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Original Research Article

Endometrial Findings in Abnormal Uterine Bleeding

Dr. K. Kasthuri Thilagam MD[1*], Dr. M. Thenmozhi[2]
[1]Associate Professor, Department of Pathology, Government Mohann Kumaramangalam Medical College, Salem, India
[2]Professor, Department of Pathology, Government Mohann Kumaramangalam Medical College, Salem, India

***Corresponding author:** Dr. K. Kasthuri Thilagam
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Abstract

Background: Alteration in the hormone status either due to primary intrinsic ovarian abnormalities or secondary disturbances in the hypothalamo - Pituitary - ovarian axis results in AUB. The etiology of AUB is often related to anovulatory disturbances and ovulatory disturbances. However, there is a need to evaluate the endometrial changes specific for ovulatory and anovulatory causes of AUB at the histological level. This study was carried out to evaluate the histomorphological pattern of endometrium in Abnormal Uterine Bleeding. ***Methods:*** This cross sectional study was carried out among 100 women who were diagnosed with AUB in our tertiary care hospital between 2012 and 2014. Endometrial biopsy sample was taken on the third week of menstrual cycle. The samples received were carefully examined for the presence of mucus & large blood clots, and, if found were removed. One slide stained with conventional Haematoxylin & Eosin, second slide with Per iodic acid Schiff method and third slide was stained with Gomeri's method for reticulin stain. ***Results:*** A total of 100 cases were analyzed in this study and majority of the cases consisted of anovulatory disturbances (62%) followed by ovulatory disturbances (34%). While majority of the anovulatory cases were irregular proliferation (27%), majority of the ovulatory cases were deficient secretory state with co-ordinated delay (11%). ***Conclusion:*** In the modern era of minimally invasive surgical and diagnostic procedures, there is a growing need for the histopathologist to develop acumen for diagnosis of AUB. This study has extensively highlighted the variations in the endometrial presentation for an in depth analysis of AUB.

Keywords: Abnormal uterine bleeding, anovulatory bleeding, histomorphology, ovulatory bleeding.

INTRODUCTION

The reproductive period of a woman ranges from 15 to 49 years. During this period, women are often faced with a wide range of problems, as an effect of either structural or functional abnormalities in the reproductive system. One of the major gynecological problems is abnormal uterine bleeding, which has significant morbidity that interferes with the quality of life of women. Abnormal Uterine Bleeding (AUB) also results in significant socioeconomic burden for women, especially in today's scenario where the proportion of working women have increased. It has been estimated that women today experience about 10 times more menstrual cycles than her ancestors during her entire reproductive period. This is mainly due to decreased parity and decrease in lactational amenorrhea. Abnormal uterine bleeding is sometimes so morbid that women suffer from social abstinence leading to major psychological problems.

Abnormal uterine bleeding (AUB) is excessive (>80 mL or a significant decrease in hemoglobin), prolonged (flow duration >7 to 10 days), or unpatterned (<21 days or >40 to 45 days in an adolescent) endometrial bleeding unrelated to structural or systemic disease. It may be described as ovulatory (e.g., heavy, cyclical bleeding) or anovulatory (e.g., irregular bleeding) [1].

The endometrium responds to every alteration in hormonal balance that occurs as a result of abuse, deficiency or excessive secretion of hormones. Alteration in the hormone status either due to primary intrinsic ovarian abnormalities or secondary disturbances in the hypothalamo - Pituitary - ovarian axis results in AUB. These hormonal changes produce arrest in follicular maturation at different stages of its development causing morphological changes that corresponds to the stage of maturation at which the follicle become impaired. Follicular arrest leads to lack of endometrial stimulation when the impairment occurs during the hormone secreting stages of Follicle or corpus luteum. When the impairment result in arrest of follicular regression with anovalation, the effect on the endometrium will be that of hyperstimulation [2-4].

The term AUB applies to any abnormal uterine bleeding including disturbances of the menstrual cycle, regular and irregular uterine bleeding and alteration in the amount or duration of menstrual loss. AUB is a group of disorder characterized dysfunction of the uterus, ovary, pituitary, hypothalamus or other part of the reproductive system, which results in abnormal or excessive uterine bleeding. The etiology of AUB is often related to anovulatory disturbances and ovulatory disturbances [5].

