeding [9,10].

However, there is limited data comparing CD34 immunoreactivity in normal endometrium with different histopathological patterns seen in AUB, particularly in the Indian population. Understanding the differences in stromal vascular expression between functional and structural causes of AUB may provide valuable insights into disease mechanisms and potential therapeutic targets.

Therefore, the present study was undertaken to evaluate CD34 protein immunoreactivity in the endometrial stroma of normal endometrium and to assess alterations in its expression across various histopathological patterns associated with abnormal uterine bleeding.

MATERIALS AND METHODS

Study Design

This was an observational study undertaken to evaluate the immunohistochemical expression of CD34 protein in cases of abnormal uterine bleeding (AUB) and to compare it with normal endometrium.

Study Period

The study was conducted over a period of 18 months, from March 2024 to September 2025.

Study Setting

The study was carried out in the Department of Pathology, Integral Institute of Medical Sciences and Research (IIMSR), Lucknow.

Study Material

The study material consisted of consecutive surgically resected endometrial biopsy and hysterectomy specimens received postoperatively from the Department of General Surgery. All specimens were fixed in 10% neutral buffered formalin and routinely processed for paraffin embedding.

Sample Processing

From each specimen, representative tissue sections were selected and cut at a thickness of 3–4 µm using a rotary microtome. Sections were mounted on glass slides for routine histopathological examination and immunohistochemical analysis.

Histopathological Evaluation

All cases were initially evaluated on hematoxylin and eosin (H&E) stained sections under light microscopy. Histopathological examination was performed to identify and categorize endometrial patterns and lesions according to standard pathological criteria.

Immunohistochemical Study

Immunohistochemical staining was performed on representative formalin-fixed paraffin-embedded tissue sections using commercially available monoclonal antibodies against CD34 (Quartett, Germany). Sections were deparaffinized and rehydrated, followed by antigen retrieval using heat-induced epitope retrieval in

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 1856-1863 | ISSN:2251-6727

Tris-EDTA buffer (pH 9). Endogenous peroxidase activity was blocked using 3% hydrogen peroxide. The sections were then incubated with the primary antibody at room temperature, followed by a polymer-based secondary antibody detection system. Visualization was achieved using diaminobenzidine (DAB) chromogen, and slides were counterstained with hematoxylin.

Interpretation and Scoring of CD34 Expression

CD34 immunoreactivity was assessed semi-quantitatively by visual estimation under light microscopy. The staining was scored based on the percentage of positive stromal cells, ranging from score 0 (absent or 1–5% staining) to score 5 (46–60% staining), as per the predefined scoring system.

Sample Size Calculation

The sample size was calculated using the formula:

$$N = \frac{Z^2 \times p(1-p)}{d^2}$$

Where p = 17.6%, confidence interval = 95%, Z = 1.96, margin of error (d) = 10%. After accounting for a 20% non-response rate, the final sample size was calculated as 65 cases.

Immunohistochemistry Protocol

Formalin-fixed paraffin-embedded tissue sections of 3 µm thickness were mounted on poly-L-lysine-coated slides and incubated at 70°C for 3 hours. Sections were deparaffinized in xylene, rehydrated through graded alcohols, and washed in distilled water. Antigen retrieval was performed in Tris-EDTA buffer (pH 9) using a decloaking chamber at 110°C for 20 minutes, followed by cooling at room temperature. After blocking endogenous peroxidase activity, sections were incubated with primary antibody for 1 hour at room temperature, followed by secondary antibody incubation. DAB was used as chromogen, and sections were counterstained with hematoxylin, dehydrated, cleared, and mounted. The stained slides were examined under light microscopy.

