"STUDY OF EXPRESSION OF ESTROGEN AND PROGESTERONE RECEPTORS AND BLOOD VESSEL DENSITY IN DYSFUNCTIONAL UTERINE BLEEDING"

By

DR. BANDARU PRATHIMA JERUSHA

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In partial fulfilment of the requirements for the award of the degree of

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IN

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Under the Guidance of

DR. MAMATHA K M.D.

Associate Professor, Department of Pathology

BLDE (DEEMED TO BE UNIVERSITY), SHRI B.M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA, 586103, KARNATAKA

CO GUIDE

DR SHREEDEVI KORI M.S.

Associate professor, Department of Obstetrics and Gynecology

BLDE (DEEMED TO BE UNIVERSITY), SHRI B.M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA, 586103, KARNATAKA

2025

BLDE (DEEMED TO BE UNIVERSITY), SHRI B.M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA, KARNATAKA

DECLARATION BY THE CANDIDATE

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Dr. BANDARU PRATHIMA JERUSHA

Postgraduate student,

Department of Pathology

BLDE (Deemed to be University)

Shri B.M. Patil Medical College, Hospital & Research Centre

Vijayapura, Karnataka

Date:

Place: VIJAYAPURA

BLDE (DEEMED TO BE UNIVERSITY), SHRI B.M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA, KARNATAKA

CERTIFICATE BY THE GUIDE

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Dr. MAMATHA. K

Associate Professor,

Department of Pathology

BLDE (Deemed to be University)

Shri B.M. Patil Medical College, Hospital & Research Centre

Vijayapura, Karnataka

Date:

Place: VIJAYAPURA

BLDE (DEEMED TO BE UNIVERSITY), SHRI B.M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA, KARNATAKA

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DR. SHREEDEVI KORI
Associate professor,
Department of Obstetrics and Gynecology
BLDE (Deemed to be University)
Shri B.M. Patil Medical College, Hospital & Research Centre
Vijayapura, Karnataka

Date:

Place: VIJAYAPURA

BLDE (DEEMED TO BE UNIVERSITY), SHRI B.M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA, KARNATAKA

ENDORSEMENT BY THE HEAD OF DEPARTMENT

This is to certify that this dissertation titled "**STUDY OF EXPRESSION OF ESTROGEN AND PROGESTERONE RECEPTORS AND BLOOD VESSEL DENSITY IN DYSFUNCTIONAL UTERINE BLEEDING**" is a bonafide and genuine research work carried out by Dr. BANDARU PRATHIMA JERUSHA, in partial fulfilment of the requirements for the degree of Doctor of Medicine (Pathology).

Dr. SUREKHA B. HIPPARGI

Professor and Head,

Department of Pathology

BLDE (Deemed to be University)

Shri B.M. Patil Medical College, Hospital & Research Centre

Vijayapura, Karnataka

Date:

Place: VIJAYAPURA

BLDE (DEEMED TO BE UNIVERSITY), SHRI B.M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA, KARNATAKA

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Dr. ARAVIND V. PATIL

Principal,

BLDE (Deemed to be University)

Shri B.M. Patil Medical College, Hospital & Research Centre

Vijayapura, Karnataka

Date:

Place: VIJAYAPURA

BLDE (DEEMED TO BE UNIVERSITY), SHRI B.M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA, KARNATAKA

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Dr. BANDARU PRATHIMA JERUSHA

Postgraduate student,

Department of Pathology

BLDE (Deemed to be University)

Shri B.M. Patil Medical College, Hospital & Research Centre

Vijayapura, Karnataka

Date:

Place: VIJAYAPURA

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Dr. BANDARU PRATHIMA JERUSHA

Postgraduate student,

Department of Pathology

BLDE (Deemed to be University)

Shri B.M. Patil Medical College, Hospital & Research Centre

Vijayapura, Karnataka

Date:

Place: VIJAYAPURA

LIST OF ABBREVIATIONS

ABBREVIATION	PARAMETER
DUB	Dysfunctional Uterine Bleeding
AUB	Abnormal Uterine Bleeding
ER	Estrogen Receptor
PR	Progesterone Receptor
FSH	Follicle Stimulating Hormone
GnRH	Gonadotropin Releasing Hormone
GnSAF	Gonadotropin Surge-Attenuating factor
LH	Luteinizing Hormone
OC	Oral contraceptives
HPO axis	Hypothalamic-Pituitary-Ovarian axis
PCOS	Polycystic ovarian syndrome
PG	Prostaglandin
EGF	Epidermal Growth Factor
TGF	Transforming Growth Factor
VEGF	Vascular Endothelial Growth Factor
ECM	Extracellular Matrix
HIF 1 α	Hypoxia Inducible Factor 1- α
bHLH	Basic helix-loop-helix
ODD	Oxygen-Dependent Degradation
DNA	Deoxyribonucleic acid
MMPs	Matrix Metalloproteinases
IHC	Immunohistochemistry

TSH	Thyroid stimulating Hormone
PT	Prothrombin Time
TAH& BSO	Total Abdominal Hysterectomy and Bilateral Salpingoopherectomy
HPR	Histopathology Reporting
H&E	Hematoxylin and Eosin

ABSTRACT

INTRODUCTION:

AUB without any associated cause is referred to as a “Dysfunctional uterine bleeding” (DUB). Proliferation and differentiation of endometrial glands and stroma are regulated by steroid hormones mainly estrogen and progesterone. There is also positive correlation between endometrial angiogenesis and menstrual disorders. Therefore, this study focuses on examining blood vessel density. Therefore, analysis of ER and PR receptors allocation in the glandular and stromal component of endometrium could facilitate medical treatment for cases of DUB thereby avoiding unnecessary surgeries. Also, as the alterations in morphology of blood vessels plays a significant role in pathogenesis of DUB, we hereby analyzed mean vascular density.

OBJECTIVES:

1. To study immunohistochemical expression of ER and PR in endometrium of women with DUB.
2. To analyze blood vessel density in cases of DUB.

MATERIALS AND METHODS:

The present study is hospital based cross – sectional study in a sample size of 82 (60 test group which included females with DUB and 22 control group included normal proliferative or secretory phase). Following the collection of endometrial samples, H and E-stained slides were analyzed for histopathological diagnosis, as well as to assess blood vessel density. Additionally, immunohistochemistry is employed to study the distribution of estrogen receptor (ER) and progesterone receptor (PR) expression

RESULTS

The current research aimed to analyze the estrogen and progesterone receptors expression in the endometrium, as well as the concentration of blood vessels in specific cases of DUB**.** Among the 60 subjects, there were 31 neoplastic cases (51.7%) and 29 nonneoplastic lesions (48.3%). Results showed notable increase in ER and PR expression in the glandular and stromal component of endometrium when compared to control groups with maximum average scoring of estrogen receptor expression in Endometrioid endometrial carcinoma NOS (6.20 ± 0.58), followed by Endometrial hyperplasia without atypia (6.40± 0.99) and Disordered endometrium (5.80 ± 0.99). Mean Alred scoring of PR in both glands and stroma showed maximum scoring in Endometrioid endometrial carcinoma NOS (6.50 ±1.15) followed by Hyperplasia without atypia (6.35 ± 1.64) and Disordered maturation (5.80 ± 1.34).

Mean blood vessel density in the cases group (58.68 ± 12.57) was highest when compared to control group (65.72 ± 16.09)

CONCLUSION

The current study was conducted emphasizing the role of ER and PR expression and vascular density in the pathogenesis of Dysfunctional Uterine Bleeding (DUB). Additionally, the study establishes a correlation between increased blood vessel density and DUB, indicating that vascular changes contribute to the pathogenesis. These results suggest that hormonal receptor profiling and blood vessel analysis can serve as valuable diagnostic and prognostic tools.

KEYWORDS: Abnormal Uterine bleeding, Immunohistochemistry, Blood vessel density, Estrogen and Progesterone receptors.

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INTRODUCTION

The term "dysfunctional uterine bleeding" (DUB) describes irregular uterine bleeding in premenopausal women that is brought on by disturbances in the endometrium's normal cyclical changes and happens for no apparent pathological reason, such as endometritis, polyps, external hormones, hyperplasia, or cancer [1].

'DUB' is a diagnosis that can only be confirmed through histopathological examination, meaning that organic causes of abnormal bleeding must be ruled out first (after confirming that pregnancy is not a factor). These causes can be classified into three primary groups: pelvic problems, systemic illnesses, and factors related to medical treatment [1].

Approximately 10% to 15% of gynecological patients are diagnosed with DUB. Research has shown that biochemical imbalances, such as increased fragility of endometrial blood vessels, disrupted endometrial angiogenesis, and inconsistencies in the supporting structures of the endometrium (including endothelial, epithelial, and stromal components), may significantly contribute to the development of DUB [2].

Local pelvic problems related to menorrhagia encompass non-cancerous conditions like fibroids, adenomyosis, endometriosis, polyps in the endometrium or cervix, cervicitis, and severe vaginal infections[2]. However, malignant causes of menorrhagia include precancerous endometrial changes, such as hyperplasia and malignancies.

Systemic diseases are also significant contributors, encompassing coagulation issues like thrombocytopathies, Von Willebrand's disease, and leukemia, along with ailments such as hypothyroidism, systemic lupus erythematosus, and cirrhosis. Furthermore, iatrogenic factors involve hormone treatments, contraceptive injections and devices, as well as drugs like tranquilizers, antidepressants, anticoagulants, and corticosteroids. If all potential causes are excluded, a diagnosis of dysfunctional uterine bleeding (DUB) can be established. DUB

encompasses more than just menorrhagia; it also involves excessively long and frequent bleeding episodes (menometrorrhagia). This condition is more prevalent in anovulatory cycles than in ovulatory ones. Anovulatory DUB occurs due to the unopposed action of estrogen on the endometrium, resulting in various changes including proliferative, disordered proliferative, hyperplastic, or neoplastic alterations[2,3].

This is a prevelant gynecological problem, accounting for one-third of appointments in gynecological outpatient clinics. It often affects women in both early and late reproductive stages, with more than 50% of those who had a hysterectomy due to menorrhagia being diagnosed with dysfunctional uterine bleeding (DUB)[4].

Both ovulatory and anovulatory cycles can give rise to dysfunctional uterine bleeding (DUB). Heavy bleeding during regular menstrual periods is a hallmark of DUB in ovulatory cycles, whereas irregular, protracted, and heavy bleeding is a hallmark of anovulatory cycles [5]. The fundamental cause of DUB is endometrial hyperplasia, a known risk factor for endometrial cancer, which results from estrogen's unopposed stimulation of the endometrium regardless of blood estrogen levels [5].

DUB has a major impact on women's health and quality of life, leading to severe anaemia and infertility as a result of anovulation. Therefore, prompt treatment is crucial [6].

The preferred method for confirming dysfunctional uterine bleeding (DUB) is through histological analysis of an endometrial biopsy sample[7].Different histological patterns can be observed in DUB, with most being successfully managed through hormonal treatment. Endometrial ablation and hysterectomy are reserved for more severe instances [8].

Comprehending steroid receptors in the endometrium is crucial, as hormone receptors play a significant role in the development and causes of dysfunctional uterine bleeding (DUB)[9]. The advent of receptor-modulating drugs and the precise identification of steroid receptor locations in the endometrium, affected by ovarian hormones, has revolutionized the medical management of patients experiencing dysfunctional uterine bleeding (DUB)[9].

Immunohistochemistry is used to identify the presence of receptors (such as estrogen and progesterone) in tissue samples[10]. Immunohistochemical analysis showed heightened levels of estrogen receptor (ER) expression during the late proliferative and early secretory phases. Although many studies have explored the cyclical variations of estrogen and progesterone receptors in healthy endometrial tissue, there is a scarcity of research examining the expression patterns of these hormonal receptors in the endometrium of individuals experiencing dysfunctional uterine bleeding (DUB) [7,8].

The endometrium is characterized by its unique ability to undergo angiogenesis, which is a crucial process in the normal endometrial cycle. Angiogenesis is the creation of new blood vessels from existing ones, along with the growth of these newly formed vessels. Angiogenesis in the endometrium usually happens as a result of spiral arteriole development during the secretory phase, vascular network restoration after menstruation, and vascular endothelial cell expansion during the proliferative phase[9,10]. According to recent research, biochemical imbalances may play a major role in the causes of dysfunctional uterine bleeding (DUB). These imbalances include abnormalities in the epithelial, endothelial, and stromal support systems in the endometrial environment, increased vulnerability of endometrial blood vessels, and disrupted angiogenesis[9,10]. Thus, the purpose of this study was to investigate blood vessel density and ER and PR expression in dysfunctional uterine hemorrhage.

AIM AND OBJECTIVES

AIM

To evaluate the level of progesterone receptor (PR) and estrogen receptor alpha (ER alpha) expression in the stroma and endometrial glands of women who have dysfunctional uterine bleeding [10].

OBJECTIVES

1. Assess immunohistochemical analysis of ER and PR in endometrium of women with Dysfunctional Uterine Bleeding (DUB) [10].
2. To analyze blood vessel density in cases of Dysfunctional Uterine Bleeding (DUB).

DYSFUNCTIONAL UTERINE BLEEDING

Definition

Dysfunctional uterine bleeding (DUB) refers to irregular uterine bleeding occurring in individuals of reproductive age, without any identifiable organic causes [11].

The term 'DUB' stands for dysfunctional uterine bleeding, which is abnormal bleeding from the uterus caused by issues with ovarian hormone production, primarily due to anovulation. All forms of irregular menstruation, with the exception of amenorrhea, are included in DUB. 75% of occurrences of this illness occur in those over 35, making it most common at the extremities of the reproductive age span[11].

DUB

Dysfunctional uterine bleeding happens when the regular menstrual cycle is interrupted, typically due to anovulation (the inability to ovulate) that is not linked to any other medical condition. Anovulation is the most prevalent cause of DUB in teenagers and women approaching perimenopause[12].

In cases of anovulatory dysfunctional uterine bleeding (DUB), estrogen is consistently produced, but no ovum is released[12]. Since there is no release of ovum, progesterone is not generated by the corpus luteum to balance the growth of the uterine lining. As a result, the uterine lining eventually exceeds its blood supply and sheds at unpredictable times[12].

Since no ovum is produced, the premenstrual and menstrual symptoms related to ovulation and progesterone are absent, resulting in typically painless uterine bleeding. Endometrial hyperplasia and cancer have been directly linked to the effects of unopposed estrogen on the uterine lining[12].

EPIDEMIOLOGY

In India, 5% of women between the ages of 30 and 49 visit their gynecologist annually due to heavy menstrual bleeding, with a prevalence rate of 17.9%. In 40-60% of these instances, no identifiable cause is determined[13].

Clinical Parameter	Descriptive term	Normal limits (5–95th percentiles)
Frequency of menses (days)	Frequent	<24
	Normal	24–38
	Infrequent	>38
Regularity of menses, cycle to cycle (Variation in days over 12 months)	Absent	No bleeding
	Regular	Variation ± 2–20 days
	Irregular	Variation >20 days
Duration of flow (days)	Prolonged	>8.0
	Normal	4.5–8.0
	Shortened	<4.5
Volume of monthly blood loss (mL)	Heavy	>80
	Normal	5–80
	Light	<5

Figure 1: Normal limits for menstrual parameters[16]

MENSTRUAL CYCLE

Phase 1: Pre-ovulatory and Endometrial Growth Phase

This is the initial phase of the menstrual cycle during which growth of follicles and thickening of endometrium occur respectively. The pre-ovulatory phase, which is variable in duration, begins on the initial day of the menstrual cycle and is marked by menstrual bleeding concluding with rupture of ovarian follicle and release of ovum[14]. This phase typically lasts from the first to the fourteenth day of a typical 28-day cycle. Concurrently, following the cessation of menstruation, the uterine proliferative phase commences and continues until ovulation14. The uterus is lined with the deeper portion of the functionalis layer and the basal layer of endometrium after menstruation. On the third or fourth day of the cycle, the uterus begins to proliferate, increasing in thickness by up to

4 or 5 mm[14].

Events during the Pre ovulatory phase: During this stage, FSH stimulates the maturation of multiple primordial follicles into Graafian follicles thereby inducing the ovaries to produce 17-β estradiol and inhibin B[14]. Studies indicate that the dominant follicle is primarily responsible for estrogen production. By approximately day 7 of the cycle, each ovary usually contains multiple antral follicles that are 9 to 10 mm in size. As these levels rise, they provide downregulation to reduce FSH levels, causing the regression of non-dominant follicles[14]

Events during the Endometrial growth phase: The endometrial glands and stroma from the decidual basalis increase when stimulated by estradiol-17β produced by the growing follicles, which also helps the vascular network to expand[14]. By the end of proliferative phase, the endometrium attains its peak thickness, which generally ranges from 8 to 12 mm, although this measurement may exhibit variability[14].

Ovulation

Ovulation usually occurs 14 days before the onset of menstruation, which means that it happens on day 14 of a normal 28-day cycle. Estradiol levels rise during the follicular phase, and toward the conclusion of this phase, 17-β estradiol stops having a negative feedback effect on the anterior pituitary and starts to have a positive one[15].

The exact processes driving this change are not completely clear and probably involve multiple factors. However, it generally happens when estradiol levels rise to a specific point. Elevated concentrations of estradiol stimulate pituitary gonadotropes in order to generate greater number of Gonadotropin-releasing hormone receptors, thereby enhancing their responsiveness to GnRH-binding sites[15]. As estradiol levels increase during the follicular phase, gonadotropin surge-attenuating factor (GnSAF) is suppressed, allowing estradiol's sensitizing effects to take effect as ovulation nears. These processes result in a notable increase in LH secretion, with LH levels rising ten times during this time, along with smaller increases in FSH levels[15].

This endocrine milieu, causes follicle to rupture and release of oocyte with the help of

plasminogen activator and various cytokines produced by mature follicle. Ovulation usually occurs around 36 to 44 hours following the start of the LH surge. 17-β estradiol decline by the end of ovulation[15].

Phase 2: Corpus Luteal and Secretory endometrium

Corpus luteal and endometrial secretory phases are subsequent stages of the menstrual cycle associated with the progesterone production by the corpus luteum and glandular activity of the mature endometrium[15]. This phase begins with rupture of graffian follicle and concludes when menstruation begins. In contrast to the fluctuating duration of the Pre-ovulatory and Endometrial Growth Phase, the Corpus Luteal and Endometrial Secretory Phases tends to be more stable for each person, usually lasting around 14 days[15]**.**

Progesterone is stimulated by luteinizing hormone throughout this stage activating formation of intricate glands, enhances glycogen storage for energy, and expands the superficial extent of vascular network[15].

Physiological Menstrual cycle

Menstruation refers to the process of endometrial sloughing due to decrease in luteal hormone and post ovulatory hormone levels at the end of the luteal phase. This process occurs during day 1 of menstrual cycle showing its occurrence in the early preovulatory phase[17].

The length of menstruation varies, but it typically does not exceed 8 days. Menstrual fluid is made up of blood, endometrial cells, vaginal secretions, and various biochemical compounds[17].

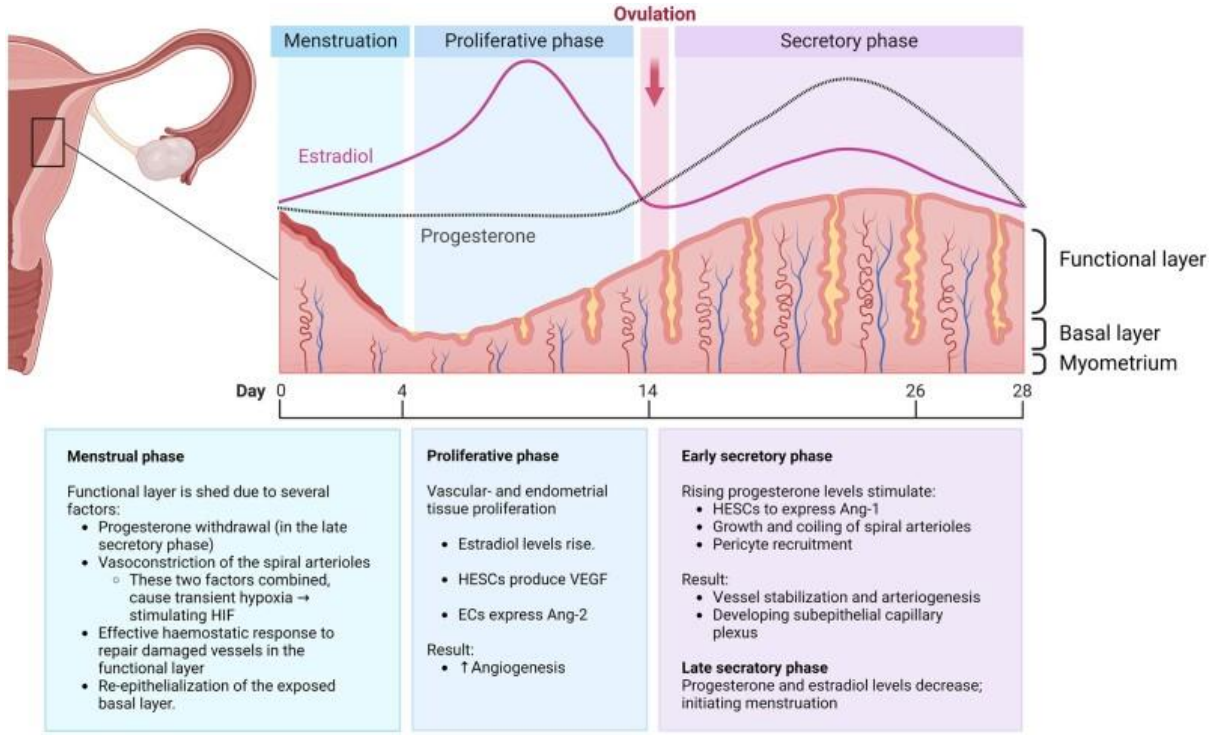


Figure 2: Human menstrual cycle[18]

HORMONAL FLUCTUATION

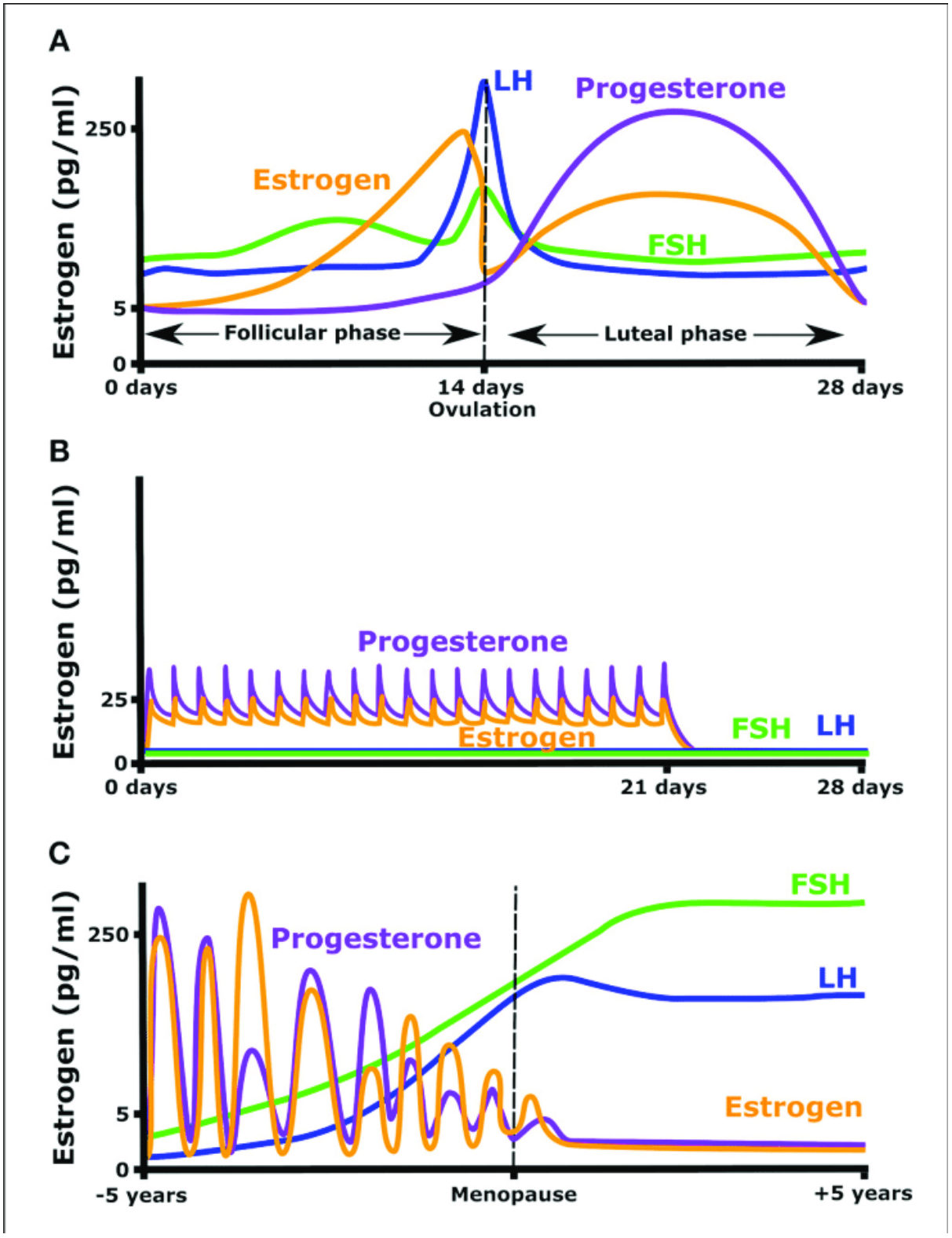


Figure 3: Hormonal fluctuations in the years leading up to and following menopause, during (A) a typical menstrual cycle, and (B) when using an oral contraceptive (OC) that contains both estrogen and progesterone[19].