For a long time the pathophysiology and pattern of AUB presentation remained unclear. Only in the later half of the last century advances in understanding of complex interrelationship of Hypothalamo – Pitutary Ovarian axis was explored. This led to the development of sensitive and specific assay method to measure the hormone and receptor status to investigate further about AUB. With today's medical advents combined with increasing awareness about gynecological problems, women gain access to most of the diagnostic and therapeutic modalities. Therefore the prevalence AUB has significantly reduced to 10% in our country. However, there is a need to evaluate the endometrial changes specific for ovulatory and anovulatory causes of AUB at the histological level. This evaluation would throw light in to the hormonal influences on the endometrium and also help formulate therapeutic strategies to manage AUB.

Objectives

This study was carried out to evaluate the histomorphological pattern of endometrium in Abnormal Uterine Bleeding.

METHODOLOGY

Study setting

This study was carried out as a cross sectional study in the Department of Pathology and Department of Gynaecology and Obstetrics in our medical college hospital, Salem for a period of two years between July 2012 and 2014.

Study participants

All the patients who were clinically diagnosed with Abnormal Uterine Bleeding during the study period were included in the study.

Inclusion Criteria

Age between 21-55 years
Willing to participate in the study

Exclusion Criteria

Adolescent girls between 10-20 years of age
Pregnant women
Women with established causes of bleeding

Ethical approval and informed consent

Approval was obtained from the Institutional Ethics Committee prior to the commencement of the study. Each participant was explained in detail about the study and informed consent was obtained prior to the data collection.

Data Collection

A structured interview schedule was used to obtain information regarding the reproductive and gynaecological history among the study participants. Endometrial biopsy sample was taken on the third week of menstrual cycle. The criteria for selection of the endometrial biopsy sample for the study were as follows:

- Sufficient sample
- Biopsy taken from the fundal mucosa
- Sample devoid of any artifact
- Samples with no evidence of degeneration and necrosis
- Properly oriented samples
- Presence of whole layer of functionalis with superficial lining

The samples received were checked for adequate fixation, fixed in 10% formalin of quantity 20 times the volume of endometrial issue fragments. The biopsy material was carefully examined for the presence of mucus & large blood clots, and, if found were removed. With the help of cloth mesh the sample was washed in physiological saline and placed in the capsule with the help of one arm of blunt forceps, and sent for tissue processing in automated tissue processor, with the conventional timing. One slide stained with conventional Haematoxylin & Eosin, second slide with Per iodic acid Schiff method and third slide was stained with Gomeri's method for reticulin stain.

Mcmanus method for pas reaction [6]

- Sections were dewaxed in xylene and brought to water.
- Treated with periodic acid, for 5 minutes
- Washed in several changes of distilled water
- Covered with schiff's solution for 15 minutes.
- Washed in running tap water for 10 minutes.
- Nucleus stained with Harris hematoxylin for 2 minutes and differentiated in acid alcohol and blueing done with tap water.
- Dehydrated in graded alcohol, cleared in xylene and mounted in resinous mountant.
- Staining was done also with controls.

Gomeri's method for reticulin fiber [7]

Sections were dewaxed and brought to water. They were treated with 1% potassium permanganate aolution for 2 minutes. After rinsing in tap water, the sections were bleached in 2% potassium metabisulphite solution and again rinsed in tap water. They were then treated with 2% iron alum for 2 minutes and further

washed in several charges of distilled water. The Sections were dewaxed and brought to water. They were treated with 1% potassium permanganate aolution for 2 minutes. After rinsing in tap water, The sections were then placed in coplin jar of silver solution for 1 minute and washed in several changes of distilled water. Further to this, they were reduced in 4% aqueous formalin solution for 3 minutes and rinsed in tap water and toned in 0.2% gold chloride solution for 10 minutes followed by rinsing in tap water. The sections were then placed in coplin jar of silver solution for 1 minute and washed in several changes of distilled water. Further to this, they were reduced in 4% aqueous formalin solution for 3 minutes and rinsed in tap water and toned in 0.2% gold chloride solution for 10 minutes followed by rinsing in tap water.