Inclusion Criteria

- Normal endometrium at different phases of the menstrual cycle
- Abnormal uterine bleeding with proliferative or secretory endometrium
- Endometrial polyps
- Neoplastic endometrial lesions
- Postmenopausal endometrium
- Adenomyotic and endometriotic lesions
- Patients with exposure to exogenous hormones less than 3 months prior to surgery

Exclusion Criteria

- Cases with a history of postmenopausal hormone replacement therapy

Statistical Analysis

Statistical analysis was performed using descriptive statistics to summarize CD34 immunoreactivity in normal endometrium and AUB cases. Comparative analyses were conducted using Student's *t*-test or Mann–Whitney *U* test, as appropriate. Correlation and regression analyses were used to assess relationships between CD34 expression and clinicopathological variables. Subgroup analyses were also performed where applicable. Statistical significance was determined based on a predefined *p*-value, and appropriate statistical tests were selected based on data distribution, with consultation from a statistician

RESULTS AND OBSERVATIONS;

Table: 1 Age-Group Wise Distribution of Cases According to Category

Age Group (years)	Normal Endometrium (n, %)	AUB (n, %)	Total Cases (n, %)
≤30	—	11 (16.92)	11 (16.92)
31–40	—	22 (33.85)	22 (33.85)
41–50	—	28 (43.08)	28 (43.08)
51–60	—	3 (4.62)	3 (4.62)
61–70	—	1 (1.53)	1 (1.53)
Total	**10 (15.4)**	**55 (84.6)**	**65 (100)**

Journal of Chemical Health Risks
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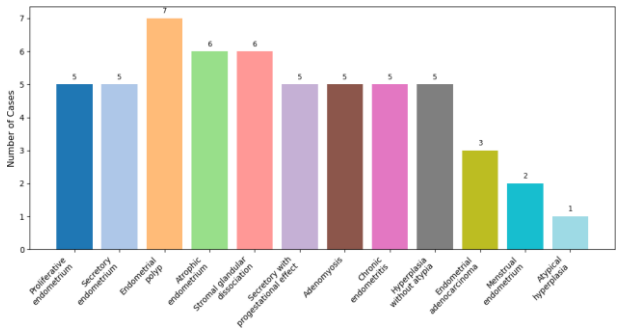
Table 2: Distribution of Cases According to Lesion Type (n=65)

Lesion Type	Number (n)	Percentage (%)
Benign	61	93.85
Premalignant	1	1.54
Malignant	3	4.61
Total	**65**	**100.0**



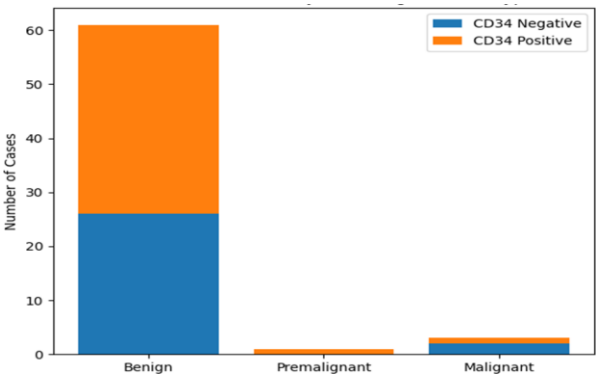
Graph 1: Distribution of Histopathological Diagnoses in AUB Category (n=55)

Table 3: Distribution of Histopathological Diagnoses Across Age Groups.

Histopathological Diagnosis	≤30	31-40	41-50	51-60	61-70	Total
Proliferative endometrium	2	4	4	0	0	10
Secretory endometrium	3	5	2	0	0	10
Endometrial polyp	3	3	0	1	0	7
Atrophic endometrium	0	0	3	2	1	6
Stromal glandular dissociation	0	0	6	0	0	6
Secretory endometrium with progestational effect	0	3	2	0	0	5
Adenomyosis	1	1	3	0	0	5
Chronic endometritis	1	2	2	0	0	5
Endometrial hyperplasia without atypia	1	1	3	0	0	5
Endometrial adenocarcinoma	0	1	2	0	0	3
Menstrual endometrium	0	2	0	0	0	2
Atypical endometrial hyperplasia	0	0	1	0	0	1
Total	**11**	**22**	**28**	**3**	**1**	**65**

Table 4: CD34 Immunoreactivity Grouped as Negative vs Positive According to Category