Cause of anovulatory bleeding

In contrast to the structured cycle of estrogen and progesterone fluctuations observed in women who ovulate regularly, women who do not ovulate experience disorganized and unpredictable hormone production, resulting in irregular menstrual bleeding [20]. Because there is no luteal phase and no ovulation, an anovulatory woman is virtually always in the follicular phase of the ovarian cycle[20]. Consequently, there is continuous estrogen stimulation, which leads to ongoing growth of the endometrium. Estrogen levels fluctuate as new groups of follicles develop and subsequently regress [20].

Although estrogen levels fluctuate, the endometrium eventually grows as a result. This layer gets progressively thicker and more fragile. Some parts of the endometrium begin to degrade and bleed when progesterone isn't there to assist organize and regulate it[20]. Some areas may shed while others may recover as a result of continuous estrogen stimulation. As the quantity of blood vessels rises, they become more delicate and prone to breaking[20]. Vasoconstriction in the basal and myometrial vessels does not occur because the tissue loss is superficial and does not impact the basal endometrial layer, which causes continuous bleeding[20]. Changes in the synthesis and balance of endometrial prostaglandins also contribute to reduced vasoconstriction and increased blood loss[20].

Classification of DUB:

1. Anovulatory
2. Ovulatory

Anovulatory DUB

- Usually stems from the hypothalamic-pituitary-ovarian (HPO) axis working abnormally [20].
- Occurs most often in women at the extreme ends of their reproductive lifetime [20].

- Since both premenarchal and perimenopausal women experience multiple anovulatory cycles annually, it is a prevalent issue in both teenage and older females[20].

Other causes/risk factors for anovulatory uterine bleeding include-

- Excessive exercise
- Emotional stress
- Eating disorders (bulimia and anorexia)
- Obesity
- Polycystic ovarian syndrome (PCOS)
- Hyperprolactinemia
- Thyroid disease

Hypothalamic-Pituitary-Ovarian (HPO) axis

GnRH is released from the hypothalamus in pulses which vary depending on the phase in the menstrual cycle.

The gonadotropins LH and FSH are released when GnRH acts on the anterior pituitary via the hypothalamo hypophyseal portal system. These then trigger the ovary to release progesterone and estrogen[20].

Anorexia nervosa is one disorder that slows GnRH pulsatility, which leads to hypogonadal hypogonadism with failure of follicular formation and amenorrhea, as well as failure of pituitary gonadotropin secretion (LH and FSH)[20].

Understanding the typical cyclic changes of the key female reproductive hormones (oestrogen and progesterone) and the two gonadotropins (luteinizing hormone [LH], follicle-stimulating hormone [FSH]) aids in comprehending the abnormalities linked to anovulation[20].

Pathophysiology of Anovulatory DUB

In cases of anovulation, estrogen levels increase as expected during the initial phase of the menstrual cycle. Since ovulation does not take place, the corpus luteum fails to develop, preventing the production of progesterone. This results in ongoing estrogen stimulation of the endometrium without opposition. Consequently, the endometrium enters a hyperproliferative phase, eventually surpassing its blood supply. This disorder results in profuse bleeding from spiral arteries that have not aged normally and uneven endometrial discharge[20].

There are two main reasons that can explain the disruption of hormonal balance:

1. Anovulation
2. Abnormal local production of prostaglandins.

Although anovulation can occur at any time during a woman's reproductive years, it usually occurs immediately following menarche and prior to menopause. In young girls, the temporary inability to ovulate is typically brief and likely linked to the underdevelopment of the hypothalamic-pituitary system responsible for releasing gonadotropins before ovulation, which matures later in puberty. When anovulation occurs, the corpus luteum follicle does not form, resulting in a lack of progesterone. This leads to unpredictable menstrual cycle lengths and frequencies, with periods that do not include premenstrual symptoms like breast tenderness or mood swings. Anovulatory cycles are characterized by the development of multiple follicles in the ovaries and a failure to produce the midcycle surge of LH [21].

FSH levels are slightly low, but the LH pulse frequency is adequate to promote estradiol secretion from the ovaries. Estrogen levels during the pre-ovulation phase are comparable in both ovulatory and anovulatory cycles. However, in anovulatory cycles, the positive feedback effect of estriol fails, leading to a lack of LH surge. The follicles keep developing until the new multiple follicles undergo spontaneous regression [22].

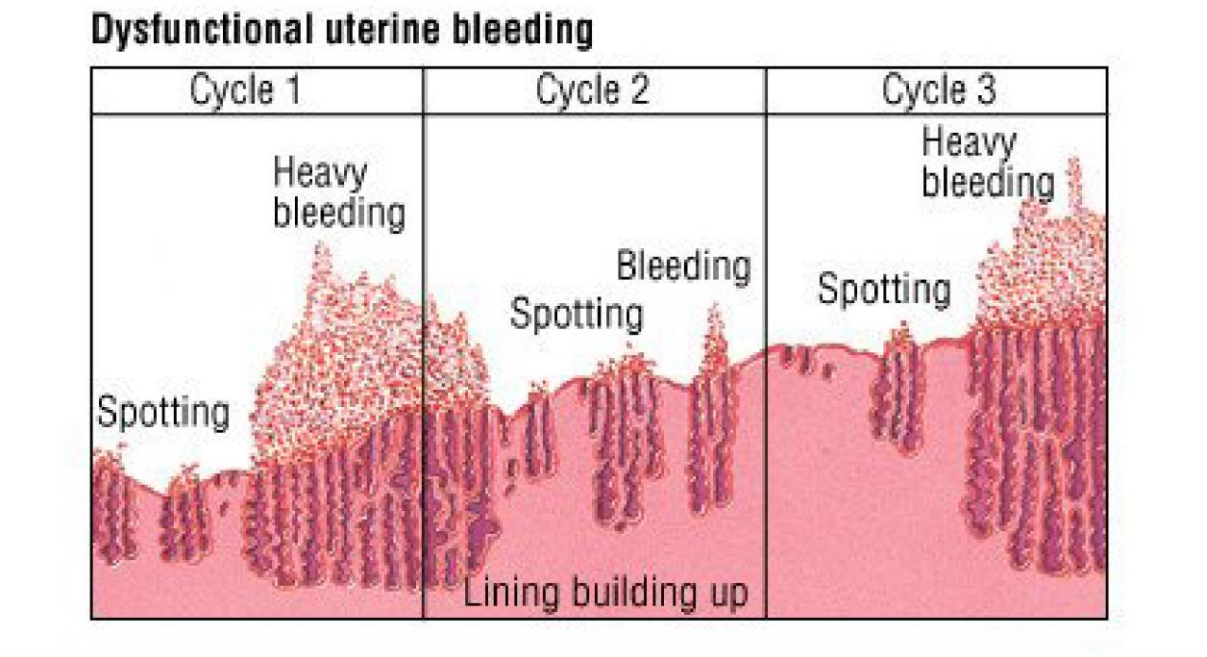


Figure 4: Pathway of action of hormones for normal menstrual function[20]

Ovulatory DUB

This kind of bleeding happens periodically and is believed to be caused by flaws in the menstrual cycle's regulation systems[20].

EFFECTS OF HORMONES ON ENDOMETRIUM

At the cost of the stroma, estrogen causes the endometrium to grow taller and have more endometrial glands. Therefore, the normal 1:1 ratio of the glandular elements to the stroma is disturbed when the endometrium is continuously stimulated by estrogen. Estrogen also increases the vascularity of the endometrium. An asynchronous development of stroma, glands, and blood vessels follows unopposed estrogen stimulation and leads to the irregular endometrial shedding that is characteristic of DUB. Shedding varies in frequency and degree, but it is often heavy and prolonged. Ovulatory DUB is most prevalent in parous women between 30 and 40 years of age. There is evidence to support the theory that aberrant bleeding in both ovular and anovular groups is caused by disruptions in local prostaglandin metabolism. PGF2 causes myometrial contraction and platelet aggregation, and is a potent vasoconstrictor, while PGE2 and prostacyclin cause

myometrial relaxation and vasodilation, and inhibit platelet aggregation. Normally, these factors are balanced, resulting in homeostatic control of the menstrual flow[23].

The endometrium from menorrhagic patients synthesizes less PGF2 and more PGE2 than normal, and it is this imbalance that leads to uncontrolled DUB[24,25].

Differential diagnosis

Pregnancy
Abortion, ectopic pregnancy, trophoblastic disease
Retained products, placental site involution, lactation
Anovulation
Perimenarchal or perimenopausal
Hyperandrogenic (PCOS, CAH, androgen-producing tumor)
Hypothyroidism
Hyperprolactinemia
Premature ovarian failure
Hypothalamic dysfunction (eg, anorexia)
Leiomyoma
Endometrial or cervical polyp
Adenomyosis
Chronic endometritis
Endometrial hyperplasia or malignancy
Cervical or vaginal neoplasia
Blood dyscrasia
Iatrogenic/medications
Systemic disease

Figure 5: Differential diagnosis of DUB[26]

Morphology of Stromal and Glandular Disintegration in Menstruation and Unusual Bleeding [27].

Various descriptive terms, including "lytic," "shedding," "slough," and "menstrual," have been used to refer to breakdown and bleeding patterns. The histological changes seen in abnormal endometrial bleeding differ slightly from those in the menstrual endometrium. This is because, in contrast to menstrual bleeding, glandular and stromal breakdown typically occurs in anovulatory cycles and happens against a nonsecretory background [28].

	Menstrual bleeding	Abnormal bleeding
Background endometrium	Late secretory	Proliferative or secretory endometrium or structural lesion
Heterogeneous background	–	+
Stromal "collapse"	+	+
Stromal cell clusters "stromal blue balls"	+/–	+
Fibrin thrombi	+/–	+
Nuclear debris at base of gland cells	+	+
Nuclear debris in stroma	–	+
Eosinophilic syncytial change	–	+
Hemosiderin	–	+
Foam cells	–	+
Stromal fibrosis and hyalinization	–	+

Figure 6: Characteristics of irregular bleeding and glandular and stromal breakdown during menstruation[29]

Bleeding Associated with Estrogen in Proliferative Endometrium with Stromal and Glandular Disintegration [30]:

One of the most often seen anomalies in biopsies taken for atypical bleeding in perimenopausal women, this disorder refers to endometrial changes brought on by anovulatory cycles[30]. Women of reproductive age may also occasionally experience anovulatory hemorrhage[31]. In most cases, younger women do not need a biopsy for this type of bleeding, as the likelihood of other conditions, such as hyperplasia or cancer, is low in this group [32].

Women who are obese, have persistent anovulation associated with polycystic ovarian syndrome (Stein-Leventhal syndrome), or have inherited cancer syndromes—especially Lynch syndrome, which increases the chance of hyperplasia or carcinoma—are exceptions to this rule [33]. When a cluster of ovarian follicles begins to mature but ovulation does not occur, an anovulatory cycle occurs[34]. Chronic anovulation can result from various conditions, including hypothalamic

dysfunction and obesity, where androgens are converted to estrogen in fat tissue and androgen production is elevated in the adrenal glands or ovaries [34].

The reasons for anovulation after follicle recruitment are intricate. They involve issues within the hypothalamic-pituitary-ovarian axis, such as elevated prolactin levels, irregular hormonal feedback mechanisms, and local ovarian factors that disrupt normal follicular development. Follicles generate estradiol, which promotes the growth of the endometrium. These developing follicles can remain for varying lengths of time before they degenerate. While the follicles are present, estradiol is released, leading to endometrial proliferation. When the follicles degenerate, there is a sharp decline in estradiol production, resulting in estrogen withdrawal bleeding. Because of this, endometrial development is no longer supported by estrogen, which destabilizes lysosomal membranes and promotes vasoconstriction, which in turn causes bleeding. On the other hand, estrogen breakthrough bleeding happens when follicles keep producing estradiol; the endometrium thickens and surpasses its structural support, causing thrombosis in expanded capillaries and localized vasoconstriction. The endometrium typically displays a proliferative phase pattern in the majority of anovulatory abnormal uterine bleeding (AUB) cases, which is marked by the disintegration of glands and stroma [34].

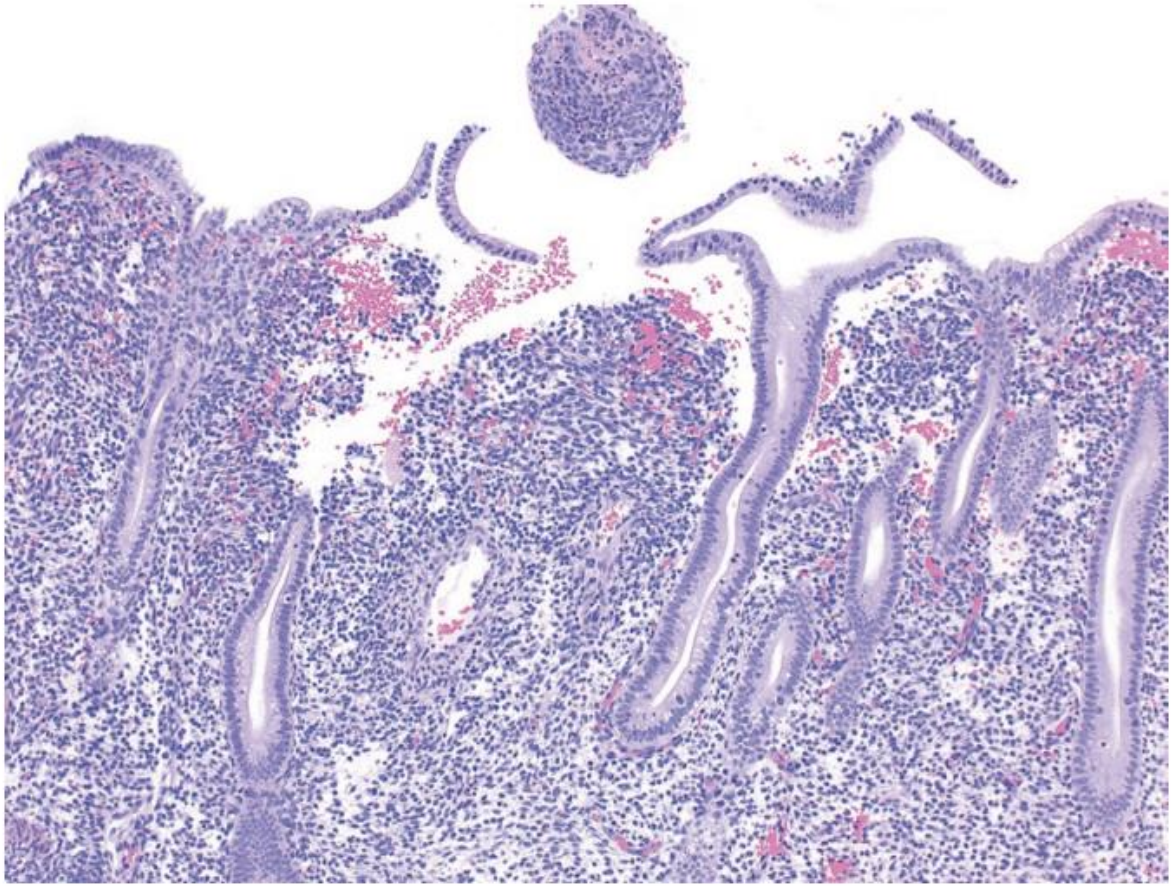


Figure 7: Proliferative endometrium with glandular and stromal breakdown[32]

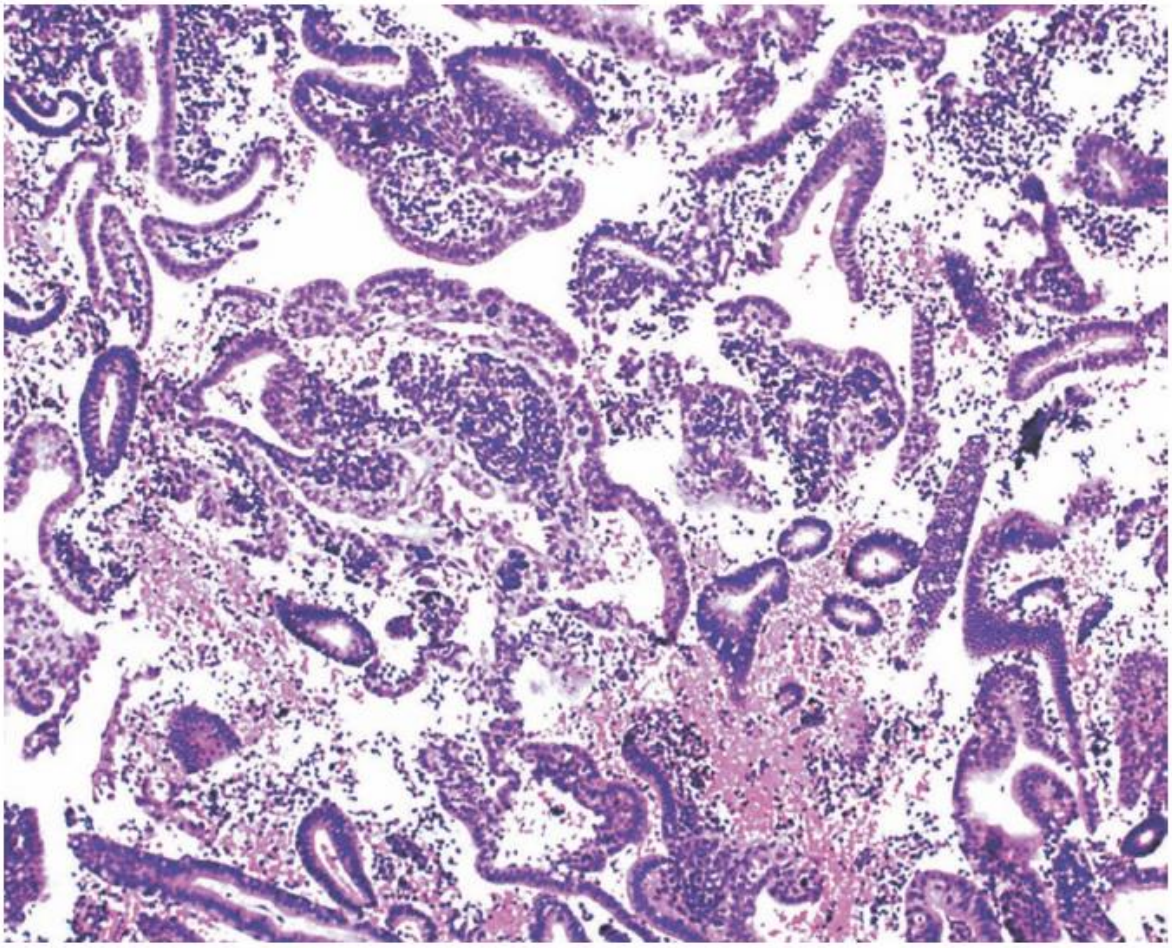


Figure 8: Detached glands showing proliferative features with tubular outlines[32]

The amount of tissue and the structural organization of the glands and stroma are determined by the duration of unopposed estrogen stimulation rather than the estrogen levels themselves. Furthermore, the degree of tissue breakdown can vary significantly, from small areas to widespread involvement of the sample. Occasional anovulation can lead to quick atresia of follicles, accompanied by bleeding due to estrogen withdrawal, which in turn causes limited endometrial growth. Consequently, a small amount of endometrial tissue with underdeveloped, weakly proliferating glands and stroma.

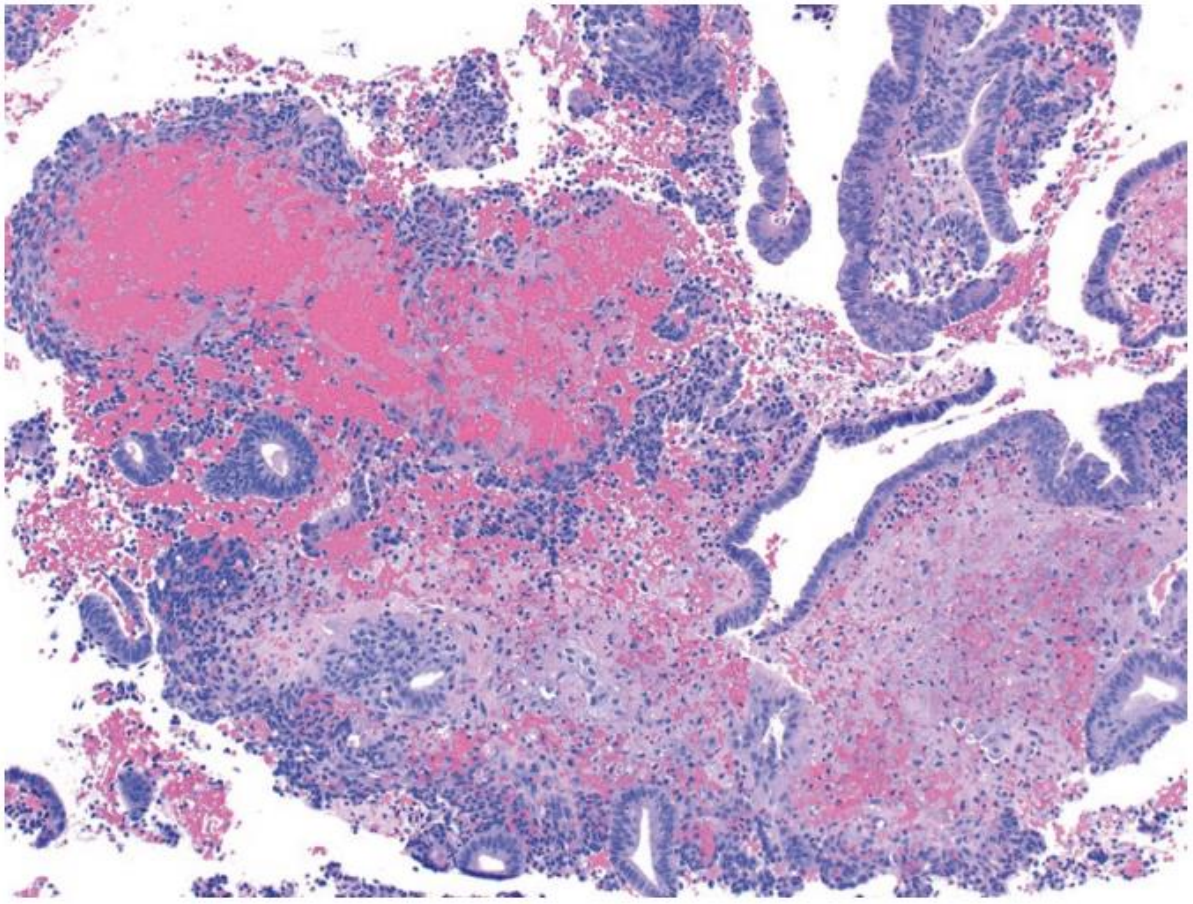


Figure 9: Weakly proliferative endometrium[32]

Due to ongoing estrogen stimulation, the tissue frequently exhibits changes in the epithelium induced by estrogen, known as "metaplasia," particularly affecting ciliated and eosinophilic cells. Although the degree and consistency of this vacuolization are not as noticeable as in normal early secretory glands, the glands may also exhibit localized subnuclear vacuolization in response to estrogen. These cytoplasmic alterations and subnuclear vacuoles can make it difficult to interpret the histological pattern, but they do not alter the diagnosis. Extended exposure to unopposed estrogen can also result in varying levels of hyperplasia, atypical hyperplasia, and potentially well-differentiated endometrioid carcinoma.