The slides were then treated with 2% potassium metabisulphite solution for 1 minute. After this, the sections were rinsed in tap water and treated with 2% sodium thiosulphate solution for 1 minute and further rinsed in tap water and counterstained with eosin. This was then dehydrated in graded alcohol, cleared in xylene and mounted in resinous mountant. Staining was done also, with the controls.

After staining slides were analysed and the sample biopsies were divided in to two groups, anovulatory and ovulatory. Then each group categorized to the sample and complete AUB classification was done and the findings were correlated with the classical histomorphological findings in AUB and analysed [8].

RESULTS

A total of 100 cases were analyzed in this study and majority of the cases consisted of anovulatory disturbances (62%) followed by ovulatory disturbances (34%) (Figure-1). The histomorphological categorization of the cases is given in Table-1. While majority of the anovulatory cases were irregular proliferation (27%), majority of the ovulatory cases were deficient secretory state with co-ordinated delay (11%).

The characteristics of anovuatory disturbances is given in Table-2. It was observed that 61.3% of the anovulatory cases consisted of abnormal morphology. The histomorphology of the anovulatory cases showed low columnar epithelium in majority of the cases (53.2%) followed by irregular in 33.9% of the cases.

The glandular presentation among the cases is given in Table-3. Glandular dilatation and mitosis were present in 74.07% of the irregular proliferation cases, while variations in the development state was observed in 90% of deficit proliferation.

The pattern of presentation of stroma is given in Table-4. It was observed that plenty of stromal mitosis was present in 70.4% of irregular proliferation while stromal edema was increased in 66.6% of irregular proliferation cases. Stromal hemorrhage was present in 20% of deficit proliferation cases.

The other histomorphological features of the AUB cases are given in Table-5. Decrease in the spiral arterioles were present in 74.07% of the irregular proliferation cases, while reticulin staining was present in 40% of deficit proliferation cases. PAS staining was positive in 20% of deficit proliferation cases.

Particulars related to glandular presentation among ovulatory disturbances are given in Table-6. Irregular glandular dilatation was present in all cases of deficit secretory state with dissociated delay and in irregular shedding cases (100%) glandular dilatation was seen with abnormal architecture of glands. Glandular mitosis was found to be 54.5% for deficient secretory state with co-ordinated delay (54.5%).

The stromal changes among ovulatory disturbances are given in Table-7. Predecidual changes were decreased in all cases of deficient secretory state with dissociated delay and in 85.7% of irregular shedding cases. Stromal edema was present in 66.6% of the deficient secretory state with dissociated delay. Endometrial granulocyte was present in 71.4% of irregular shedding cases while PAS positivity was present in all cases of late secretory phase, deficient secretory phase with dissociated delay and irregular shedding (100%).