CD34 Expression	Normal (n=10)	AUB (n=55)	p value
Negative (Score 0)	0 (0.0)	28 (50.90)	$p < 0.001$
Positive (Score 1-5)	10 (100.0)	27 (49.09)	
Total	**10 (100%)**	**55 (100%)**	
Comparison between Normal and AUB groups was performed using the Chi-square test			



Graph 2: Stacked bar chart showing CD34 immunoreactivity grouped as negative and positive according to lesion type

Table 5: CD34 Immunoreactivity Across Histopathological Diagnoses (Category-wise)

Category	Histopathological Diagnosis	0	1+	2+	3+	4+	5+	Total
Normal	Proliferative endometrium	0	0	0	5	0	0	5
	Secretory endometrium	0	5	0	0	0	0	5

Journal of Chemical Health Risks

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AUB	Proliferative endometrium	3	2	0	0	0	0	5
	Secretory endometrium	5	0	0	0	0	0	5
	Endometrial polyp	5	1	1	0	0	0	7
	Atrophic endometrium	6	0	0	0	0	0	6
	Stromal glandular dissociation	0	3	3	0	0	0	6
	Secretory with progestational effect	0	5	0	0	0	0	5
	Adenomyosis	2	0	0	0	3	0	5
	Chronic endometritis	0	0	0	3	2	0	5
	Endometrium Hyperplasia without atypia	3	0	1	0	0	2	5
	Atypical endometrial hyperplasia	0	0	0	1	0	0	1
	Endometrial adenocarcinoma	2	0	0	1	0	0	3
	Menstrual endometrium	2	0	0	0	0	0	2

Table 6: CD34 Immunoreactivity Across Histopathological Diagnoses (Category-wise, Negative vs Positive)

Category	Histopathological Diagnosis	CD34 Negative n	CD34 Positive n	Total
Normal	Proliferative endometrium	0	5	5
	Secretory endometrium	0	5	5
AUB	Proliferative endometrium	3	2	5
	Secretory endometrium	5	0	5
	Endometrial polyp	5	2	7
	Atrophic endometrium	6	0	6
	Stromal glandular dissociation	0	6	6
	Secretory endometrium with progestational effect	0	5	5
	Adenomyosis	2	3	5
	Chronic endometritis	0	5	5
	Hyperplasia without atypia	3	2	5
	Atypical endometrial hyperplasia	0	1	1
	Endometrial adenocarcinoma	2	1	3
	Menstrual endometrium	2	0	2

CD34 immunoreactivity was categorised as negative (score 0) and positive (scores 1+ to 5+) for analytical purposes

Table 7: CD34 Immunoreactivity in Functional vs Structural Causes of AUB

AUB Type	CD34 Negative n	CD34 Positive n	Total	p value
Functional AUB	10	2	12	p=0.002
Structural AUB	18	25	43	
Total	**25**	**30**	**55**	

Functional AUB: Proliferative endometrium (AUB), Secretory endometrium (AUB), Menstrual endometrium

Structural AUB: Endometrial polyp, Adenomyosis, Hyperplasia, Carcinoma, Atrophic endometrium, Stromal-glandular dissociation, Chronic endometritis, Progestational endometrium

CD34 negative = score 0; CD34 positive = scores 1+ to 5+

DISCUSSION

Abnormal uterine bleeding (AUB) is a frequent clinical problem, particularly in women of late reproductive and perimenopausal age, and is often associated with

Journal of Chemical Health Risks
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JCHR (2026) 16(1), 1856-1863 | ISSN:2251-6727

significant histopathological and vascular alterations within the endometrium. The present observational study evaluated CD34 protein immunoreactivity in the endometrial stroma of normal endometrium and compared it with various histopathological patterns observed in AUB, highlighting the role of stromal vascular changes in the pathogenesis of abnormal bleeding.

In the present study, the majority of cases (76.93%) were clustered in the 31–50-year age group, with a peak incidence in the 41–50-year age range. This age distribution is consistent with previous studies, which have reported a higher prevalence of AUB during the perimenopausal period due to hormonal imbalance, anovulatory cycles, and increased susceptibility to structural uterine pathology [11,12]. The predominance of AUB cases (84.6%) over normal endometrium further reflects the clinical burden of this condition in the study population.