When there is breakdown and bleeding in proliferative endometrium, it typically indicates anovulatory cycles. However, external estrogens can produce similar symptoms, so a thorough clinical history is essential to confirm that the bleeding is indeed a result of anovulation. The differential diagnosis for the endometrium during the proliferative phase, characterized by the

breakdown of glands and stroma, encompasses conditions like inflammation, polyps, and leiomyomas. Accurate diagnosis in these situations is facilitated by identifying particular characteristics, such as the thick-walled arteries and dense stroma linked to polyps or the presence of plasma cells in chronic endometritis[32].

Progesterone-Related Bleeding

Biopsy samples from women of reproductive age and those in perimenopause sometimes exhibit unusual secretory phase patterns, which can lead to nonmenstrual breakdown and bleeding. The ovaries' synthesis of progesterone is responsible for the secretory pattern in these cases, but the stromal and glandular alterations are usually less developed than those observed in a typical late secretory endometrium[34]. No particular day in the typical luteal phase corresponds with the endometrial pattern. The stroma does not demonstrate major predecidual change, and the glands may show secretory modifications but not significant twisting or evidence of secretory depletion[35]. No particular date during the typical luteal phase corresponds with the endometrial pattern. Although there may be secretory changes in the glands, there is no discernible twisting or indication of secretory depletion, and there are no noticeable predecidual changes in the stroma[35].

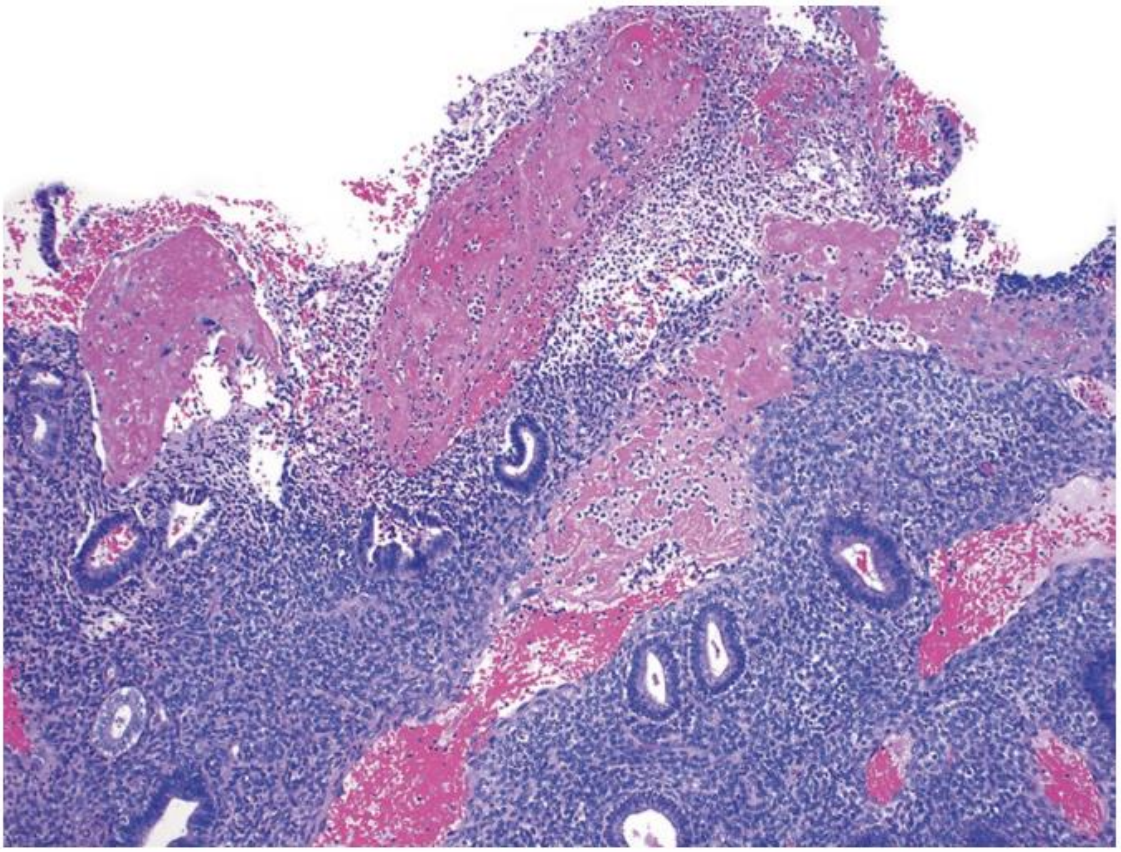


Figure 10: Abnormal secretory phase with glandular and stromal breakdown[32]

The causes and prevalence of abnormal bleeding linked to luteal phase issues are unclear, as these conditions seem to occur sporadically and do not last long enough for clinical-pathological connections to be made. Experimental studies have thoroughly documented structural changes in the endometrium resulting from fluctuations in estrogen and progesterone levels. Therefore, it is probable that irregular secretory bleeding patterns are partly due to ovulatory issues related to the luteal phase [36].

BLOOD VESSEL DENSITY IN DUB

Angiogenesis

When there is unchecked cell development, this process - which creates new blood arteries by growing capillaries from preexisting ones is crucial. This process depends on the interplay of various regulatory molecules and signaling proteins.

Numerous vital growth regulators that are necessary for angiogenesis are produced by the endometrium, such as vascular endothelial growth factor (VEGF) and epidermal growth factor

(EGF). A careful balance between angiogenic factors (growth factors) and their interactions with the extracellular matrix (ECM) is necessary for the process of angiogenesis [37]. The ECM serves as a storage site for substances that encourage angiogenesis while also housing numerous inhibitors of angiogenesis [37].

Angiogenesis and DUB

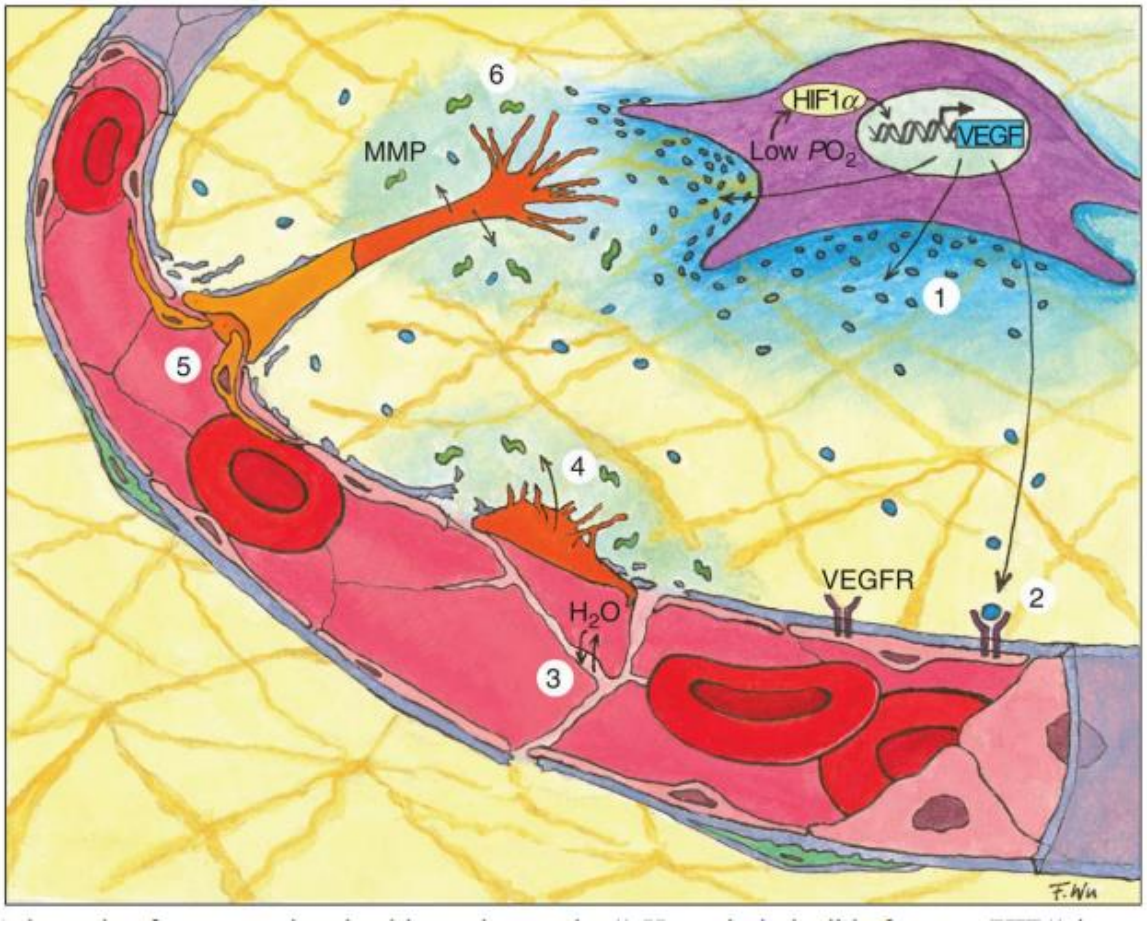


Figure 11: Schematic of processes involved in angiogenesis[38]

Hypoxia inducible factor 1-α

The α and β subunits that make up the heterodimer HIF-1 are both members of the growing family of basic helix-loop-helix (bHLH) transcription factors[39].

HIF 1α functions and their expression in endometrium

In the uterus, HIF and a number of its primary target genes have been found. Studies reveal that the endometrium contains both HIF1α and HIF1β, and that the expression levels of these proteins vary during the menstrual cycle[40]. Notably, HIF1α is most highly expressed in the endometrial

layers that are discarded during menstruation, particularly during the perimenstrual phase. L. Erichaung and associates demonstrated that an oxygen-dependent degradation (ODD) domain in HIF-1α controls its instability, and that the ubiquitin-proteasome pathway is responsible for its degradation[40]. When the entire ODD domain was removed, HIF-1α became stable even in the presence of oxygen, leading to autonomous heterodimerization, DNA binding, and transactivation without relying on hypoxia signaling [40].

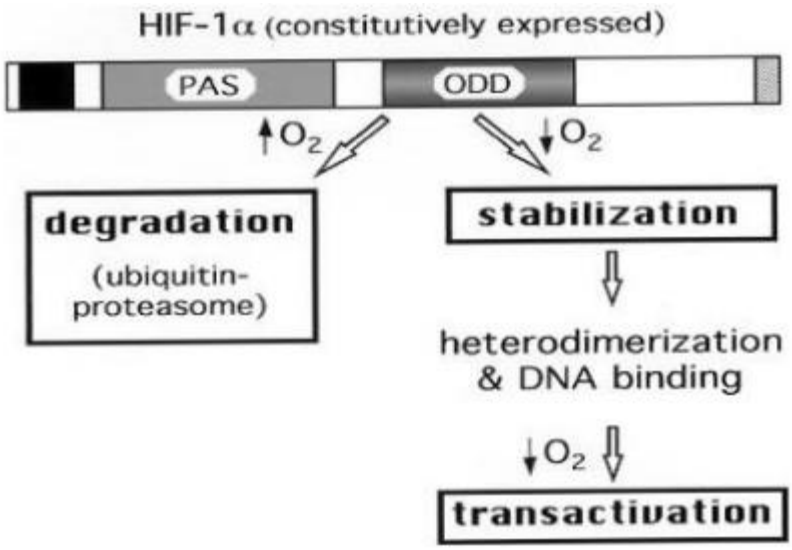


Figure 12: Model for hypoxia induced activation of HIF-1[38]

Matrix Metalloproteinases (MMPs) expression in endometrium [40]

Many matrix metalloproteinases (MMPs) have been identified in the human endometrium, with the majority showing higher expression during menstruation [40]. Specifically, collagenase-1 (MMP-1) and stromelysin-1 (MMP-3) and -2 (MMP-10) were predominantly expressed in the menstrual phase, while gelatinases A (MMP-2) and B (MMP-9) were present throughout the menstrual cycle but were more prevalent during menstruation [40]. Research using in situ hybridization and Northern blotting showed that MMP-1, MMP-3, and MMP-10 expression was mostly restricted to the perimenstrual phase[40].

MMP-1 and MMP-9 were specifically expressed in the functionalis layer, which was later eliminated. The involvement of MMPs, especially MMP-1 and MMP-9, in breaking down the endometrial matrix was strongly indicated by the regulated expression, secretion, and activation of

these enzymes by sex hormones. Throughout menstruation, MMP-9 messengerRNA was only found in the human endometrium; it was not present throughout the proliferative, secretory, or late secretory stages of the normal menstrual cycle[41].

Role of IHC in DUB

The Estrogen Receptor (ER) and Progesterone Receptor (PR) are two distinct intracellular hormone receptors that ovarian steroid hormones connect to in order to produce their effects[42]. These receptors can be found in the epithelial and mesenchymal cells of the endometrium [42]. ER and PR are part of the nuclear hormone receptor superfamily, through which estrogen and progesterone exert their effects via both cytoplasmic and membrane-bound receptors [42]. The PR receptor has two variations, PR A and PR B, and the ER has two main types, ER-α and ER-β [42]. IHC is advantageous due to its ability to localize hormones within tissues, helping to evaluate the distribution and intensity in both glandular and stromal cells. It can serve as a valuable tool when combined with pelvic ultrasound and histopathological analysis of endometrial biopsies for managing dysfunctional uterine bleeding (DUB) in women of reproductive age [43].

Kavitha M et al, studied Estrogen and Progesterone receptor expression in 50 DUB patients, in which they concluded that hyperplasia without atypia showed maximum expression of ER and PR and minimum expression in atypical hyperplasia [44].

Ahemed M. Mostafa et al, studied Estrogen and Progesterone receptor expression in 60 DUB patients, which exhibited a notable increase in Estrogen alpha and Progesterone expression in the glandular component when compared to stromal component [45].

Jayanti Mala et al, studied Estrogen and Progesterone receptor expression in 266 DUB patients and observed mean Alred scoring of estrogen receptors and progesterone receptors in endometrial glands was highest among neoplastic cases and showed significant correlation. They also observed average blood vessel density higher among hyperplasia without atypia followed by polyp, endometrial carcinoma and atypical hyperplasia respectively [46].

Pradyot singh et al, analyzed estrogen and progesterone receptor expression in 266 DUB patients out of which observed highest average Alred scoring in neoplastic cases such as Endometrial carcinoma (7±0, 7.67±2.92) followed by Hyperplasia without atypia (6.33±0.58, 7.67±2.92) respectively[47].

Aparna Bhattacharya et al examined the expression of the estrogen and progesterone receptors in 50 DUB patients, selecting 25 cases of abnormal uterine bleeding caused by uterine fibroids as disease controls and another 25 cases as non-disease controls who had no prior history of abnormal bleeding. They found that strong progesterone receptor positivity was present in 70% of DUB cases. In 75% of the fibroid cases, there was a strong positive result for estrogen[48].

Dr.V. Kalyan Chakravarthy et.al conducted a study on Estrogen and progesterone receptor expression in 60 clinically diagnosed DUB patients. They observed that out of all histopathological pattern, proliferative phase is seen in 40% followed by disordered proliferative endometrium in 13.3% and concluding higher ER and PR levels in endometrial hyperplasia & endometrial carcinoma compared to disordered endometrium [49].

Siva Kaliyamoorthy et al studied Estrogen and progesterone receptor expression in 100 case

samples collected from subjects of endometrial curettings and hysterectomy diagnosed as endometrial hyperplasia. They observed that DUB (75%) was the most common diagnosis in the women of reproductive age group and among the perimenopausal age group. They have compared estrogen and progesterone receptor status (H scores) both in gland and stroma with different types of hyperplasia and concluded that progesterone receptor positivity is marginally higher than the estrogen receptor status in cases of hyperplasia without atypia [50].

Dr. Vanesa John et al studied microvascular density and vascular changes in 200 endometrial samples of clinically diagnosed DUB patients, where they found variable patterns such as proliferative pattern, secretory pattern/ therapy related changes, disordered proliferative endometrium, endometrial hyperplasia and hyperplasia with atypia thereby assessing blood vessel density out of which maximum mean vessel density noted in endometrial hyperplasia with atypia [51].

Samhitha Chakraborthy et al assessed immunohistochemical expression of ER and PR in 50 endometrial samples of women presented with dysfunctional uterine bleeding. They concluded that maximum alred scoring of ER and PR is noted in endometrial hyperplasia without atypia followed by disordered endometrium when compared to control group [52].

Brijesh Thakur et al. [53] studied Estrogen and progesterone receptor expression in hundred and fifty endometrial samples of patients with DUB and infertility out of which 107 were DUB cases and 23 were infertility cases. The secretory phase endometrial accounted for the largest percentage of DUB cases (36.4%) out of 107 instances, followed by disordered endometrium (26.2%) and proliferative phase (18.7%). Out of these cases they have concluded that HPR diagnosis with proliferative phase showed average PR expression was 84.0% HPR diagnosis with secretory phase showed average expression of 63.13% and mean total ER expression in cases of DUB was high in the proliferative phase (86.50%) while in the secretory phase was 59.68%[53].

MATERIAL AND METHODS

Source of data

Patients reporting with complaints of DUB to "Department of Obstetrics and Gynecology" in "Shri B. M. Patil Medical College, Hospital and Research Centre", Vijayapura

Study period: May, 2023 to December 2024

Methods of Data Collection

Study Design: Hospital based cross - sectional study

Study Population: All patients reporting with complaint of DUB

Sample Size: Using G*Power ver. 3.1.9.4 software for sample size calculation, The optical staining intensity for Control group (Mean=85.97, SD=6.51) and DUB group (Mean=92.73, SD=10.52), this study requires a sample size of 82(DUB group - 60, control group - 22).

Therefore, in order to detect a difference with a 93% power, T-tests Means: The difference, at the 5% level of significance, between two independent means (two groups).

Selection Criteria:

Inclusion Criteria: Patients who have experienced menstrual irregularities such as irregular cycles and excessive or prolonged natural bleeding, along with a clinically confirmed diagnosis of Dysfunctional Uterine Bleeding (DUB).

Exclusion Criteria: Individuals who are taking oral contraceptives, intrauterine contraceptives, hormone replacement therapy, or long-term aspirin medication. Women who have a history of bleeding disorders (shown by abnormal PT levels) or thyroid dysfunction (shown by high TSH levels) were excluded from the study .

Data Collection Procedure:

- The institutional ethical clearance certificate was taken.

- Study purpose was explained to the patient & consent was taken.
- Detailed history was elicited.
- The study incorporated cases of biopsies/curettage / TAH& BSO specimen of DUB patients which were received in the Department of Pathology.
- Complete history, menstrual history, physical examination findings (general, per abdomen, per vaginal, per speculum) and ultrasound findings were noted.
- The material underwent standard processing and was preserved in 10% formalin. The majority of the appropriate tissue block was used to create sections that were 4 microns thick. Hematoxylin and Eosin was used to stain one segment in order to determine the blood vessel density and make a morphologic diagnosis. Two sections were mounted on poly L lysine coated slides, which was subjected to ER alpha and PR immunohistochemical staining.

STATISTICAL ANALYSIS:

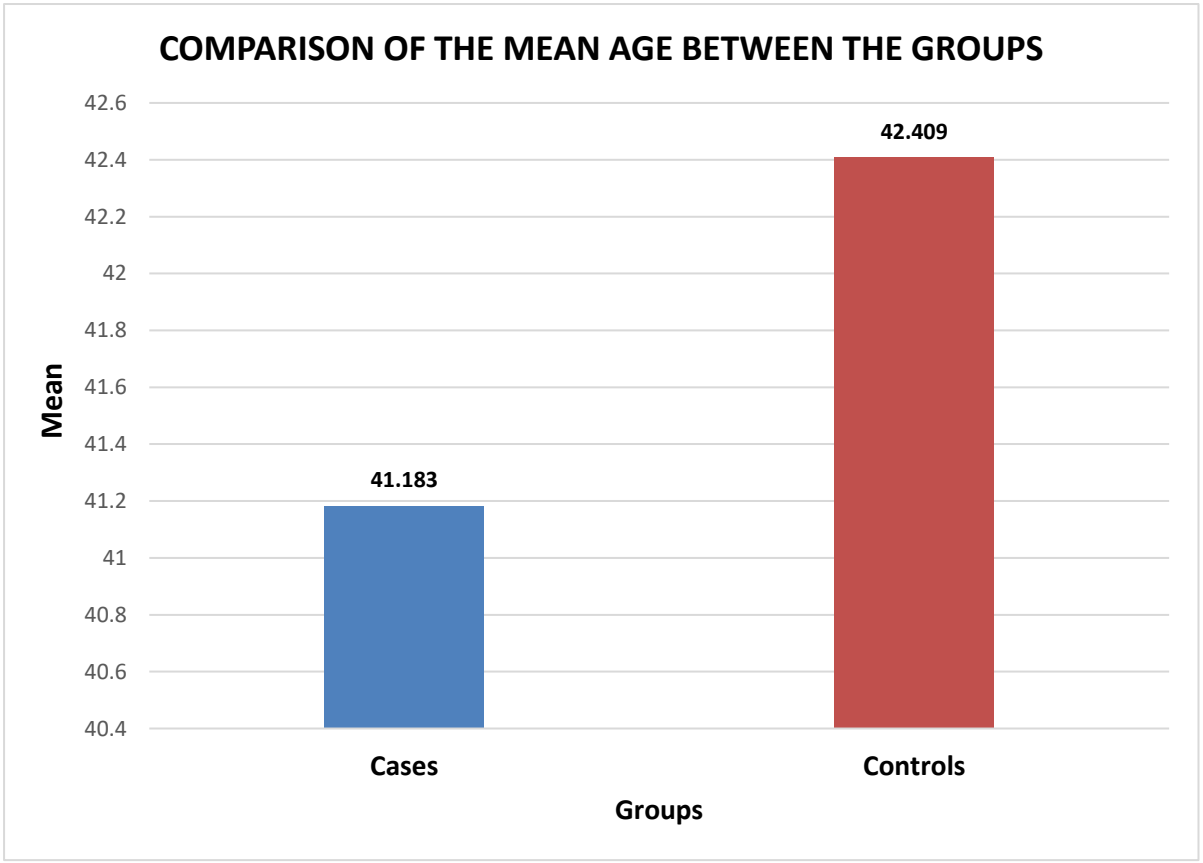
The data obtained was entered in a Microsoft Excel sheet, and statistical analyses were performed using a "Statistical package for the social sciences (SPSS) (Version 20)". Results were presented as Mean, SD, counts and percentages, and diagrams. For normally distributed continuous variables between the two groups were compared using an independent sample t-test. For not normally distributed variables, the Mann-Whitney U .test was used. For categorical variables between the two groups were compared using the Chi-square test/Fishers exact test. If more than two groups we used ANOVA. For not normally distributed, Kruskal-Walli H Test. "P=0.05" was considered statistically significant. All statistical analyses were conducted using a two-tailed approach.

RESULTS

The present study was conducted at the "Department of Pathology, B.L.D.E. (Deemed to be University)", "Shri B.M. Patil Medical College, Hospital & Research Centre, Vijayapura, Karnataka". In this study, we analyzed the distribution of ER and PR receptor expression through Immunohistochemistry among tests and control groups. Also we evaluated blood vessel density done on H&E slides.

TABLE 1: USING THE INDEPENDENT SAMPLE T TEST, COMPARISON OF THE MEAN AGE OF THE GROUPS.

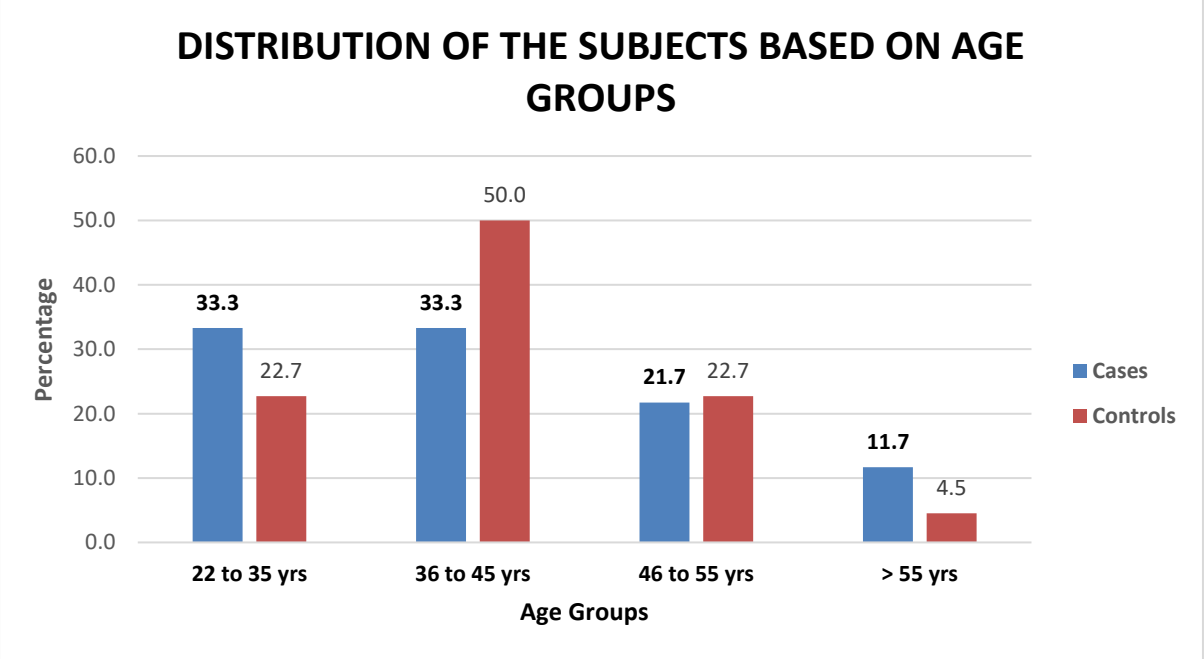
Groups	N	Minimum	Maximum	Mean	S.D	Mean diff	p value
Cases	60	22.0	74.0	41.183	10.8526	-1.22	0.628
Controls	22	29.0	56.0	42.409	7.6945		



The mean age of the patients was 41.18 ± 10.85 years in the test group and in the control group was 42.40 ± 7.69 years. Using independent sample t test, the comparison of mean age between the groups was statistically not significant. (p=0.62)

TABLE 2: SUBJECTS' DISTRIBUTION ACCORDING TO AGE GROUPS.

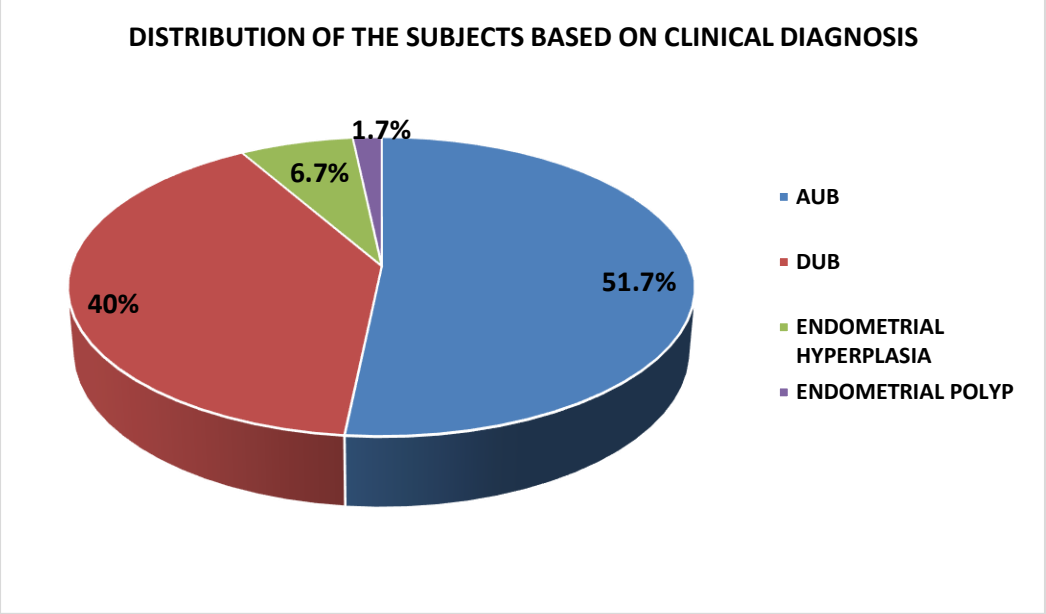
Age Groups		Groups		Total
		Cases	Controls	
22 to 35 yrs	Count	20	5	25
	%	33.3%	22.7%	30.5%
36 to 45 yrs	Count	20	11	31
	%	33.3%	50.0%	37.8%
46 to 55 yrs	Count	13	5	18
	%	21.7%	22.7%	22.0%
> 55 yrs	Count	7	1	8
	%	11.7%	4.5%	9.8%
Total	Count	60	22	82
	%	100.0%	100.0%	100.0%
Chi-square value-2.62				



Out of 82 patients, **25 patients** (30.5%) were in the age group of 22 to 35 years, of which, 20 (33.3%) were cases and 5 (22.7%) were controls. **31 patients** (37.8%) were in the age group of 36 to 45 years, of which, 20 (33.3%) were cases and 11 (50%) were controls. **18 patients** (22%) were in the age group of 46 to 55 years of which, 13 (21.7%) were cases and 5 (22.7%) were control groups. 8 patients (9.8%) were in the age group of above 55 years, of which, 7 (11.7%) were cases and 1 (4.5%) was a control.

TABLE 3: DISTRIBUTION OF THE SUBJECTS BASED ON CLINICAL DIAGNOSIS

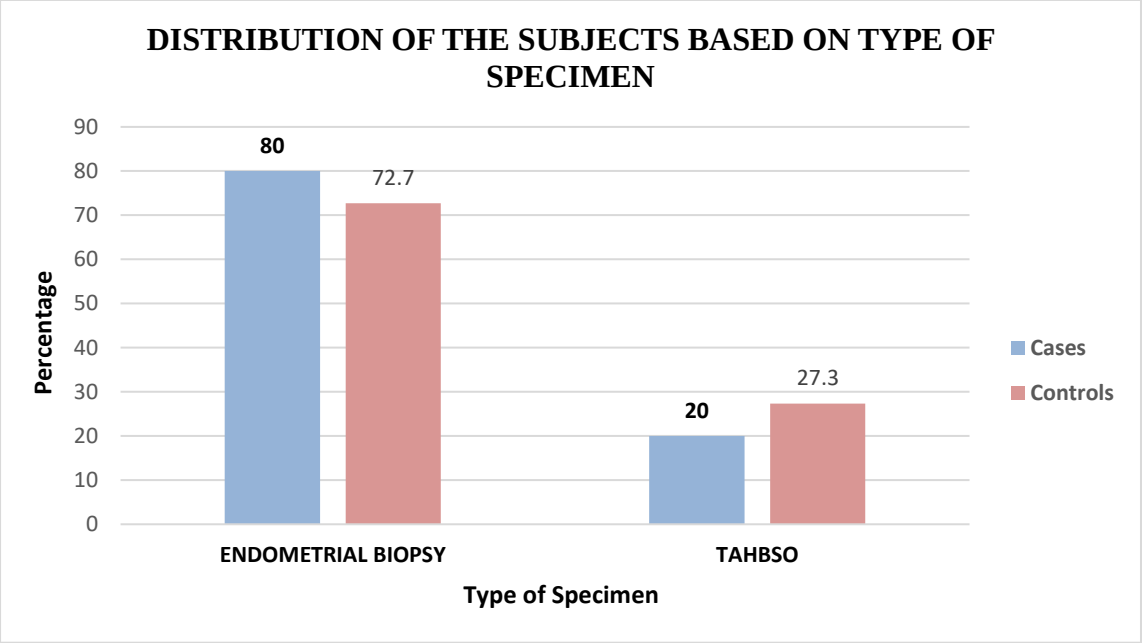
CLINICAL DIAGNOSIS	Cases		Total
	Count	%	
AUB	31	51.7%	31
DUB	24	40.0%	24
ENDOMETRIAL HYPERPLASIA	4	6.7%	4
ENDOMETRIAL POLYP	1	1.7%	1
Total	60	100.0%	60



Clinical diagnosis was AUB in 31 patients (51.7%) followed by DUB in 24 patients (40%), endometrial hyperplasia in 4 patients (6.7%) and 1 patient (1.7%) was diagnosed with an endometrial polyp.

TABLE 4: DISTRIBUTION OF THE SUBJECTS BASED ON TYPE OF SPECIMEN

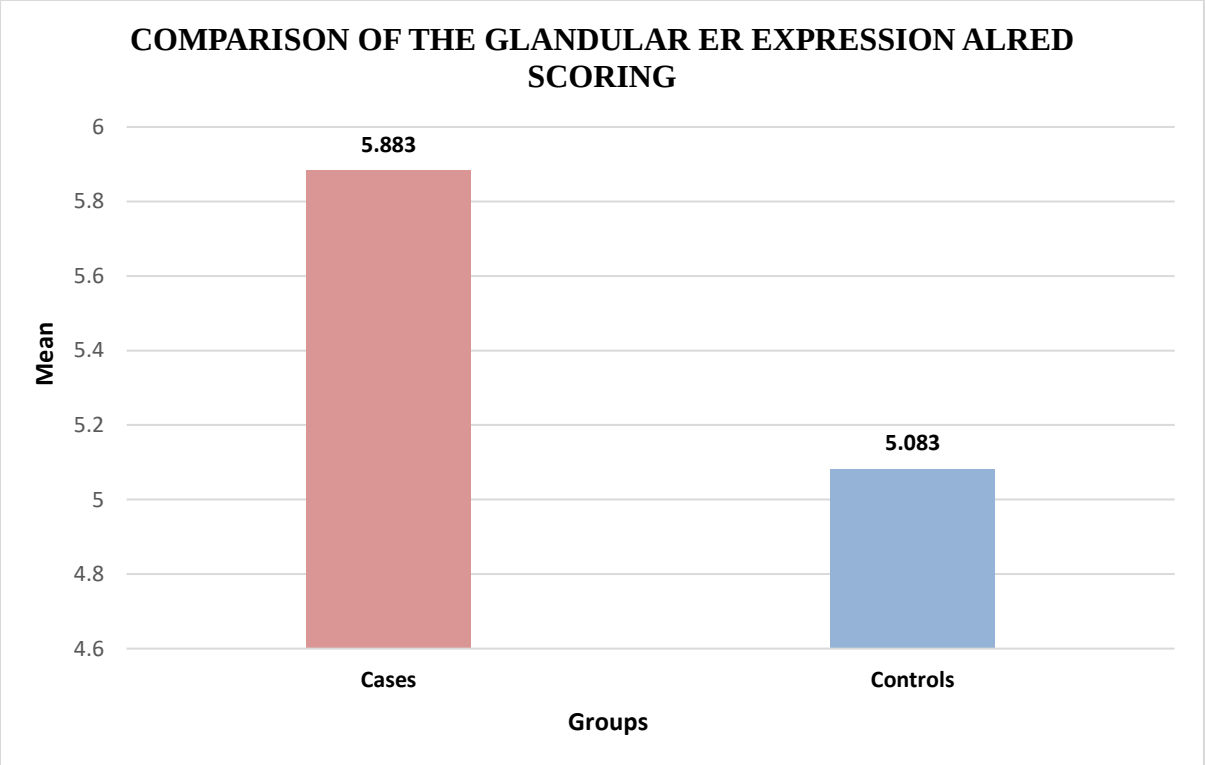
TYPE OF SPECIMEN		Groups		Total
		Cases	Controls	
ENDOMETRIAL BIOPSY	Count	48	16	64
	%	80%	72.7%	76.8%
TAHBSO	Count	12	6	18
	%	20.0%	27.3%	22.0%
Total	Count	60	22	82
	%	100.0%	100.0%	100.0%
Chi-square value- 0.82				



Type of specimen was endometrial biopsy in 63 patients (76.8%) of which, 48 (80%) were cases and 16 (72.7%) were controls. Type of specimen was TAHBSO in 18 patients (22%), of which, 12 (20%) were cases and 6 (27.3%) were controls.

TABLE 5: COMPARISON OF THE GLANDULAR ER EXPRESSION ALRED SCORING USING INDEPENDENT SAMPLE T TEST

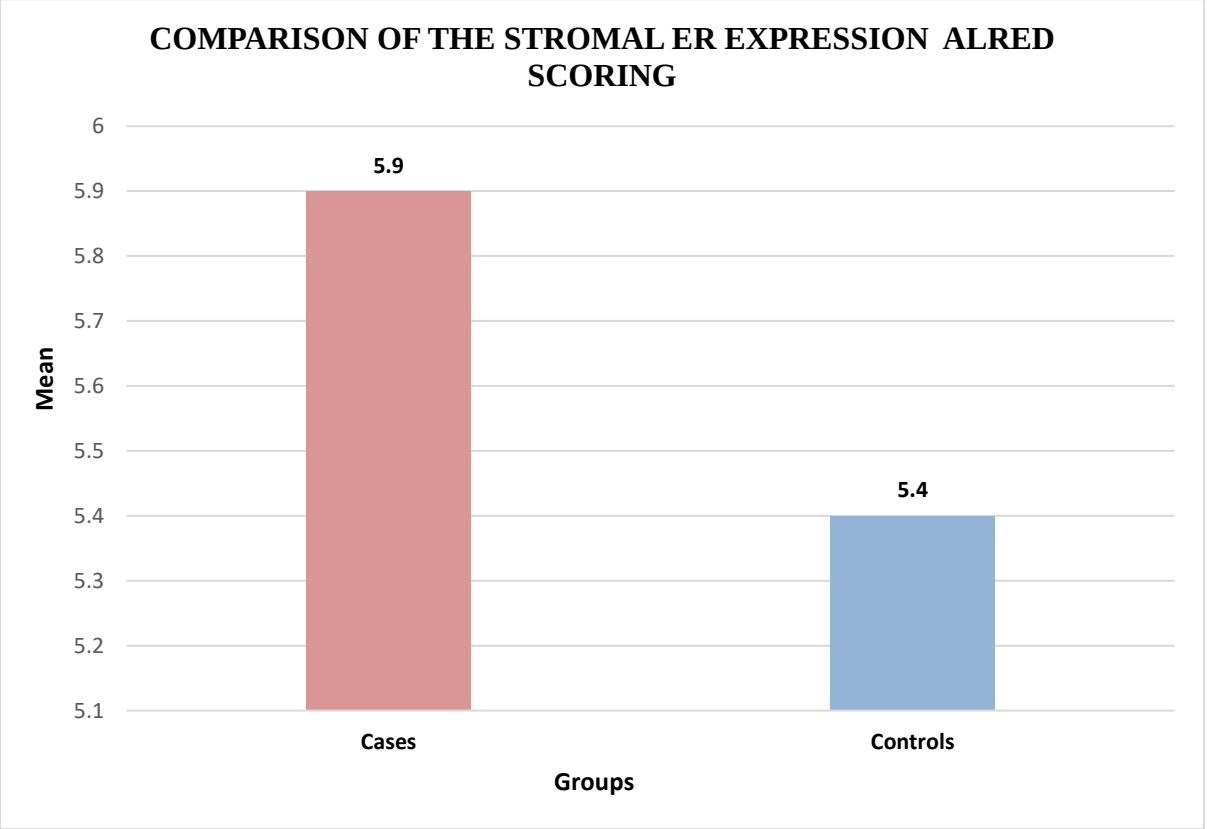
Glandular ER expression Alred scoring	N	Minimum	Maximum	Mean	S.D	Mean diff	p value
Cases	60	3.0	8.0	5.883	1.1802	0.800	0.030*
Controls	22	2.0	7.0	5.083	1.2105		



Using independent sample t test, mean glandular ER expression Alred scoring in cases group was 5.88 ± 1.18 and in control group was 5.08 ± 1.21. The comparison of glandular ER expression Alred scoring was statistically significant. (p=0.03)

TABLE 6: COMPARISON OF THE STROMAL ER EXPRESSION ALRED SCORING USING INDEPENDENT SAMPLE T TEST

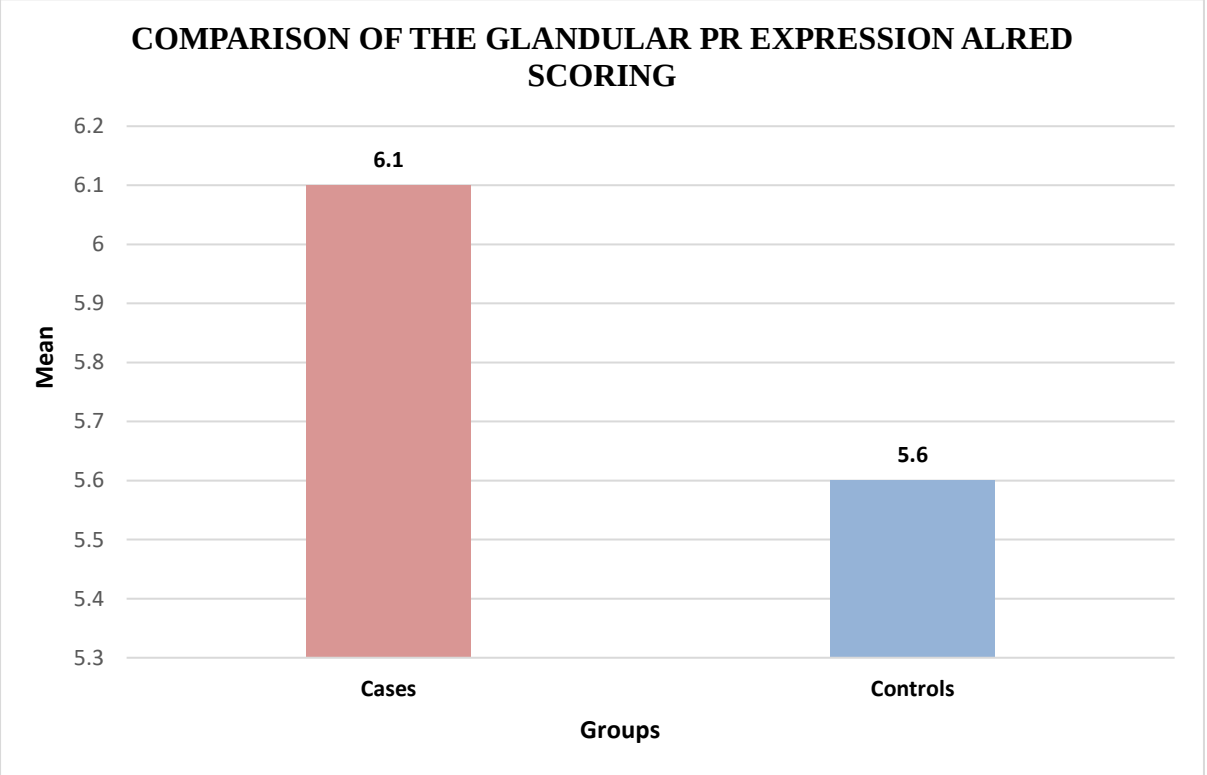
Stromal ER expression Alred scoring	N	Minimum	Maximum	Mean	S.D	Mean diff	p value
Cases	60	3.0	7.0	5.900	1.0813	0.500	0.040*
Controls	22	2.0	7.0	5.400	1.1843		



Using independent sample t test, mean stromal ER expression Alred scoring in cases group was 5.90 ± 1.08 and in control group was 5.40 ± 1.18. The comparison of ER expression stromal Alread scoring was statistically significant. (p=0.04)

TABLE 7: COMPARISON OF THE GLANDULAR PR EXPRESSION ALRED SCORING USING INDEPENDENT SAMPLE T TEST

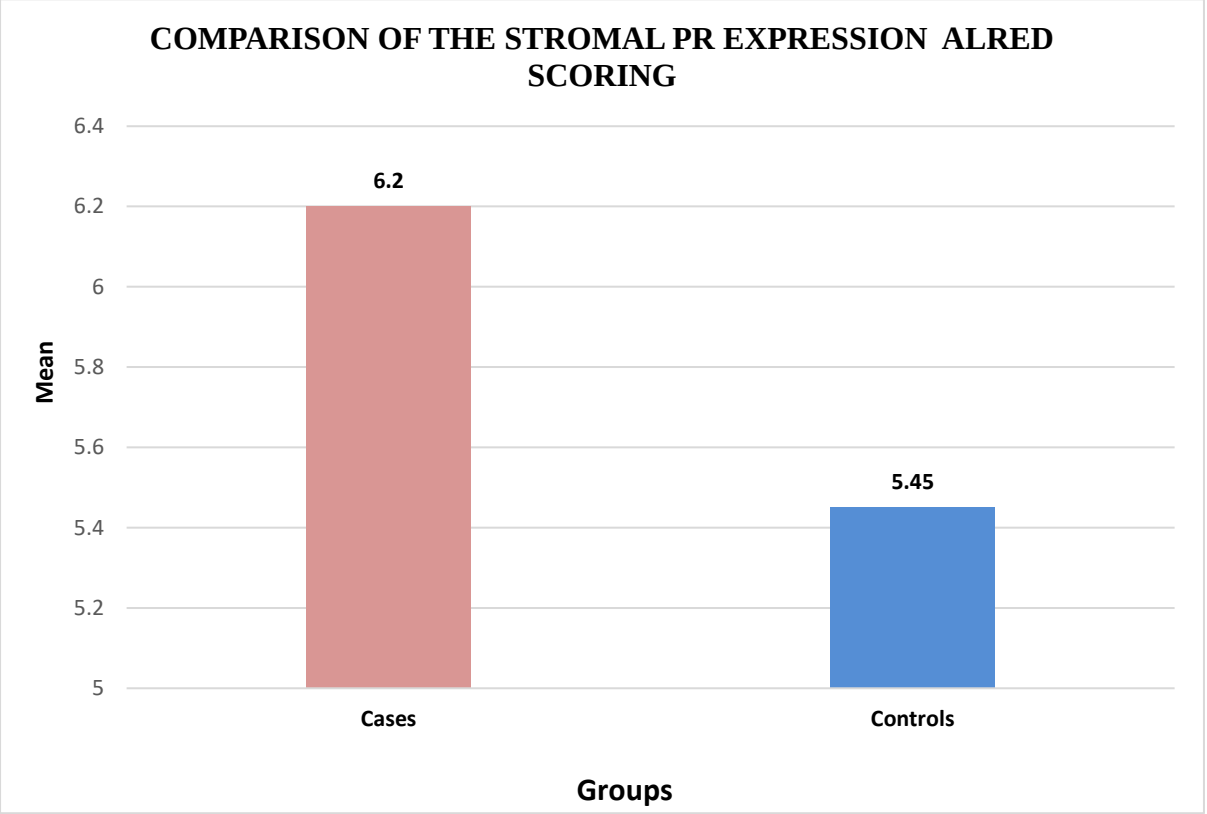
Glandular PR expression Alred scoring	N	Minimum	Maximum	Mean	S.D	Mean diff	p value
Cases	60	3.0	8.0	6.100	1.5835	0.500	0.025*
Controls	22	3.0	7.0	5.600	1.3159		