Histopathological analysis revealed that benign lesions constituted the majority of cases (93.85%), with only a small proportion of premalignant and malignant lesions. Similar findings have been reported in earlier studies, where benign endometrial patterns such as proliferative and secretory endometrium, polyps, adenomyosis, and chronic endometritis were the most common causes of AUB [13,14]. The low incidence of malignancy in the present study underscores the importance of histopathological evaluation in excluding serious pathology, particularly in perimenopausal and postmenopausal women.

Among the AUB cases, proliferative and secretory endometrium were the most frequently observed patterns, followed by endometrial polyps, atrophic endometrium, stromal-glandular dissociation, and adenomyosis. Functional causes of AUB predominated over structural causes, a finding that aligns with previous observations suggesting that ovulatory dysfunction and endometrial factors play a major role in AUB, especially in younger and perimenopausal women [15].

A key finding of the present study was the significant difference in CD34 immunoreactivity between normal endometrium and AUB cases. All normal endometrial samples demonstrated positive CD34 expression, whereas nearly half of the AUB cases (50.9%) showed complete absence of CD34 staining. This difference was statistically significant ($p < 0.001$), indicating a strong association between altered stromal vascularity and abnormal uterine bleeding. These findings support earlier reports that normal endometrium exhibits well-organized stromal vasculature, whereas AUB is associated with disrupted angiogenesis and vascular integrity [16,17].

Evaluation of CD34 expression across different histopathological diagnoses revealed distinct patterns. Normal proliferative endometrium showed strong CD34 positivity, reflecting active angiogenesis during the proliferative phase, while normal secretory endometrium exhibited comparatively lower but consistent expression. These cyclical variations in vascular density have been well documented and are believed to be hormonally regulated [18].

In contrast, AUB-associated proliferative and secretory endometrium frequently demonstrated negative or weak CD34 expression, suggesting impaired angiogenic response or defective stromal vascular remodeling. Similar alterations have been described by Hickey et al., who proposed that abnormal angiogenesis leads to fragile and unstable blood vessels, contributing to excessive or irregular bleeding [19].

Structural lesions such as endometrial polyps, adenomyosis, and endometrial hyperplasia showed variable CD34 expression. Polyps and atrophic endometrium predominantly exhibited negative staining, possibly due to reduced vascular activity or stromal fibrosis. Conversely, adenomyosis and chronic endometritis demonstrated higher CD34 positivity, which may be attributed to inflammatory-mediated angiogenesis and increased stromal vascular proliferation [20].

Notably, malignant and premalignant lesions exhibited altered and heterogeneous CD34 expression. Endometrial adenocarcinoma showed reduced CD34 positivity, consistent with previous studies reporting aberrant and disorganized neovascularization in malignant endometrium [21]. These findings suggest that CD34 expression may reflect not only vascular density but also the functional integrity of the endometrial microvasculature.

Journal of Chemical Health Risks

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When CD34 immunoreactivity was analyzed in functional versus structural causes of AUB, functional AUB cases showed a significantly higher proportion of CD34 negativity compared to structural AUB ($p = 0.002$). This observation supports the hypothesis that functional AUB is primarily related to defective endometrial angiogenesis rather than gross anatomical abnormalities [22].

Overall, the findings of the present study emphasize the crucial role of stromal vascular remodeling in maintaining endometrial hemostasis. Altered CD34 expression appears to be a common feature in AUB and may serve as a useful adjunct marker in understanding the underlying pathophysiology. However, the study is limited by its sample size and lack of correlation with hormonal and molecular angiogenic factors, which warrants further investigation.

CONCLUSION

CD34 immunoreactivity in the endometrial stroma differs significantly between normal endometrium and abnormal uterine bleeding. Normal endometrium shows consistent CD34 positivity, while many AUB cases demonstrate reduced or absent expression, indicating altered stromal vascularity. Functional AUB is more commonly associated with CD34 negativity than structural causes. CD34 immunohistochemistry is a useful adjunct in understanding endometrial vascular changes in AUB.

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