Using independent sample t test, mean glandular PR expression Alred scoring in cases group was 6.10 ± 1.58 and in control group was 5.60 ± 1.31. The comparison of PR expression glandular Alread scoring was statistically significant. (p=0.02)

TABLE 8: COMPARISON OF THE STROMAL PR EXPRESSION ALRED SCORING USING INDEPENDENT SAMPLE T TEST

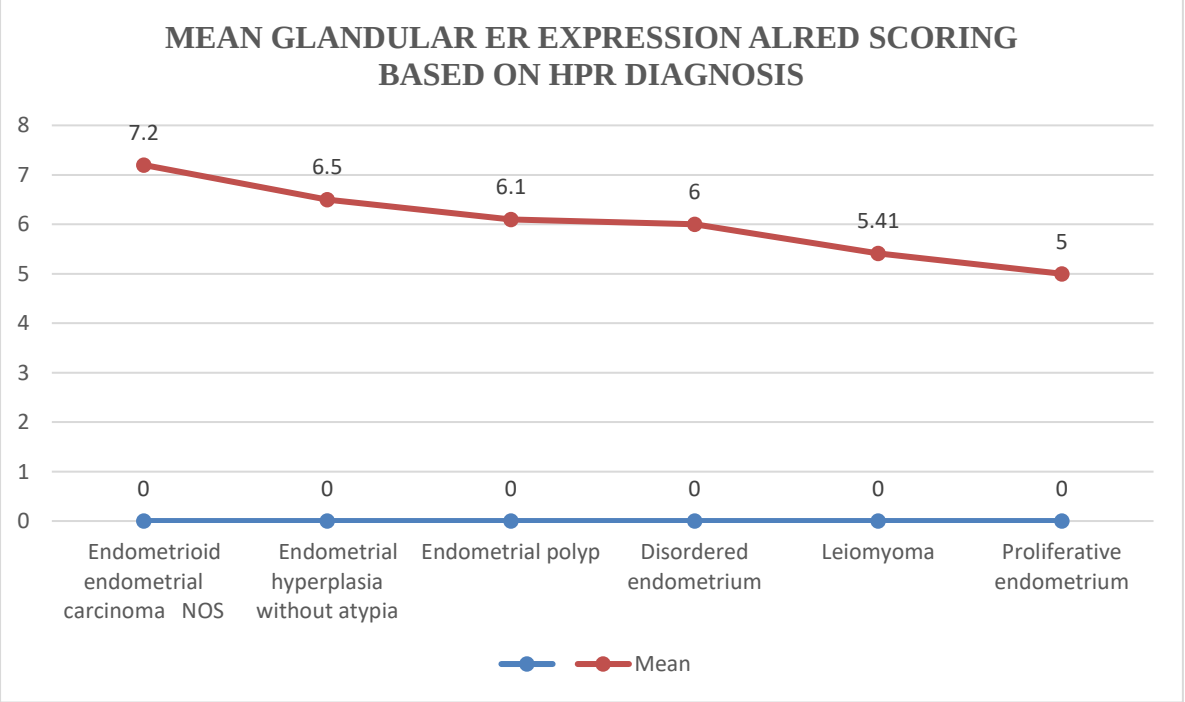
Stromal PR expression Alred scoring	N	Minimum	Maximum	Mean	S.D	Mean diff	p value
Cases	60	3.0	8.0	6.200	1.3339	0.750	0.022*
Controls	22	3.0	8.0	5.450	1.3683		



Using independent sample t test, mean stromal PR expression Alred scoring in cases group was 6.2 ± 1.33 and in control group was 5.40 ± 1.36. The comparison of PR expression stromal Alred scoring was statistically significant. (p=0.02)

TABLE 9: MEAN GLANDULAR ER EXPRESSION ALRED SCORING BASED ON HPR DIAGNOSIS

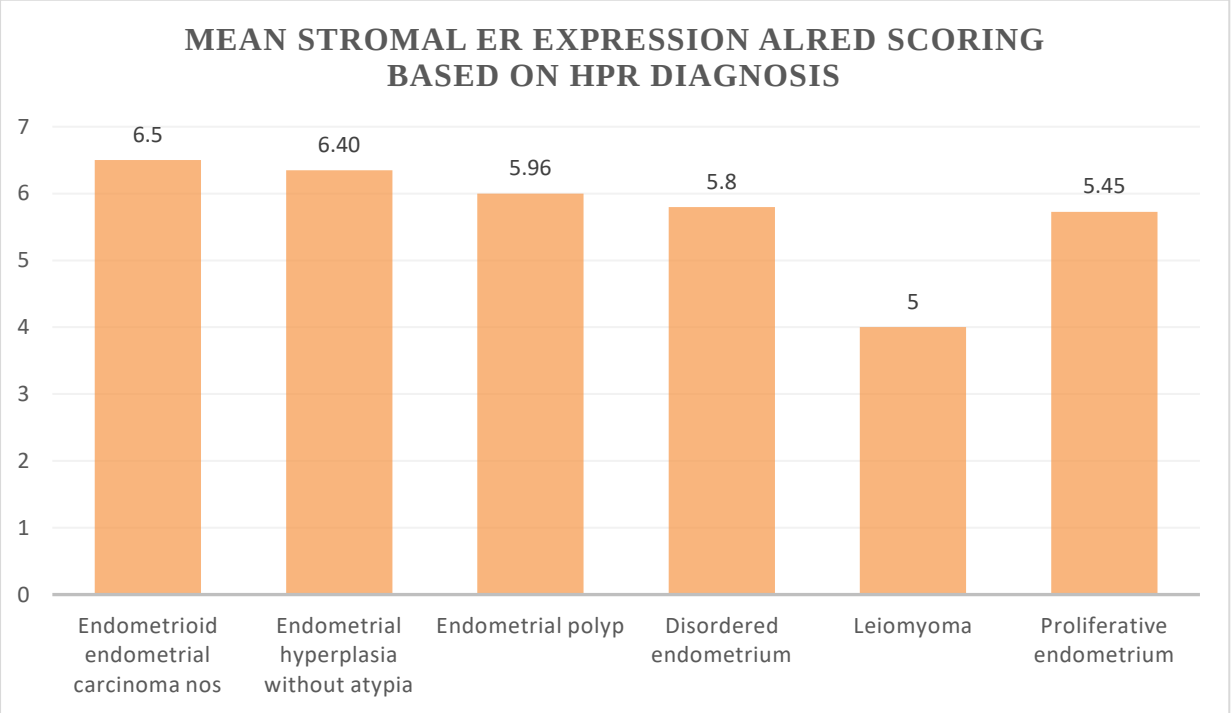
HPR DIAGNOSIS	N	Minimum	Maximum	Mean	S.D
Endometrioid endometrial carcinoma NOS	3	5.0	6.0	6.20	0.58
Endometrial hyperplasia without atypia	8	3.0	8.0	6.17	1.15
Endometrial polyp	19	4.0	7.0	6.10	1.16
Disordered endometrium	29	3.0	8.0	6.00	1.28
Leiomyoma	1	5.0	5.0	5.00	-
Proliferative endometrium	22	2.0	7.0	5.32	1.21



Mean glandular ER expression Alred scoring in Endometrioid Endometrial carcinoma NOS was 6.20 ± 0.58, Endometrial hyperplasia without atypia was 6.17 ± 1.15, benign endometrial polyp was 6.10 ± 1.16. In disordered endometrium mean ER expression was 6.00 ± 1.28. In leiomyoma, mean ER expression was 5.00 ± 0.00. In proliferative endometrium, mean ER expression was 5.32 ± 1.21.

TABLE 10: MEAN STROMAL ER EXPRESSION ALRED SCORING BASED ON HPR DIAGNOSIS

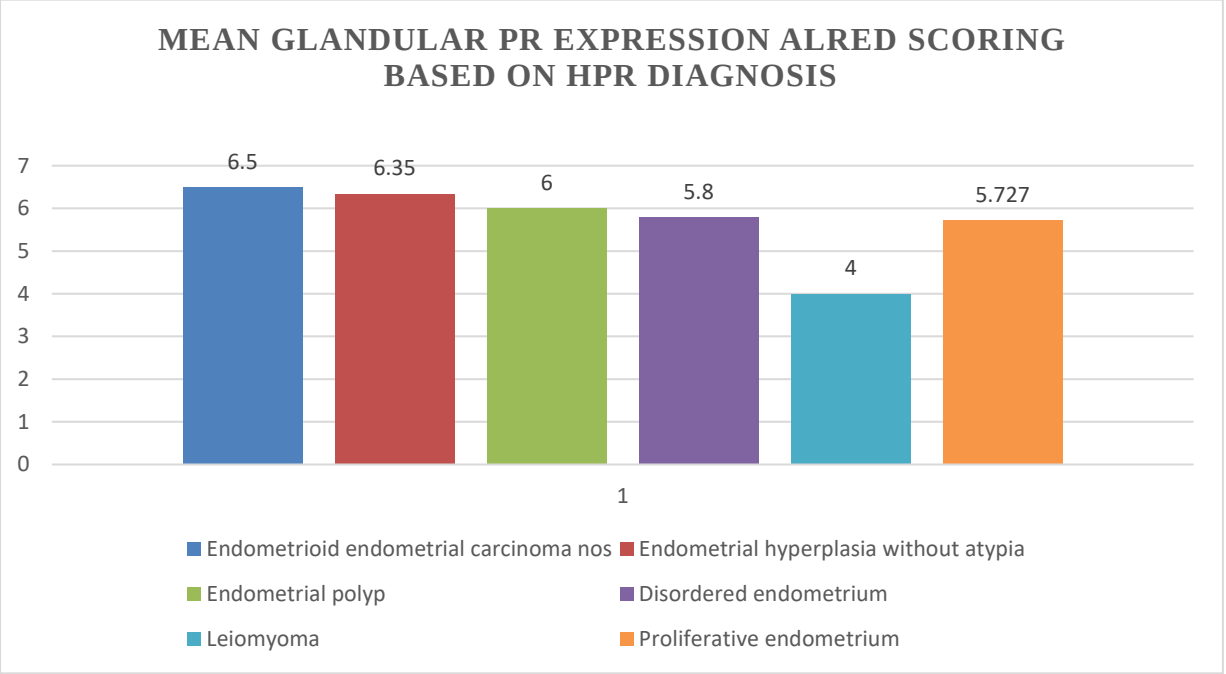
HPR DIAGNOSIS	N	Minimum	Maximum	Mean	S.D
Endometrioid endometrial carcinoma nos	3	5.0	7.0	6.50	1.00
Endometrial hyperplasia without atypia	8	4.0	7.0	6.40	0.9910
Endometrial polyp	18	3.0	7.0	5.967	1.2217
Disordered endometrium	29	3.0	7.0	5.80	.9994
Leiomyoma	1	5.0	5.0	5.000	-
Proliferative endometrium	22	2.0	7.0	5.545	1.1843



Mean stromal ER expression Alred scoring in Endometrioid Endometrial carcinoma NOS, mean ER expression was 6.50 ± 1.00, endometrial hyperplasia without atypia was 6.40± 0.99, benign endometrial polyp was 5.96 ± 1.22. In disordered endometrium mean ER expression was 5.80 ± 0.99, leiomyoma, mean ER expression was 5.00 ± 0.00. In proliferative endometrium, mean ER expression was 5.54 ± 1.18

TABLE 11: MEAN GLANDULAR PR EXPRESSION ALRED SCORING BASED ON HPR DIAGNOSIS

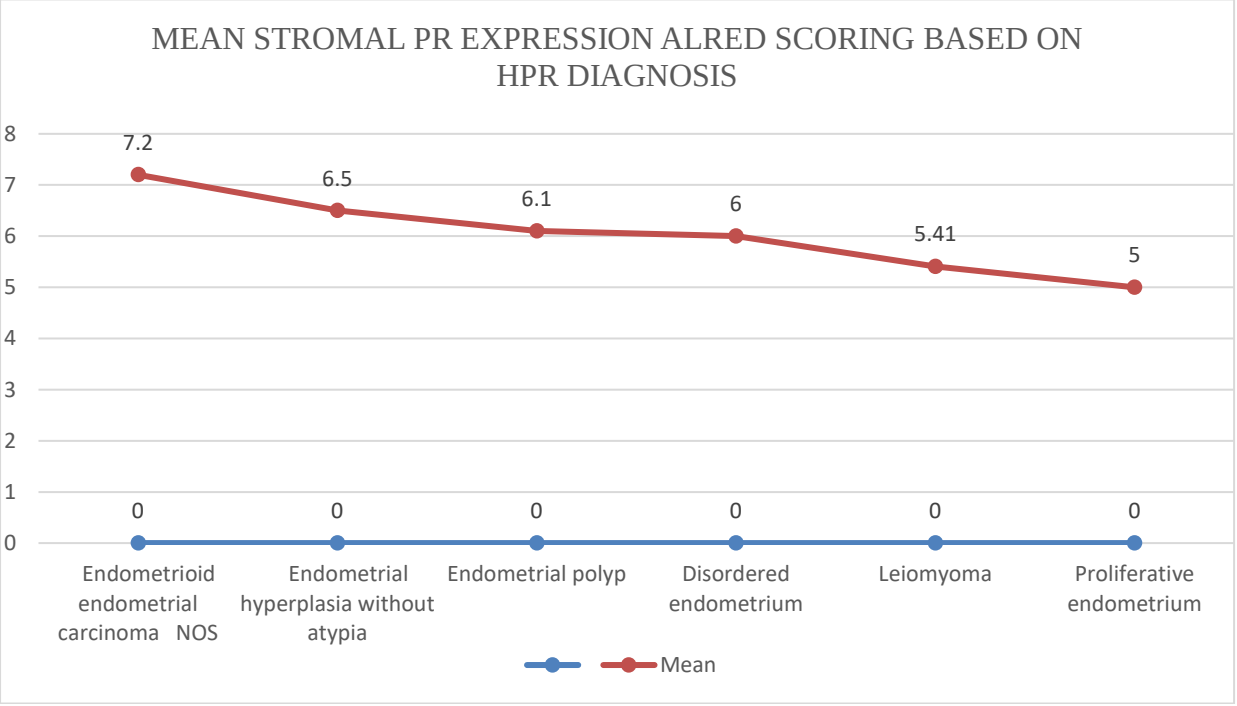
HPR DIAGNOSIS	N	Minimum	Maximum	Mean	S.D
Endometrioid Endometrial Carcinoma nos	3	5.0	8.0	6.50	1.1547
Endometrial hyperplasia without atypia	8	4.0	8.0	6.35	1.6421
Endometrial polyp	18	3.0	8.0	6.00	1.8068
Disordered endometrium	29	3.0	8.0	5.80	1.3476
Leiomyoma	1	4.0	4.0	4.000	-
Proliferative endometrium	22	3.0	7.0	5.727	1.3159



Mean glandular PR expression Alred scoring in Endometrioid Endometrial carcinoma NOS, mean PR expression was 6.50 ±1.15, endometrial hyperplasia without atypia was 6.35 ± 1.64, benign endometrial polyp was 6.00 ± 1.80. Disordered endometrium mean PR expression was 5.80 ± 1.34. In leiomyoma, mean PR expression was 4.00 ± 0.00 and in proliferative endometrium, mean PR expression was 5.72 ± 1.31.

TABLE 12: MEAN STROMAL PR EXPRESSION ALRED SCORING BASED ON HPR DIAGNOSIS

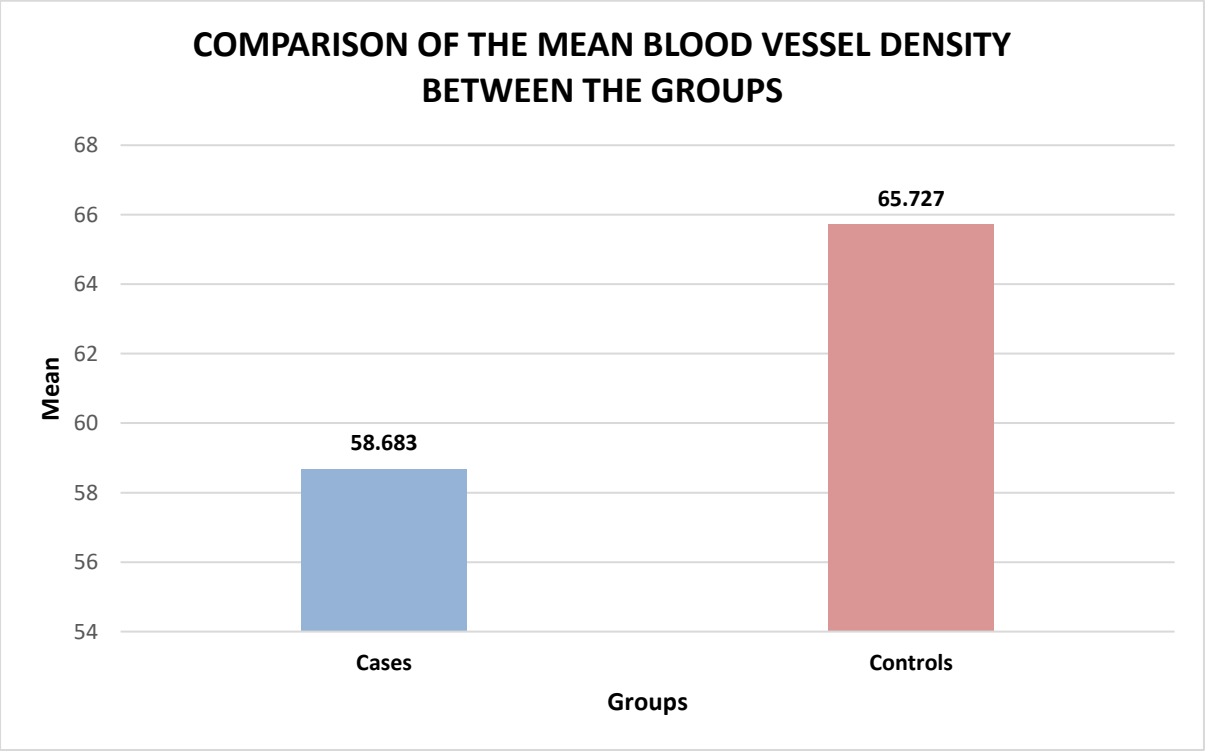
HPR DIAGNOSIS	N	Minimum	Maximum	Mean	S.D
Endometrioid endometrial carcinoma nos	3	5.0	8.0	7.20	1.00
Endometrial hyperplasia without atypia	8	5.0	7.0	6.50	0.99
Endometrial polyp	18	3.0	8.0	6.10	1.41
Disordered endometrium	29	3.0	7.0	6.00	1.30
Leiomyoma	1	3.0	8.0	5.41	-
Proliferative endometrium	22	5.0	5.0	5.00	1.37



Mean stromal PR expression Alred scoring in Endometrioid Endometrial carcinoma NOS, mean PR expression was 7.20 ± 1.00, endometrial hyperplasia without atypia was 6.50 ± 0.99, Benign endometrial polyp was 6.10± 1.41. In Disordered endometrium mean PR expression was 6.00± 1.30, leiomyoma was 5.41 ± 0.00. In proliferative endometrium, mean PR expression was 5.00 ± 1.37.

TABLE 13: COMPARISON OF THE MEAN BLOOD VESSEL DENSITY BETWEEN THE GROUPS USING INDEPENDENT SAMPLE T TEST

Groups	N	Minimum	Maximum	Mean	S.D	Mean diff	p value
Cases	60	35.0	89.0	65.727	12.5732	-7.043	0.041*
Controls	22	38.0	94.0	58.683	16.0955		



Mean blood vessel density in the cases group was 58.68 ± 12.57 and mean blood vessel density in the control group was 65.72 ± 16.09. Using independent sample t test, the comparison of mean blood vessel density between the groups was statistically significant. (p=0.04)

TABLE 14: ASSOCIATION OF BLOOD VESSEL DENSITY AND NEOPLASTIC/ NON-NEOPLASTIC CATEGORIES

Blood Vessel Density		Neoplastic/Non neoplastic		Total
		Neoplastic	Non-neoplastic	
35 to 45/10hpf	Count	4	8	12
	%	7.8%	25.8%	14.6%
46 to 55/10hpf	Count	15	8	23
	%	29.4%	25.8%	28.0%
56 to 65/10/hpf	Count	12	10	22
	%	23.5%	32.3%	26.8%
66 to 75/10hpf	Count	6	4	10
	%	11.8%	12.9%	12.2%
76 to 85/10hpf	Count	9	1	10
	%	17.6%	3.2%	12.2%
86 to 95/10hpf	Count	5	0	5
	%	9.8%	0.0%	6.1%
Chi-square value-11.23				
p value-0.047*				

*significant

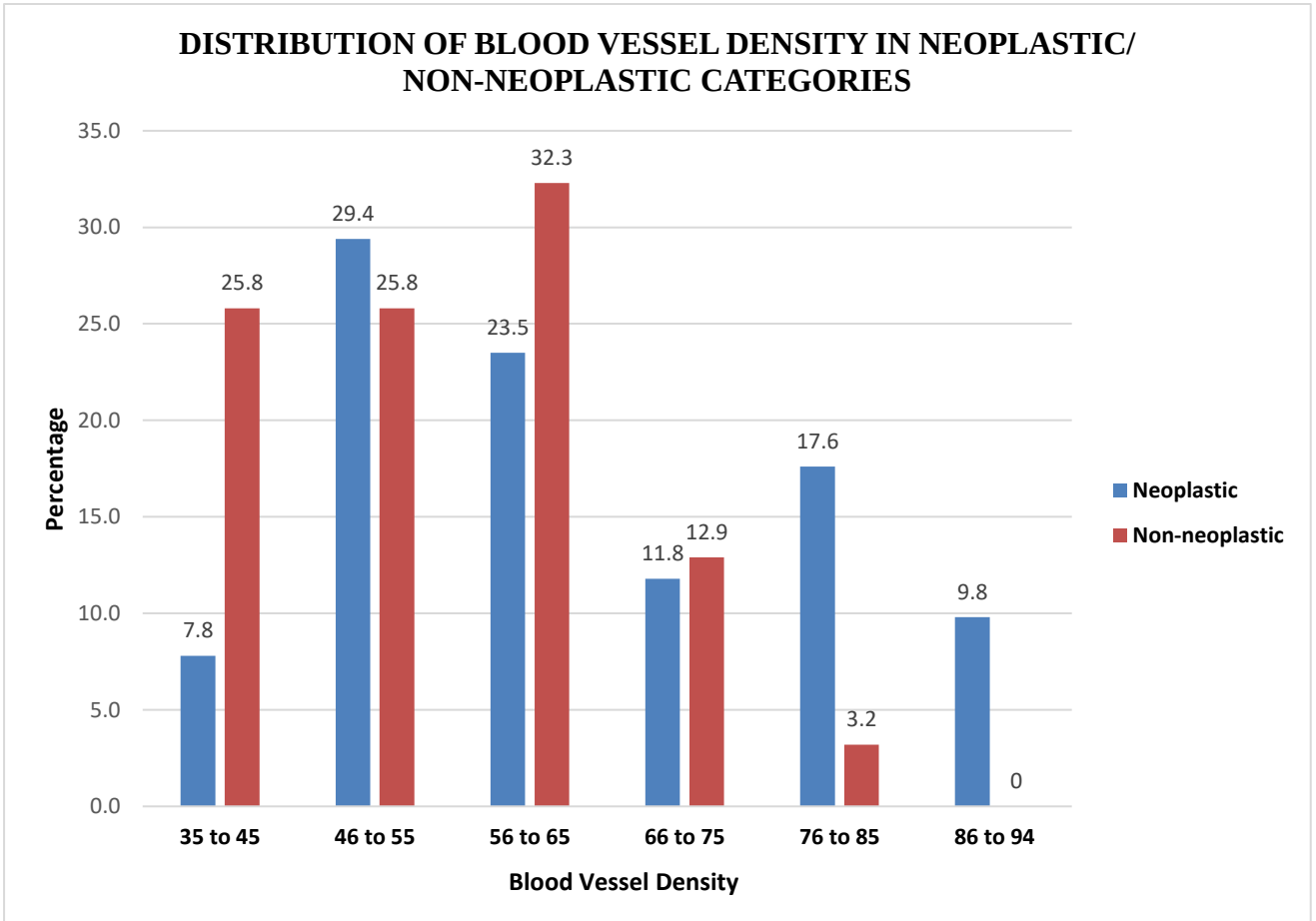
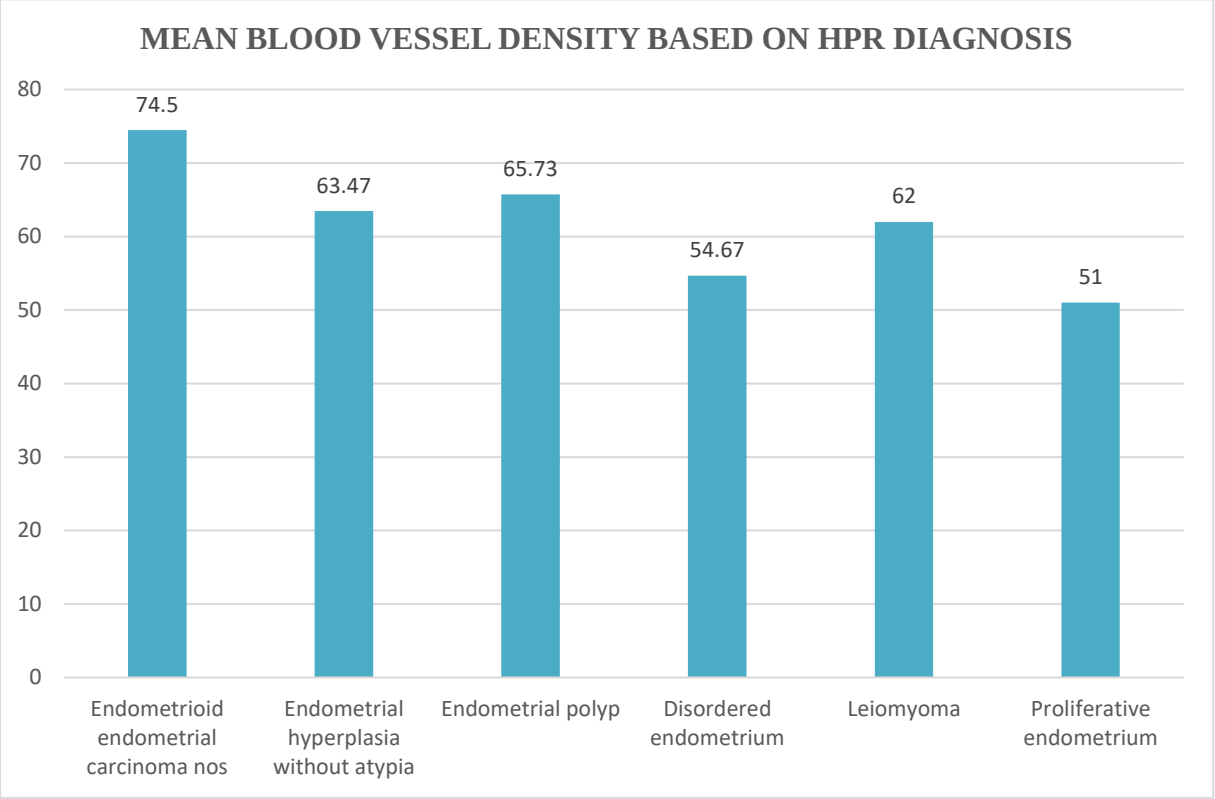


TABLE 15: MEAN BLOOD VESSEL DENSITY BASED ON HPR DIAGNOSIS

HPR DIAGNOSIS	N	Minimum	Maximum	Mean	S.D
Endometrioid endometrial carcinoma nos	3	42	65	74.5	-
Endometrial hyperplasia without atypia	8	46	89	63.47	14.48
Endometrial polyp	18	38	94	65.73	16.10
Disordered endometrium	30	35	76	54.67	10.60
Leiomyoma	1	62	62	62.00	18.01
Proliferative endometrium	22	51	51	51.00	-



Mean blood vessel density based on HPR diagnosis. Out of 60 test groups, Neoplastic lesions showed highest blood vessel density such as Endometrioid endometrial carcinoma (74.5 ± 0.00) followed by endometrial hyperplasia without atypia (63.47 ± 14.48), endometrial polyp (65.73 ± 16.10), and leiomyoma (62.0 ± 18.01). Non neoplastic lesions such as disordered endometrium showed blood vessel density of 54.67 ± 10.60, and proliferative endometrium showed 51.00 ± 0.00.

DISCUSSION

Dysfunctional Uterine Bleeding (DUB) is a significant gynaecological condition constituting 90% anovulatory cases and 10% ovulatory, affecting many women, particularly during their reproductive years and the perimenopausal phase [48]. Understanding steroid receptors in the endometrium is essential, as hormone receptors significantly influence the development and causes of DUB. Additionally, because endometrial angiogenesis and menstruation problems are positively correlated, changes in blood vessel density and morphology are also important pathophysiological factors[52].

Therefore, the current study is to analyze blood vessel density in patients presenting with dysfunctional uterine bleeding as well as the level of expression of progesterone receptors (PR) and estrogen receptor alpha (ER alpha) in the stroma and endometrial glands of females with DUB.

The mean age of the patients was 41.18 ± 10.85 years and in the control group was 42.40 ± 7.69 years. (p=0.62). Similarly, a study by Khan R et al analyzed 160 samples and observed that majority of the patients (32.5%) were in the age group of 30-39 years followed by patients between 40 to 49 years (29.1%) [54]. On the contrary, Gleeson et al in his study has analysed 110 samples and reported an age range of 28 years to 49 years as the most common group [55].

Both ovulatory and anovulatory menstrual cycles can cause DUB, while the etiology of this condition is mainly unknown. Anovulatory bleeding is a systemic illness that arises due to endocrine, neurochemical, or pharmacological processes, whereas ovulatory dysfunctional bleeding is caused by deficiencies in local endometrial hemostasis[54]. In the present study, most common presenting complaint being DUB was diagnosed in 31 patients (51.7%), followed by menorrhagia diagnosed in 24 patients (40%), oligomenorrhoea was diagnosed in 4 patients (6.7%) and 1 patient (1.7%) was diagnosed with polymenorrhagia. Similarly, in a study done by Sajitha K et al evaluated 156 endometrial samples and out of those, the most frequently reported symptom

was menorrhagia (47%), followed by oligomenorrhea (11.6%), polymenorrhagia (6.6%), and menometrorrhagia (6.6%) [54]. Similarly, another study by Singh P et al assessed 550 samples and noted that majority of patients experienced menorrhagia (84%), followed by abnormal uterine bleeding (12%) and persistent menstrual bleeding (4%) [55].

In the present study, type of specimen included endometrial biopsies and TAHBSO, out of which 64(78%) of them were endometrial biopsies, of which, 48 (75%) were cases and 16 (25%) were controls and 18(22%) subjects were TAHBSO, of which 11(61%) were cases, 7(39%) were control groups. Similarly a study by Siva Kaliyamoorthy et.al analyzed 100 samples out of which 75 of them were endometrial curettings and 25 were hysterectomy specimens.

In our study, HPR diagnosis was disordered endometrium in 30 patients (50%) which is the commonest pattern, benign endometrial polyp in 18 patients (30%), Endometrial hyperplasia without atypia in 8 patients (13.3%), endometrioid endometrial carcinoma NOS in 3 patients (5%) and leiomyoma in 1 patient (1.7%). In contrast, Sajitha K et al. examined 156 samples and found that the most prevalent histological pattern was endometrial hyperplasia in 39 patients (25%) followed by secretory endometrium in 26 patients (16.7%), proliferative and dysregulated endometrial proliferation in 19 patients (12.2%) each, and endometrial carcinoma in 7 (4.5%) cases[58].

The research of endometrial hormone-binding receptors is important for DUB because it supports the pathophysiological involvement of hormone receptors. It was suggested that an elevated concentration of ER and PR in the endometrial glandular and stromal component may amplify the effects of estrogen and progesterone in DUB, thereby contributing to the development of irregular uterine bleeding[59].

In the present study, the mean glandular ER expression Alred scoring in cases group was 5.88 ± 1.18 and in control group was 5.08 ± 1.21. Moreover, the mean stromal ER expression Alred scoring in cases group was 5.90 ± 1.08 and in control group was 5.40 ± 1.18. (p=0.04)

The mean glandular PR expression Alred scoring in cases group was 6.10 ± 1.58 and in control

group was 5.60 ± 1.31. (p=0.02) The mean stromal PR expression Alred scoring in cases group was 6.2 ± 1.33 and in control group was 5.40 ± 1.36. (p=0.02). The comparison of ER and PR expression glandular and stromal Alred scoring was statistically significant.

These findings were in concordance with the findings of Singh P et al who evaluated 600 endometrial samples and concluded that on comparison of DUB group with control group showed statistically significant difference for ERα and PR receptor showing highest expression of ER and PR in Hyperplasia without atypia followed by disordered endometrium and proliferative endometrium both in glandular and stromal components [57].

Table 16: Comparison of ER and PR expression between studies

Present study			
ER	Control	Cases	p-value
Glandular	5.88 ± 1.18	5.08 ± 1.21	p=0.03
Stromal	5.90 ± 1.08	5.88 ± 1.18	
PR			
Glandular	5.60 ± 1.31	6.10 ± 1.58	p=0.02
Stromal	5.40 ± 1.36	6.2 ± 1.33	
Singh P et al[57]			
ER			
Glandular	3.6(2.85)	6.86(3.89)	0.0005
Stromal	1.95(1.85)	5.27(3.92)	0.0003
PR			
Glandular	4.5(3.12)	6.61(4.15)	0.0334
Stromal	2.6(2.69)	7.40(3.63)	0.0001
Ahmed M. Mostafa et al			

ER			
Glandular	5.64	2.99	0.004
Stromal	3.67	4.42	0.001
PR			
Glandular	2.42	2.17	0.034
Stromal	4.67	5.85	< 0.001

In the present study, the mean glandular ER expression, with a maximum Alred score, was observed in endometrioid endometrial carcinoma at 6.20 ± 0.58 followed by endometrial hyperplasia without atypia with mean Alred scoring of 6.17±1.15, subsequently in endometrial polyp with mean ER expression of 6.10 ± 1.28, followed by disordered endometrium with mean ER expression of 6.00 ± 1.28 followed by leiomyoma with mean ER expression was 5.00 ± 0.00 and proliferative endometrium with mean ER expression of 5.32 ± 1.21.

Mean stromal ER expression Alred scoring was highest in Endometrioid Endometrial carcinoma with 6.50 ± 1.00 followed by endometrial hyperplasia without atypia showing mean Alred scoring of 6.40 ± 0.9910, endometrial polyp with mean ER expression of 5.967 ±1.221 subsequently followed by disordered endometrium with mean ER expression of 5.80 ± 0.9994 followed by leiomyoma showing mean ER expression of 5.00 ± 0.00 and proliferative endometrium showing mean ER expression of 5.54 ± 1.18.

Mean glandular PR expression Alred scoring showed maximum in cases of Endometrioid Endometrial carcinoma NOS with mean PR expression of 6.50±1.15 followed by endometrial hyperplasia without atypia with mean PR expression of 6.35 ± 1.64, Benign endometrial polyp showing mean PR expression of 6.00 ± 1.80, subsequently disordered endometrium showing mean PR expression of 5.80±1.34 followed by leiomyoma, with mean PR expression of 4.00 ± 0.00 and proliferative endometrium showing mean PR expression of 5.72 ± 1.31.

Mean stromal PR expression Alred scoring was highest in Endometrioid Endometrial carcinoma

NOS with mean PR expression of 7.20 ± 1.0 followed by Endometrial hyperplasia without atypia with PR expression showing 6.50± 0.99, benign endometrial polyp showing mean PR expression of 6.10 ± 1.41 followed by disordered endometrium with mean PR expression of 6.00 ± 1.30, leiomyoma showing mean PR expression of 5.41 ± 0.00 followed by proliferative endometrium, with mean PR expression of 5.00 ± 1.37. The highest expression of ER and PR glandular and stromal component was observed in neoplastic lesions such as Endometrial endometrioid carcinoma followed by endometrial hyperplasia when compared to non neoplastic lesion such as disordered endometrium.

Another study by Jayanthi Mala et al analyzed 266 samples which showed that Estrogen and Progesterone receptor expression were showing significant values in neoplastic lesions such as Endometrial carcinoma and endometrial hyperplasia as compared to non-neoplastic cases such as disordered endometrium. Also they observed that higher glandular activity suggests a higher sensitivity of these structures to steroid hormones[60].

Similar findings were found in a study by V Kalyan Chakravarthy et al. on 60 clinically diagnosed DUB cases. Of these, 40% had a proliferative phase as their most common histopathologic diagnosis, 13.3% had disordered proliferative endometrium, 31.6% had secretory endometrium, 10% had endometrial hyperplasia without atypia, and 1.6% had endometrial hyperplasia with atypia[61]. They have concluded that estrogen receptor expression in glands and stroma is markedly increased in Hyperplasia with atypia followed by endometrial hyperplasia without atypia and disordered proliferation of endometrium likewise progesterone receptor in stroma and glands showed increased expression in endometrial hyperplasia with atypia and endometrial carcinoma followed by disordered proliferation and endometrial hyperplasia without atypia[61].

Table 17: Comparison of ER and PR expression in different spectrum of uterine pathologies between studies

Present study			**Jayanthi et al[60]**	
ER	Glandular	Stromal	Glandular	Stromal
Endometrial polyp	6.10 ± 1.16	5.96 ± 1.21	6.3	4.7
Disordered Endometrium	6.00 ± 1.28	5.80 ± 0.99	5.5	3.3
Endometrioid Endometrial Carcinoma	6.20 ± 0.58	6.50 ± 1.00	7.0	2.0
Leiomyoma	5.00 ± 0.00	5.00 ± 0.00	4.8	4.8
Proliferative endometrium	5.32 ± 1.21	5.54 ± 1.18	4.1	4.1
PR	**Glandular**	**Stromal**	**Glandular**	**Stromal**
Endometrial polyp	6.00 ± 1.80	6.10 ± 1.41	6.0	4.0
Disordered endometrium	5.80± 1.34	6.00 ± 1.30	4.0	2.0
Endometrioid Endometrial carcinoma	6.50 ± 1.15	7.20 ± 1.00	8.0	4.0
Leiomyoma	4.00 ± 0.00	5.41 ± 0.00	6.5	2.0
Proliferative endometrium	5.72 ± 1.31	5.00 ± 1.37	6.0	2.0

In the present study, notable increase in Estrogen and Progesterone receptor expression was observed in the glandular component of endometrium. We observed that patients presenting with DUB showed greater endometrial thickness and elevated levels of estrogen and progesterone. Because it sheds light on the hormones' true effects, measuring the expression of the estrogen and progesterone receptors is therefore more useful than biochemical study of the hormones themselves. Therefore, it serves as a valuable investigation for conducting clinical trials to explore medical treatment options for DUB in the reproductive age group.

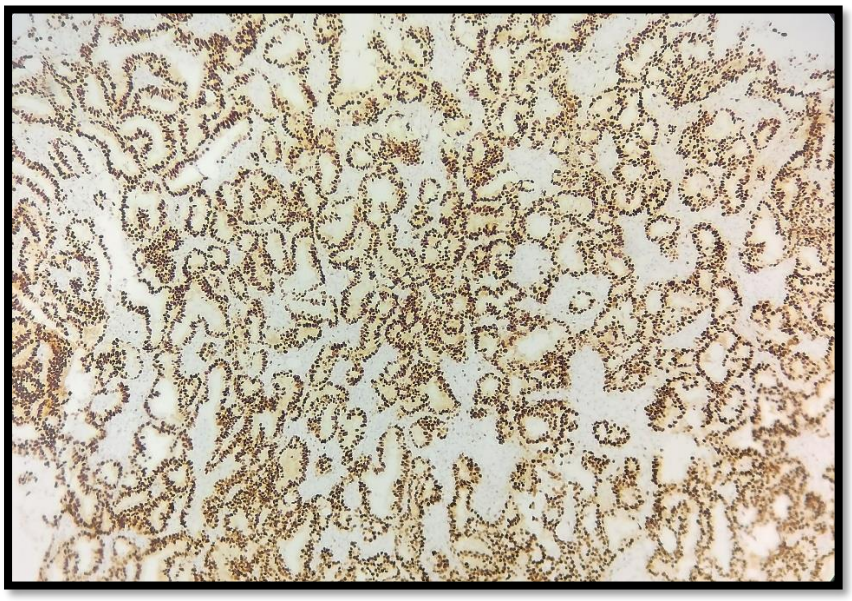


Fig 13 : IHC(20x) Progesterone receptor expression in endometrial glands and stroma of endometrial carcinoma

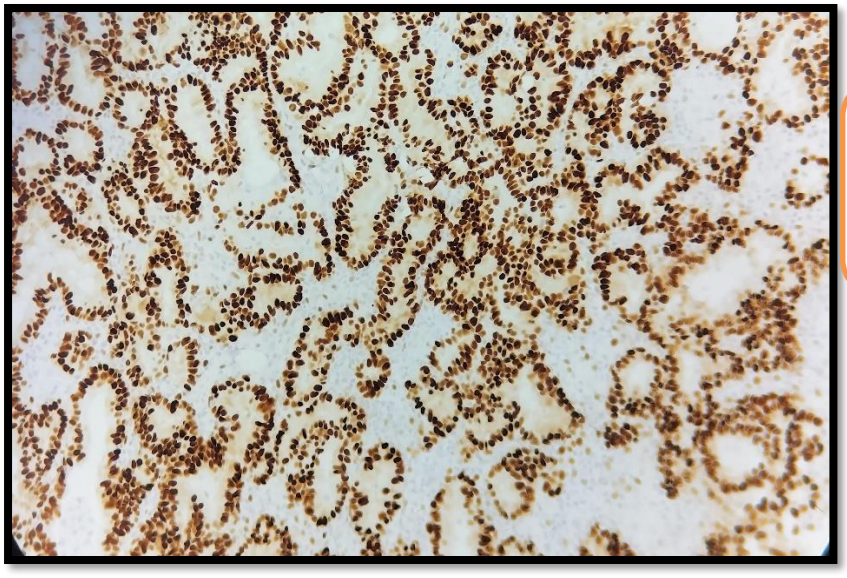


Fig 14: IHC(20x) Estrogen receptor expression in endometrial glands and stroma of endometrial carcinoma

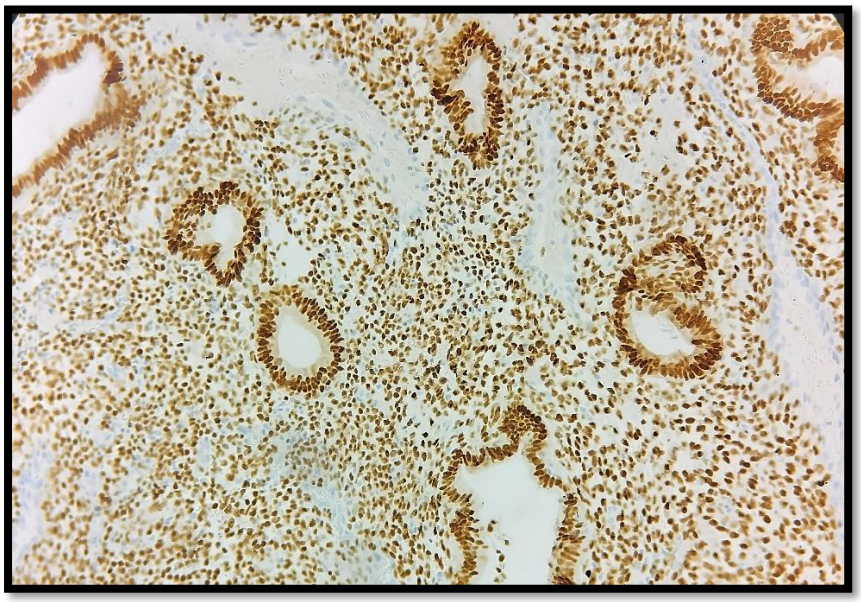


Fig15: IHC(40x) Estrogen receptor expression in endometrial glands and stroma of Disordered endometrium

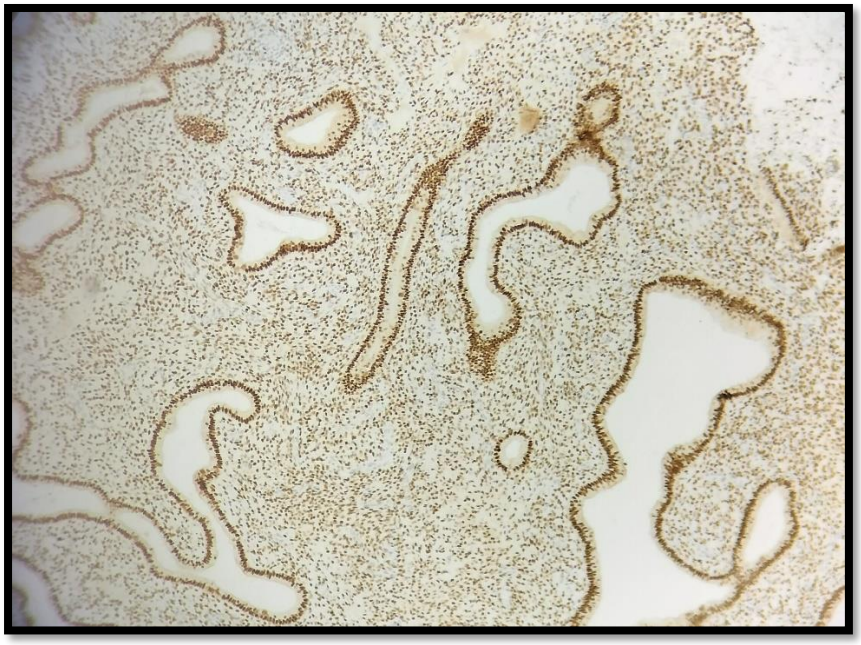


Fig16: IHC(20x) Progesterone receptor expression in endometrial glands and stroma of Disordered endometrium

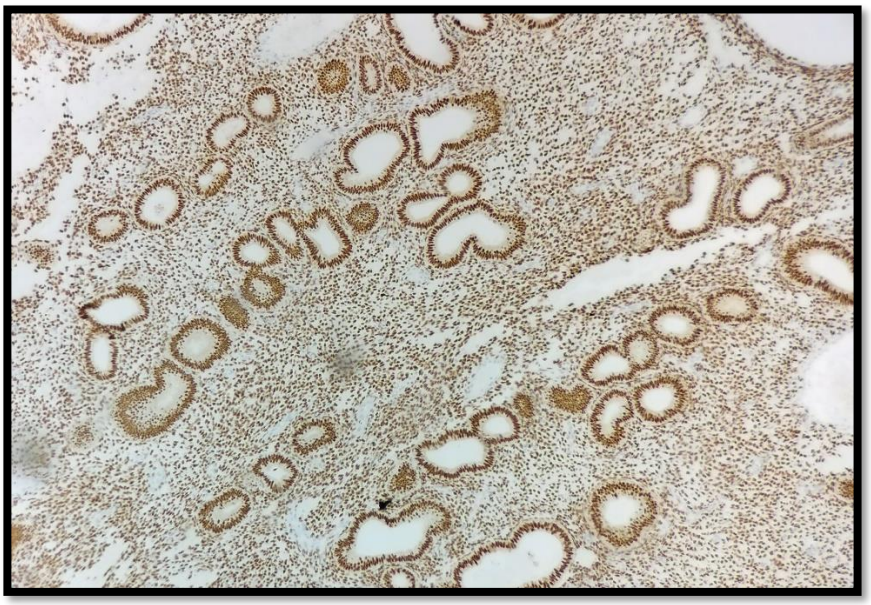


Fig 17: IHC(20x) Estrogen receptor expression in endometrial glands and stroma of Endometrial hyperplasia without atypia

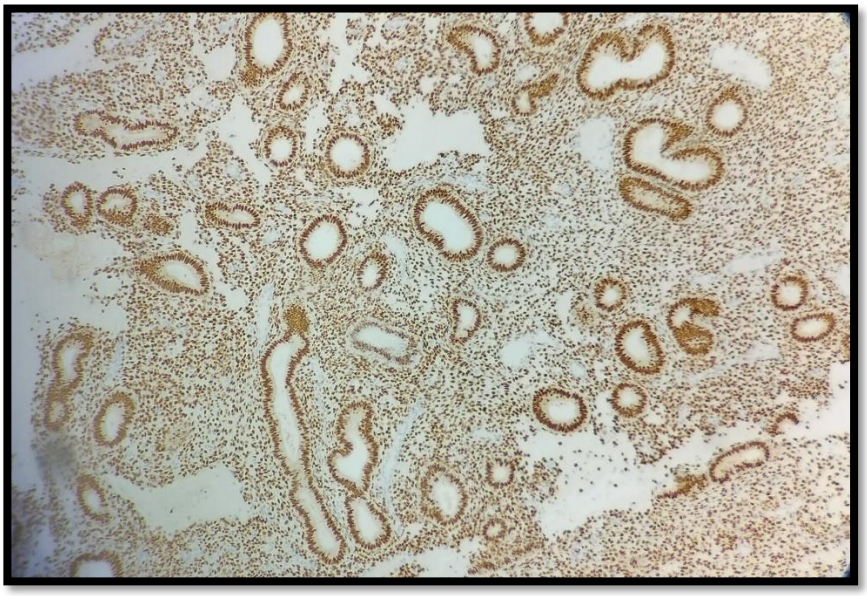


Fig 18: IHC(20x) Progesterone receptor expression in endometrial glands and stroma of Endometrial hyperplasia without atypia

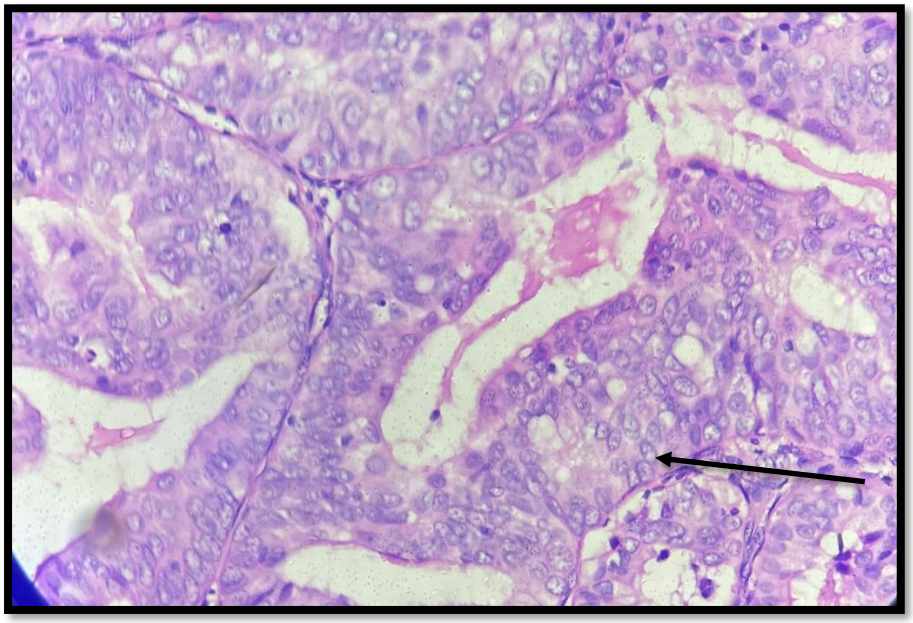


Fig 19: H&E(40x) showing features of endometrial carcinoma with tumor tissue arranged in glandular pattern.

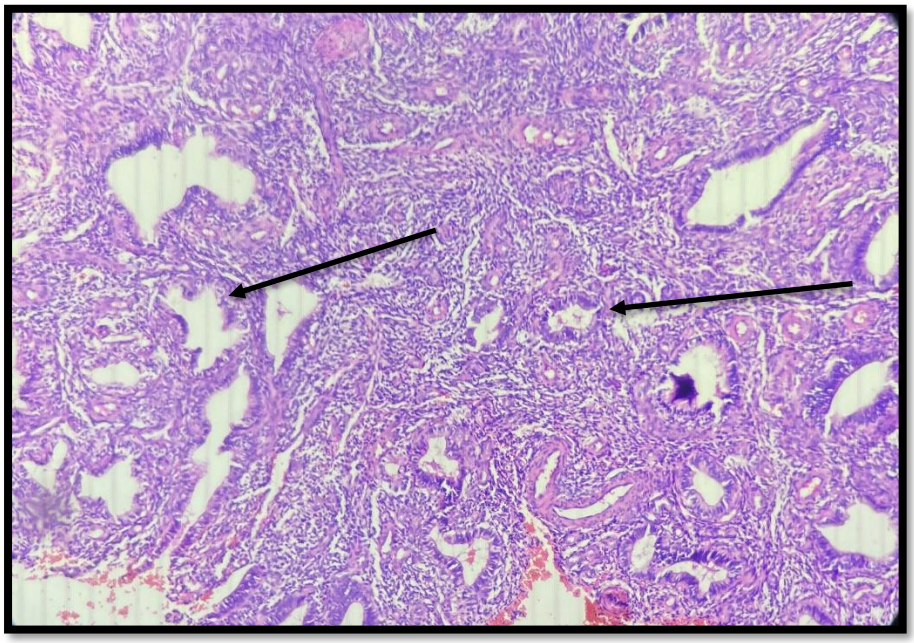


Fig 20: H&E(20x) showing glands in disordered phase with few round to tubular glands and few tortous

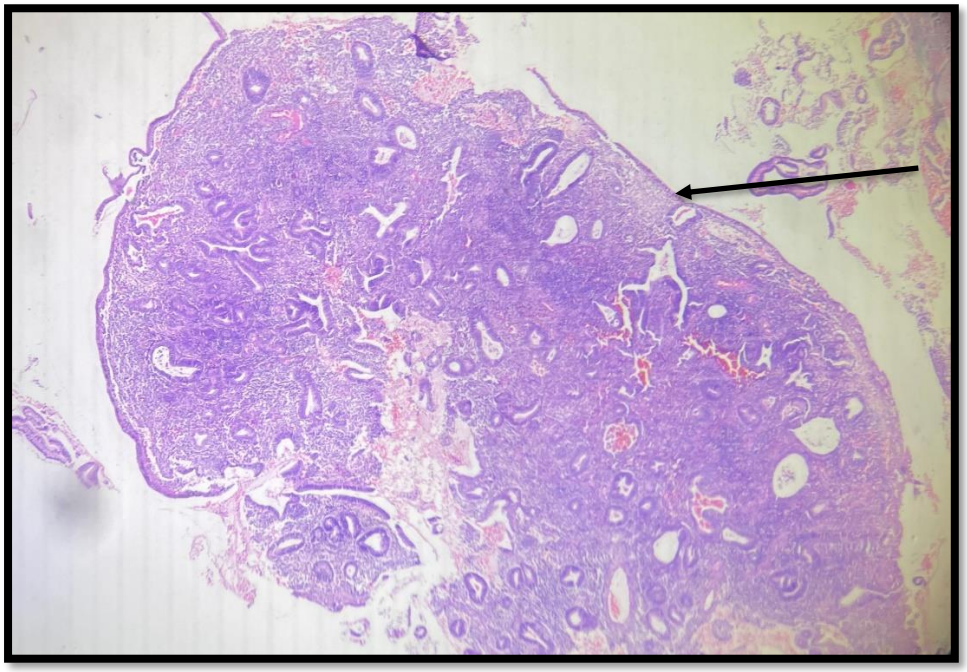


Fig 21: H&E(scanner) showing endometrial polyp lined by cuboidal to low columnar epithelium

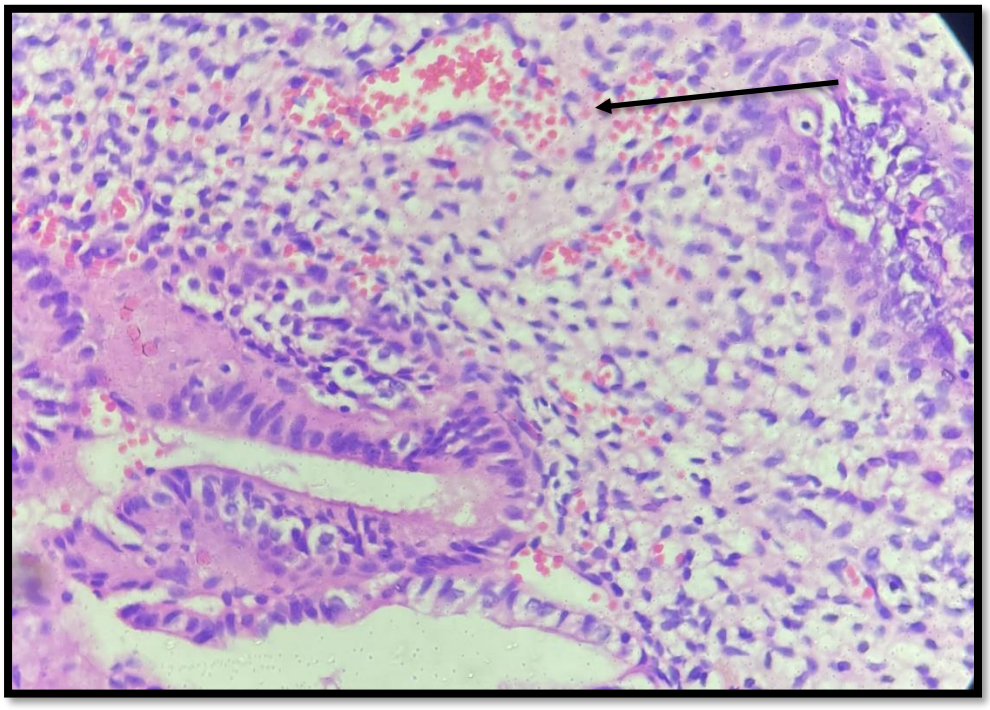


Fig 22: Microphotograph of HPE(40x) showing average blood vessels of 3-4/10HPF

Blood vessel density

In the present study, mean blood vessel density in the cases group was 58.68 ± 12.57 and mean blood vessel density in the control group was 65.72 ± 16.09. Among the neoplastic group, 4 patients (7.8%) had blood vessel density of 35 to 45/10hpf, 15 patients (29.4%) had blood vessel density of 46 to 55/10hpf, 12 patients (23.5%) had blood vessel density of 56 to 65/10hpf, 6 patients (11.8%) had blood vessel density of 66 to 75/10hpf, 9 patients (17.6%) had blood vessel density of 76 to 85/10hpf, 5 patients (9.8%) had blood vessel density of 86 to 95/10hpf. Among the non-neoplastic group, 8 patients (25.8%) had blood vessel density of 35 to 45/10hpf, 8 patients (25.8%) had blood vessel density of 46 to 55/10hpf, 10 patients (32.3%) had blood vessel density of 56 to 65/10hpf, 4 patients (12.9%) had blood vessel density of 66 to 75/10hpf and 1 patient (3.2%) had blood vessel density of 76 to 85/10hpf. The overall average blood vessel density was highest among neoplastic lesions compared to nonneoplastic lesions and the association of blood vessel density in neoplastic and non- neoplastic groups was statistically significant. (p=0.04).

In a similar way, Jayanti Mala et al. examined 266 individuals and determined that hyperplasia without atypia had the highest mean vascular density (40–49 blood vessels/10 HPF) at 42.60±3.21, followed by polyps (38.80±3.78) and disorganized maturation (20.17±3.81)[60].

Makhija et al. evaluated 500 endometrial specimens for alterations in blood vessels in various phases of the menstrual cycle, menstrual disturbances which included 437 cases and 63 controls in the study concluding that maximum number and congestion of blood vessels was found in in cases of DUB.

In contrast, Khan et al. observed that the average number of blood vessels/10HPF (mean vascular density) in the non-secretory group was considerably elevated in complex hyperplasia with atypia but negligible in proliferative, simple hyperplasia, and complex hyperplasia without atypia. The irregular ripening was considerably elevated in the secretory group, while the secretory pattern was negligible (p>0.05)[54].

SUMMARY:

- This was a hospital based cross - sectional study in patients reporting with complaint of DUB to Department of Obstetrics and Gynaecology in Shri B. M. Patil Medical College, Hospital and Research Centre, Vijayapura to examine the degree of expression of Estrogen Receptor alpha (ER alpha) and Progesterone Receptors (PR) in the endometrium glands and stroma along with mean blood vessel density of females presenting with dysfunctional uterine bleeding.
- These were the following findings:

  Using independent sample t test, the comparison of mean age between the groups was done with mean age of the patients in cases being 41.18 ± 10.85 years and in the control group being 42.40 ± 7.69 years.
- Out of 60 cases, the commonest HPR diagnosis noted was disordered endometrium in 30 patients (50%) followed by benign endometrial polyp in 18 patients (30%) followed by endometrial hyperplasia without atypia in 8 patients (13.3%), endometrioid endometrial carcinoma NOS in 3 patients (5%) and leiomyoma in 1 patient (1.7%).

Mean glandular ER expression Alred scoring was done which showed significant correlation between cases (5.88 ± 1.18) and control groups (5.08 ± 1.21).Mean stromal ER expression Alred scoring also showed significant correlation between cases (5.90 ± 1.08) and control groups(5.40 ± 1.18). Mean glandular PR expression Alred scoring was also done between cases and control groups with average score being 6.10 ± 1.58 and 5.60 ± 1.31 respectively showed statistically significance. Mean stromal PR expression Alred scoring showed significant correlation between cases group (6.2 ± 1.33) and control groups.

Mean glandular ER and PR expression Alred scoring was done in neoplastic and non-neoplastic cases.The highest expression of ER and PR glandular and stromal component was observed in neoplastic lesions such as Endometrial endometrioid carcinoma followed by endometrial hyperplasia when compared to nonneoplastic lesion such as disordered endometrium.

Mean blood vessel density also showed significant correlation between cases group (58.68 ±12.57) and control group (65.72 ± 16.09). Hence this study highlights the significance of ER and PR expression and vascular density in disease progression emphasizing the role of hormonal profiling thereby avoiding unnecessary surgical interventions.

CONCLUSION

The findings of this study indicate that the Immunohistochemical expression of Estrogen receptors (ER) and Progesterone receptors (PR) in the endometrium is valuable for understanding the trends and underlying mechanisms of dysfunctional uterine bleeding (DUB). Additionally, the density of blood vessels may allow for microscopic quantification of the structural changes in blood vessels associated with pathological processes and could help to predict angiogenesis in DUB. Furthermore, morphometric assessments could enhance diagnosis and treatment options for DUB cases. Consequently, investigating ER and PR expression along with blood vessel density could provide a basis for clinical trials aimed at exploring medical treatments for DUB in women of reproductive age.

STUDY LIMITATIONS

1. SAMPLE SIZE - The sample size was relatively small, which may limit the generalizability of the findings to a larger population.
2. SINGLE CENTRE STUDY – The current study has been conducted at only one location, institution, or facility, which may limit the generalizability of its findings to broader populations.

FUTURE RECOMMENDATIONS

Future research should use a larger and more diverse sample to improve generalizability. Longitudinal studies can provide deeper insights into long-term trends, while mixed-method approaches combining qualitative and quantitative techniques can enhance analysis. Future research trials are needed to enhance medical treatments and reduce unnecessary surgeries.

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ANNEXURE - I

BLDE
(DEEMED TO BE UNIVERSITY)
Declared as Deemed to be University u/s 3 of UGC Act, 1956
Accredited with 'A' Grade by NAAC (Cycle-2)
The Constituent College
SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA

BLDE (DU)/IEC/ 934/2023-24 10/4/2023

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this University met on **Saturday, 18th March, 2023 at 11.30 a.m. in the CAL Laboratory, Dept. of Pharmacology,** scrutinized the Synopsis/ Research Projects of Post Graduate Student / Under Graduate Student /Faculty members of this University /Ph.D. Student College from ethical clearance point of view. After scrutiny, the following original/ corrected and revised version synopsis of the thesis/ research projects has been accorded ethical clearance.

TITLE: "STUDY OF EXPRESSION OF ESTROGEN & PROGESTERONE RECEPTORS & BLOOD VESSEL DENSITY IN DYSFUNCTIONAL UTERINE BLEEDING".

NAME OF THE STUDENT/PRINCIPAL INVESTIGATOR: DR. PRATHIMA JERUSHA BANDARU.

NAME OF THE GUIDE: DR.MAMATHA K., ASSOCIATE PROFESSOR
DEPT. OF PATHOLOGY.

Dr. Santoshkumar Jeevangi
Chairperson
IEC, BLDE (DU),
VIJAYAPURA
Chairman,
Institutional Ethical Committee,
BLDE (Deemed to be University)
Vijayapura

Dr.Akram A. Naikwadi
Member Secretary
IEC, BLDE (DU),
VIJAYAPURA
MEMBER SECRETARY
Institutional Ethics Committee
BLDE (Deemed to be University)
Vijayapura-586103. Karnataka

Following documents were placed before Ethical Committee for Scrutinization.

- Copy of Synopsis/Research Projects
- Copy of inform consent form
- Any other relevant document

Smt. Bangaramma Sajjan Campus, B. M. Patil Road (Sholapur Road), Vijayapura - 586103, Karnataka, India.
BLDE (DU): Phone: +918352-262770, Fax: +918352-263303, Website: www.bldedu.ac.in, E-mail:office@bldedu.ac.in
College: Phone: +918352-262770, Fax: +918352-263019, E-mail: bmpmc.principal@bldedu.ac.in

ANNEXURE – II

B.L.D.E. (DEEMED TO BE UNIVERSITY)

SHRI B.M. PATIL MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTER, VIJAYAPUR-586103

INFORMED CONSENT FOR PARTICIPATION IN DISSERTATION/RESEARCH

I, the undersigned,_____ ,S/O D/O W/O _____,aged____years, ordinarily resident of _____do hereby state/declare that Dr. _____ of _____ Hospital has examined me thoroughly on _____ at _____ (place) and it has been explained to me in my own language that I am suffering from _____ disease (condition) and this disease/condition mimic following diseases . Further Doctor informed me that he/she is conducting dissertation/research titled "**STUDY OF EXPRESSION OF ESTROGEN AND PROGESTERONE RECEPTORS AND BLOOD VESSEL DENSITY IN DYSFUNCTIONAL UTERINE BLEEDING"** under the guidance of Dr Mamatha K requesting my participation in the study. Apart from routine treatment procedure, the pre-operative, operative, post-operative and follow-up observations will be utilized for the study as reference data.

Doctor has also informed me that during conduct of this procedure like adverse results may be encountered. Among the above complications most of them are treatable but are not anticipated hence there is chance of aggravation of my condition and in rare circumstances it may prove fatal in spite of anticipated diagnosis and best treatment made available. Further Doctor has informed me that my participation in this study help in evaluation of the results of the study which is useful reference to treatment of other similar cases in near future, and also I may be benefited in getting relieved of suffering or cure of the disease I am suffering.

The Doctor has also informed me that information given by me, observations made/ photographs/ video graphs taken upon me by the investigator will be kept secret and not assessed by the person other than me or my legal hirer except for academic purposes.

The Doctor did inform me that though my participation is purely voluntary, based on information given by me, I can ask any clarification during the course of treatment / study related to diagnosis, procedure of treatment, result of treatment or prognosis. At the same time I have been informed that I can withdraw from my participation in this study at any time if I want or the investigator can terminate me from the study at any time from the study but not the procedure of treatment and follow-up unless I request to be discharged.

After understanding the nature of dissertation or research, diagnosis made, mode of treatment, I the undersigned Shri/Smt _____ under my full conscious state of mind

agree to participate in the said research/dissertation.

Signature of patient:

Signature of doctor:

Witness: 1.

2.

Date:

Place:

B.L.D.E (DEEMED TO BE UNIVERSITY) ಶ್ರೀ ಬಿ.ಎಂ.ಪಟ್ಟೀಲ್ ಮೆಡಿಕಲ್ ಕಾಲೇಜು, ಆಸ್ಪತ್ರೆ ಮತ್ತು ಸಂಶೋಧನಾ ಕೇಂದ್ರ, ವಿಜಯಪುರ-586103

ಪ್ರಬಂಧ/ಸಂಶೋಧನೆಯಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳಲು ಮಾಹಿತಿ ಪಡೆದ ಸಮ್ಮತಿ

ನಾನು, ಕೆಳಗಿನವರು_____ ಸಹಿಯಿಟ್ಟವರು, ಮಗ/ಮಗಳು/ಪತ್ನಿಯ _____ ವಯಸ್ಸು _____ವರ್ಷಗಳು, ಸಾಮಾನ್ಯವಾಗಿ ನಿವಾಸಿಸುವ ಸ್ಥಳದ ಹೆಸರು_____, ಇಲ್ಲಿ ಹೇಳಿದ್ದೇನೆ/ಘೋಷಿಸುತ್ತೇನೆ ಡಾಕ್ಟರ್ ಹೆಸರು_____ ಅವರು ಆಸ್ಪತ್ರೆ ಹೆಸರು_____ ಅವರು ನನ್ನನ್ನು ಪೂರ್ಣವಾಗಿ ಪರೀಕ್ಷಿಸಿದರು ದಿನಾಂಕದಲ್ಲಿ_____ ಸ್ಥಳ ಹೆಸರು_____ ಮತ್ತು ನನಗೆ ನನ್ನ ಭಾಷೆಯಲ್ಲಿ ವಿವರಿಸಲಾಗಿದೆ ನಾನು ಒಂದು ರೋಗ (ಸ್ಥಿತಿ) ಅನುಭವಿಸುತ್ತಿದ್ದೇನೆ. ಮುಂದುವರಿದು ಡಾಕ್ಟರ್ ನನಗೆ ತಿಳಿಸಿದ್ದಾರೆ ಅವರು ಒಂದು ಪದ್ಧತಿ/ಸಂಶೋಧನೆ ನಡೆಸುತ್ತಿದ್ದಾರೆ ಶೀರ್ಷಿಕೆಯುಳ್ಳ **STUDY OF EXPRESSION OF ESTROGEN AND PROGESTERONE RECEPTORS AND BLOOD VESSEL DENSITY IN DYSFUNCTIONAL UTERINE BLEEDING** ಡಾಕ್ಟರ್ Dr Mamatha K ಮಾರ್ಗದರ್ಶನದಲ್ಲಿ ನನ್ನ ಪಾಲ್ಗೊಳ್ಳುವಿಕೆಯನ್ನು ಕೇಳಿದ್ದಾರೆ ಅಧ್ಯಯನದಲ್ಲಿ.

ಡಾಕ್ಟರ್ ನನಗೆ ಇದನ್ನು ಕೂಡಾ ತಿಳಿಸಿದ್ದಾರೆ ಈ ಕ್ರಮದ ನಡುವಲ್ಲಿ ಪ್ರತಿಕೂಲ ಫಲಿತಾಂಶಗಳನ್ನು ಎದುರಿಸಬಹುದು. ಮೇಲೆ ಹೇಳಿದ ಪ್ರಕಟಣೆಗಳಲ್ಲಿ, ಅಧಿಕಾಂಶವು ಚಿಕಿತ್ಸಿಸಬಹುದಾದರೂ ಅದನ್ನು ನಿರೀಕ್ಷಿಸಲಾಗುತ್ತಿಲ್ಲ ಆದ್ದರಿಂದ ನನ್ನ ಸ್ಥಿತಿಯ ಹಿರಿದಾಗುವ ಅವಕಾಶವಿದೆ ಮತ್ತು ಅಪರೂಪದ ಸಂದರ್ಭಗಳಲ್ಲಿ ಅದು ಮರಣಕಾರಕವಾಗಿ ಪರಿಣಮಿಸಬಹುದು ಹೊಂದಿದ ರೋಗನಿರ್ಧಾರ ಮತ್ತು ಯಥಾಶಕ್ತಿ ಚಿಕಿತ್ಸೆ ಮಾಡಲು ಹೊಂದಿದರೂ, ಮುಂದುವರಿದು ಡಾಕ್ಟರ್ ನನಗೆ ತಿಳಿಸಿದ್ದಾರೆ ನನ್ನ ಪಾಲ್ಗೊಳ್ಳುವಿಕೆ ಈ ಅಧ್ಯಯನದ ಫಲಿತಾಂಶಗಳ ಮೌಲ್ಯಮಾಪನದಲ್ಲಿ ಸಹಾಯಕವಾಗುತ್ತದೆ ಇತರ ಸಮಾನ ಪ್ರಕರಣಗಳ ಚಿಕಿತ್ಸೆಗೆ ಉಪಯುಕ್ತ ಉಲ್ಲೇಖವಾಗಿದೆ, ಮತ್ತು ನಾನು ಅನುಭವಿಸುವ ರೋಗದಿಂದ ವಿಮುಕ್ತಿ ಅಥವಾ ಗುಣಮುಖಗೊಳ್ಳುವಲ್ಲಿ ನನಗೆ ಪ್ರಯೋಜನವಾಗಬಹುದು.

ಡಾಕ್ಟರ್ ನನಗೆ ಇದನ್ನು ಕೂಡಾ ತಿಳಿಸಿದ್ದಾರೆ ನನ್ನಿಂದ ನೀಡಿದ ಮಾಹಿತಿ, ಮಾಡಿದ ಪರಿಶೀಲನೆಗಳು / ಫೋಟೋಗ್ರಾಫ್‌ಗಳು / ವೀಡಿಯೋ ಗ್ರಾಫ್‌ಗಳು ನನ್ನ ಮೇಲೆ ತೆಗೆದುಕೊಳ್ಳಲಾಗುವ ಅನ್ವೇಷಕರು ರಹಸ್ಯವಾಗಿ ಇಡುವರು ಮತ್ತು ನಾನು ಅಥವಾ ನನಗೆ ಕಾನೂನು ದೃಷ್ಟಿಯಲ್ಲಿ ಸಂಬಂಧಿತರನ್ನು ಹೊರತುಪಡಿಸಿ ಇತರ ವ್ಯಕ್ತಿಯಿಂದ ಮೌಲ್ಯಮಾಪನ ಮಾಡಲಾಗುವುದಿಲ್ಲ. ಡಾಕ್ಟರ್ ನನಗೆ ತಿಳಿಸಿದ್ದಾರೆ ನನ್ನ ಪಾಲ್ಗೊಳ್ಳುವಿಕೆ ಶುದ್ಧವಾಗಿ ಸ್ವೇಚ್ಛಾಯಿತ, ನನ್ನಿಂದ ನೀಡಿದ ಮಾಹಿತಿಯ ಆಧಾರದ ಮೇಲೆ, ಚಿಕಿತ್ಸೆ / ಅಧ್ಯಯನದ ಸಂಬಂಧದಲ್ಲಿ ರೋಗನಿರ್ಧಾರ, ಚಿಕಿತ್ಸೆಯ ವಿಧಾನ, ಚಿಕಿತ್ಸೆಯ ಫಲಿತಾಂಶ ಅಥವ ಭವಿಷ್ಯದ ಪ್ರವೃತ್ತಿಗಳು ಬಗ್ಗೆ ಯಾವುದೇ ಸ್ಪಷ್ಟತೆ ಕೇಳಬಹುದು. ಅದೇ ಸಮಯದಲ್ಲಿ ನನಗೆ ತಿಳಿಸಲಾಗಿದೆ ನಾನು ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಈ ಅಧ್ಯಯನದಲ್ಲಿ ನನ್ನ ಪಾಲ್ಗೊಳ್ಳುವಿಕೆಯನ್ನು ನಿಲ್ಲಿಸಬಹುದು ನಾನು ಬಯಸಿದರೆ ಅಥವಾ ಅನ್ವೇಷಕರು ಅಧ್ಯಯನದಿಂದ ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ನನ್ನನ್ನು ನಿಲ್ಲಿಸಬಹುದು.

ಪ್ರಬಂಧ ಅಥವಾ ಸಂಶೋಧನೆಯ ಸ್ವಭಾವ, ಮಾಡಿದ ರೋಗನಿರ್ಧಾರ ಮತ್ತು ಚಿಕಿತ್ಸೆಯ ವಿಧಾನವನ್ನು ಅರ್ಥಮಾಡಿಕೊಂಡು, ನಾನು ಕೆಳಗಿನ ಶ್ರೀ / ಶ್ರೀಮತಿ_____ ನನ್ನ ಪೂರ್ಣವಾದ ಪ್ರಜ್ಞೆಯ ಸ್ಥಿತಿಯಲ್ಲಿ ಹೇಳಿದ ಸಂಶೋಧನೆ / ಪ್ರಬಂಧದಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳಲು ಒಪ್ಪುತ್ತೇನೆ.

ರೋಗಿಯ ಸಹಿ ಡಾಕ್ಟರನ ಸಹಿ

ಸಾಕ್ಷಿಗಳು

1)
2)

ANNEXURE – III

PROFORMA

NAME	:	OP/IP No.	:
AGE	:		
SEX	:	D.O.A	:
RELIGION	:	D.O.D	:
OCCUPATION	:		
RESIDENCE	:		

Presenting Complaints :
Past history :
Personal history :
Family history :
Treatment history :

General physical examination:

Pallor	present/absent
Icterus	present/absent
Clubbing	present/absent
Lymphadenopathy	present/absent
Edema	present/absent
Built	poor/average/well

VITALS: PR: RR: BP:

TEMPERATURE: WEIGHT:

SYSTEMIC EXAMINATION:

Cardiovascular system:

Respiratory system:

Per Abdomen:

Central nervous system:

Clinical Diagnosis:

INVESTIGATIONS:

IHC :

ER aplha expression :

PR expression :

MICROSCOPY

Blood vessel density :

MASTER CHART

Sr No	Age	Clinical Diagnosis	HPR No	Type of Specimen	HPR Diagnosis	ER (Alred scoring)	PR (Alred scoring)	Blood vessel density
1	45	AUB	3570/23	ENDOMETRIAL BIOPSY	DISORDERED ENDOMETRIUM	Positive (7+6)	Positive (7+6)	43/10hpf
2	27	ENDOMETRIAL POLYP	3808/23	ENDOMETRIAL BIOPSY	BENIGN ENDOMETRIAL POLYP	Positive (7+7)	Positive (8+7)	51/10hpf
3	49	DUB	3817/23	ENDOMETRIAL BIOPSY	DISORDERED ENDOMETRIUM	Positive (6+6)	Positive (6+6)	56/10hpf
4	48	ENDOMETRIAL HYPERPLASIA	3936/23	ENDOMETRIAL BIOPSY	ENDOMETRIAL HYPERPLASIA WITHOUT ATYPIA	Positive (7+7)	Positive (8+8)	78/10hpf
5	48	ENDOMETRIAL HYPERPLASIA	3948/23	ENDOMETRIAL BIOPSY	ENDOMETRIAL HYPERPLASIA WITHOUT ATYPIA	Positive (5+6)	Positive (4+7)	69/10hpf
6	37	ENDOMETRIAL HYPERPLASIA	4000/23	ENDOMETRIAL BIOPSY	DISORDERED ENDOMETRIUM	Positive (5+6)	Positive (3+5)	45/10hpf
7	40	ENDOMETRIAL HYPERPLASIA	4833/23	ENDOMETRIAL BIOPSY	ENDOMETRIAL POLYP	Positive (8+7)	Positive (8+7)	54/10hpf
8	56	DUB	2178/24	ENDOMETRIAL BIOPSY	ENDOMETRIAL POLYP	Positive (4+4)	Positive (4+4)	65/10hpf
9	49	AUB	2228/24	ENDOMETRIAL BIOPSY	DISORDERED PROLIFEARTIVE ENDOMETRIUM	Positive (7+6)	Positive (7+6)	76/10hpf
11	28	AUB	2284/24	ENDOMETRIAL BIOPSY	ENDOMETRIAL POLYP	Positive (5+5)	Positive (3+5)	52/10hpf
12	32	AUB	2288/24	ENDOMETRIAL BIOPSY	ENDOMETRIAL POLYP	Positive (6+4)	Positive (5+3)	46/10hpf
13	27	DUB	2354/24	ENDOMETRIAL BIOPSY	ENDOMETRIAL POLYP	Positive (5+6)	Positive (4+5)	78/10hpf
14	27	AUB	2479/24	ENDOMETRIAL BIOPSY	ENDOMETRIAL POLYP	Positive (7+6)	Positive (7+6)	63/10hpf
15	34	AUB	2521/24	ENDOMETRIAL BIOPSY	ENDOMETRIAL POLYP	Positive (6+5)	Positive (4+7)	52/10hpf
16	22	DUB	2720/24	ENDOMETRIAL BIOPSY	DISORDERED PROLIFEARTIVE ENDOMETRIUM	Positive (3+3)	Positive (3+3)	53/10hpf
17	55	AUB	2730/24	TAHBSO	LEIOMYOMA	Positive (6+5)	Positive (4+6)	69/10hpf
18	56	AUB	2780/24	ENDOMETRIAL BIOPSY	ENDOMETRIAL POLYP	Positive (7+6)	Positive (5+4)	56/10hpf
19	74	AUB	2923/24	ENDOMETRIAL TISSUE	ENDOMETRIALCARCINOMA	Positive (5+7)	Positive (6+5)	71/10hpf

20	43	AUB	5853/24	ENDOMETRIAL BIOPSY	ENDOMETRIAL HYPERPLASIA	Positive (6+7)	Positive (7+5)	68/10hpf
21	51	DUB	6071/24	ENDOMETRIAL BIOPSY	DISORDERED ENDOMETRIUM	Positive (8+7)	Positive (8+7)	52/10hpf
22	47	AUB	6137/24	ENDOMETRIAL BIOPSY	ENDOMETRIAL POLYP	Positive (6+5)	Positive (4+3)	89/10hpf
23	48	DUB	6217/24	ENDOMETRIAL BIOPSY	DISORDERED ENDOMETRIUM	Positive (6+7)	Positive (5+6)	50/10hpf
24	35	AUB	6459/24	TAHBSO	ENDOMETRIAL POLYP	Positive (6+5)	Positive (8+6)	46/10hpf
25	49	DUB	6489/24	ENDOMETRIAL BIOPSY	DISORDERED ENDOMETRIUM	Positive (7+6)	Positive (7+6)	61/10hpf
26	37	AUB	6596/24	ENDOMETRIAL BIOPSY	ENDOMETRIAL HYPERPLASIA WITHOUT ATYPIA	Positive (6+6)	Positive (6+6)	40/10hpf
27	29	DUB	6606/24	ENDOMETRIAL BIOPSY	ENDOMETRIAL POLYP	Positive (3+3)	Positive (5+7)	85/10hpf
28	33	AUB	425/24	ENDOMETRIAL BIOPSY	DISORDERED ENDOMETRIUM	Positive (7+6)	Positive (6+7)	64/10hpf
29	57	DUB	359/24	ENDOMETRIAL BIOPSY	ENDOMETRIAL HYPERPLASIA WITHOUT ATYPIA	Positive (6+6)	Positive (7+7)	55/10hpf
30	50	AUB	360/24	ENDOMETRIAL BIOPSY	DISORDERED ENDOMETRIUM	Positive (5+5)	Positive (3+5)	43/10hpf
31	58	AUB	426/24	ENDOMETRIAL BIOPSY	DISORDERED ENDOMETRIUM	Positive (3+4)	Positive (3+4)	68/10hpf
32	32	AUB	612/24	ENDOMETRIAL BIOPSY	DISORDERED ENDOMETRIUM	Positive (5+6)	Positive (4+5)	59/10hpf
33	42	DUB	687/24	ENDOMETRIAL BIOPSY	DISORDERED ENDOMETRIUM	Positive (7+7)	Positive (7+7)	54/10hpf
34	52	AUB	1091/24	ENDOMETRIAL BIOPSY	DISORDERED ENDOMETRIUM	Positive (6+5)	Positive (4+3)	43/10hpf
35	38	DUB	1623/24	ENDOMETRIAL BIOPSY	ENDOMETRIAL HYPERPLASIA WITHOUT ATYPIA	Positive (6+6)	Positive (7+7)	65/10hpf
36	32	AUB	1782/24	ENDOMETRIAL BIOPSY	DISORDERED ENDOMETRIUM	Positive (5+4)	Positive (6+7)	57/10hpf
37	54	AUB	1863/24	TAHBSO	WELL DIFFERENTIATED ENDOMETRIAL ADENOCARCINOMA	Positive (6+7)	Positive (6+7)	75/10hpf
38	28	DUB	1983/24	TAHBSO	DISORDERED ENDOMETRIUM	Positive (5+4)	Positive (4+5)	52/10hpf
39	48	DUB	6217/24	ENDOMETRIAL BIOPSY	DISORDERED ENDOMETRIUM	Positive (5+6)	Positive (7+7)	50/10hpf
40	35	AUB	6459/24	TAHBSO	ENDOMETRIAL POLYP	Positive (5+6)	Positive (8+7)	46/10hpf

41	49	DUB	6489/24	ENDOMETRIAL BIOPSY	DISORDERED ENDOMETRIUM	Positive (7+7)	Positive (8+7)	61/10hpf
42	37	AUB	6596/24	ENDOMETRIAL BIOPSY	ENDOMETRIAL HYPERPLASIA WITHOUT ATYPIA	Positive (7+7)	Positive (8+7)	40/10hpf
43	29	DUB	6606/24	ENDOMETRIAL BIOPSY	ENDOMETRIAL POLYP	Positive (7+7)	Positive (8+7)	85/10hpf
44	33	AUB	425/24	ENDOMETRIAL BIOPSY	DISORDERED ENDOMETRIUM	Positive (7+7)	Positive (8+7)	64/10hpf
45	57	DUB	359/24	ENDOMETRIAL BIOPSY	ENDOMETRIAL HYPERPLASIA WITHOUT ATYPIA	Positive (7+7)	Positive (8+7)	55/10hpf
46	50	AUB	360/24	ENDOMETRIAL BIOPSY	DISORDERED ENDOMETRIUM	Positive (7+7)	Positive (8+7)	43/10hpf
47	58	AUB	426/24	ENDOMETRIAL BIOPSY	DISORDERED ENDOMETRIUM	Positive (7+7)	Positive (8+7)	68/10hpf
48	32	AUB	612/24	ENDOMETRIAL BIOPSY	DISORDERED ENDOMETRIUM	Positive (7+7)	Positive (8+7)	59/10hpf
49	42	DUB	687/24	ENDOMETRIAL BIOPSY	DISORDERED ENDOMETRIUM	Positive (7+7)	Positive (8+7)	54/10hpf
50	52	AUB	1091/24	ENDOMETRIAL BIOPSY	DISORDERED ENDOMETRIUM	Positive (7+7)	Positive (8+7)	43/10hpf
51	38	DUB	1623/24	ENDOMETRIAL BIOPSY	ENDOMETRIAL HYPERPLASIA WITHOUT ATYPIA	Positive (7+7)	Positive (8+7)	65/10hpf
52	32	AUB	1782/24	ENDOMETRIAL BIOPSY	DISORDERED ENDOMETRIUM	Positive (7+7)	Positive (8+7)	57/10hpf
53	54	AUB	1863/24	TAHBSO	ENDOMETRIAL ADENOCARCINOMA	Positive (7+7)	Positive (8+7)	75/10hpf
54	28	DUB	1983/24	TAHBSO	DISORDERED ENDOMETRIUM	Positive (7+7)	Positive (8+7)	52/10hpf
55	35	DUB	2701/24	ENDOMETRIAL BIOPSY	ENDOMETRIAL HYPERPLASIA	Positive (7+7)	Positive (8+7)	61/10hpf
56	31	AUB	3316/24	ENDOMETRIAL BIOPSY	ENDOMETRIAL HYPERPLASIA WITHOUT ATYPIA	Positive (7+7)	Positive (8+7)	58/10hpf
57	41	DUB	3402/24	ENDOMETRIAL BIOPSY	DISORDERED ENDOMETRIUM	Positive (7+7)	Positive (8+7)	42/10hpf
58	50	AUB	3363/23	TAHBSO	DISORDERED ENDOMETRIUM	Positive (7+7)	Positive (8+7)	72/10hpf
59	30	DUB	290/25	ENDOMETRIAL BIOPSY	DISORDERED ENDOMETRIUM	Positive (7+7)	Positive (8+7)	65/10hpf
60	42	DUB	235/25	ENDOMETRIAL BIOPSY	DISORDERED ENDOMETRIUM	Positive (7+7)	Positive (8+7)	75/10hpf

MASTER CHART- CONTROL GROUPS

Sr No	Age	Clinical Diagnosis	HPR No	Type of Specimen	HPR Diagnosis	ER (Alred scoring)	PR (Alred scoring)	Blood vessel density
1	40	DUB	3339/24	ENDOMETRIAL BIOPSY	PROLIFERATIVE ENDOMETRIUM	Positive **5+6**	Positive **7+5**	65/10hpf
2	45	DUB	3357/24	ENDOMETRIAL BIOPSY	PROLIFERATIVE ENDOMETRIUM	Positive **6+5**	Positive **4+5**	76/10hpf
3	40	AUB	3373/24	ENDOMETRIAL BIOPSY	PROLIFERATIVE ENDOMETRIUM	Positive **5+7**	Positive **6+6**	52/10hpf
4	48	AUB	3386/24	ENDOMETRIAL BIOPSY	PROLIFERATIVE ENDOMETRIUM	Positive **5+6**	Positive **7+6**	46/10hpf
5	29	AUB	3441/24	ENDOMETRIAL BIOPSY	PROLIFERATIVE ENDOMETRIUM	Positive **5+6**	Positive **6+5**	78/10hpf
6	54	AUB	3458/24	ENDOMETRIAL BIOPSY	PROLIFERATIVE ENDOMETRIUM	Positive **7+6**	Positive **5+4**	63/10hpf
7	35	AUB	3464/24	ENDOMETRIAL BIOPSY	PROLIFERATIVE ENDOMETRIUM	Positive **5+6**	Positive **7+6**	52/10hpf
8	37	ENDOMETRIAL HYPERPLASIA	3468/24	ENDOMETRIAL BIOPSY	PROLIFERATIVE ENDOMETRIUM	Positive **5+4**	Positive **6+4**	53/10hpf
9	32	DUB	3472/24	BIOPSY	PROLIFERATIVE ENDOMETRIUM	Positive **3+4**	Positive **5+4**	69/10hpf
10	45	AUB	6689/24	ENDOMETRIAL BIOPSY	PROLIFERATIVE ENDOMETRIUM	Positive **5+6**	Positive **4+3**	56/10hpf
11	45	AUB	6635/24	ENDOMETRIAL BIOPSY	PROLIFERATIVE ENDOMETRIUM	Positive **5+4**	Positive **6+4**	71/10hpf
12	42	AUB WITH CHRONIC PID	6627/24	ENDOMETRIAL BIOPSY	PROLIFERATIVE ENDOMETRIUM	Positive **5+6**	Positive **4+5**	38/10hpf
13	46	AUB	6697/24	ENDOMETRIAL BIOPSY	PROLIFERATIVE ENDOMETRIUM	Positive 3+4	Positive 4+5	54/10hpf

14	30	AUB WITH CHRONIC PID	6566/24	ENDOMETRIAL BIOPSY	PROLIFERATIVE ENDOMETRIUM	Positive **6+4**	Positive **5+4**	61/10hpf
15	54	DUB	6542/24	TAHBSO	PROLIFERATIVE ENDOMETRIUM	Positive **5+4**	Positive **5+6**	45/10hpf
16	35	AUB	6689/24	ENDOMETRIAL BIOPSY	PROLIFERATIVE ENDOMETRIUM	Positive **6+5**	Positive **5+4**	53/10hpf
17	42	DUB	6455/24	TAHBSO	PROLIFERATIVE ENDOMETRIUM	Positive **4+5**	Positive **6+5**	78/10hpf
18	56	DUB	6268/24	TAHBSO	PROLIFERATIVE ENDOMETRIUM	Positive **5+3**	Positive **5+4**	89/10HPF
19	53	AUB	6595/24	ENDOMETRIAL BIOPSY	PROLIFERATIVE ENDOMETRIUM	Positive **5+4**	Positive **6+5**	78/10hpf
20	45	DUB	6115/24	TAHBSO	PROLIFERATIVE ENDOMETRIUM	Positive **6+5**	Positive **4+5**	89/10HPF
21	39	DUB	5395/24	TAHBSO	PROLIFERATIVE ENDOMETRIUM	Positive **5+6**	Positive **7+6**	94/10hpf
22	41	DUB	3402/24	TAHBSO	PROLIFERATIVE ENDOMETRIUM	Positive **4+5**	Positive **6+5**	86/10hpf

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