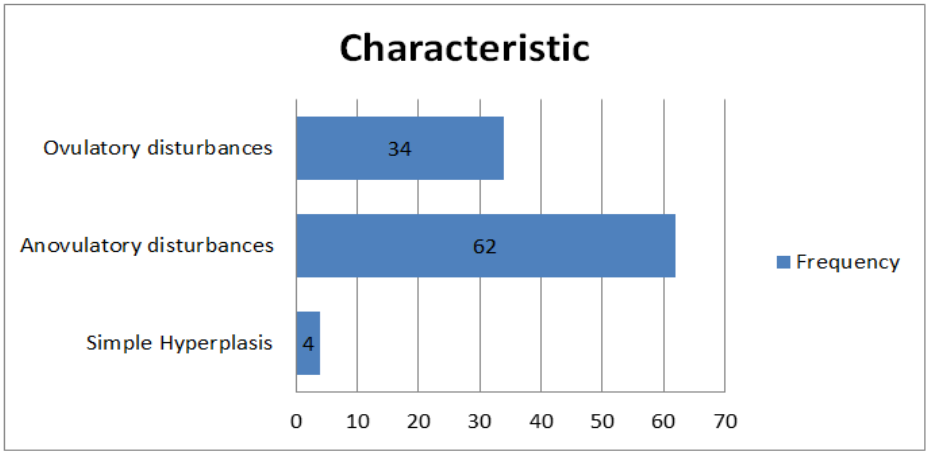

Fig-1: Distribution of participants by pathology

Table-1: Histomorphological Categories

S. No	Categories	Frequency (n=100)	Percentage (%)
1	**Anovulatory/ / proliferative**		
1.1	Early Proliferative	9	9
1.2	Mid Proliferative	10	10
1.3	Late Proliferative	5	5
1.4	Atrophy	1	1
1.5	Deficient Proliferation	10	10
1.6	Irregular proliferation	27	27
2	**Ovulatory/- secretory**		
2.1	Late Secretory phase	6	6
2.2	Deficient Secretory state with co-ordinated delay	11	11
2.3	Deficient Secretory state with dissociated delay	9	9
2.4	Irregular shedding	7	7
2.5	Dysmenorrhea Membranacea	1	1
3	Simple Hyperplasia	4	4

Table-2: Characteristics of anovulatory cases

S. No	Characteristic	Frequency N (62)	Percentage (%)
1	**Patten in anovulatory disturbances**		
	Abnormal morphology	38	61.30
	Normal morphology	24	38.70
2	**Histomorphology of anovulatory**		
	Pecudostrstisfied	8	12.9
	Irregular	21	33.9
	Low columnar	33	53.2

Table-3: Glandular presentation among the anovulatory cases

S. No	Characteristic	N	Frequency	Percentage (%)
1	**Glandular dilatation**			
	Irregular proliferation	27	20	74.07
	Deficit proliferation	10	1	10.0
2	**Glandular mitosis**			
	Irregular proliferation	27	20	74.05
	Mild proliferative	10	7	70.0
	Deficit proliferation	10	8	80
3	**Glandular secretary activity**			
	Irregular proliferation	27	0	0.0
	Deficit proliferation	10	2	20.0
4	**Variation in development state observed**			
	Deficit proliferation	10	9	90.0
	Irregular proliferation	27	12	44.45

Table-4: Stromal presentation among the anovulatory cases

S. No	Characteristic	N	Frequency	Percentage (%)
1	**Stromal mitosis plenty**			
	Irregular proliferation	27	19	70.4
	Deficit proliferation	10	3	30.00
2	**Stromal oedema increased**			
	irregular proliferation	27	18	66.6
	Deficit proliferation	10	2	20.0
3	**Stromal haemorrhage**			
	Deficit proliferation	10	2	20.0
	Irregular proliferation	27	3	11.11
4	Stromal cells deficit proliferation	10	10	100.0
5	Stromal cell in irregular proliferation	27	27	100.0

Table-5: Histomorhphological features of anovulatory disturbances

S. No	Characteristic	N	Frequency	Percentage (%)
1	**Spiral arteriole decreased**			
	Deficit proliferation	10	2	20.0
	Irregular proliferation	27	20	74.07
2	**Inflammatory cells**			
	Irregular proliferation	27	1	3.7
	Deficit proliferation	10	0	0.0
3	**Reticulin stain**			
	Focal dissolution of Deficit proliferation	10	4	40.0
	Irregular proliferation	27	3	11.11
4	**PAS stain positivity**			
	Deficit proliferation	10	2	20.0
	Irregular proliferation	27	0	0.0

Table-6: Glandular presentation among ovulatory disturbances

S. No	Characteristic	N	Frequency	Percentage (%)
1	**Irregular glandular dilatation**			
	Deficient secretory state with co-ordinated delay	11	4	36.3
	Deficient secretory state with dissociated delay	9	9	100.0
	Irregular shedding,	7	7	100.0
2	**Glandular mitosis**			
	Deficient secretory state with co-ordinated delay	11	6	54.5
	Deficient secretory state with dissociated delay	9	2	22.2
	Irregular shedding	7	3	42.8
3	**Glandular secretory activity**			
	Deficient secretory state with co-ordinated delay	11	4	36.35
	Deficient secretory state with dissociated delay	9	8	88.88
	Irregular shedding	7	7	100.0
4	**Development of the glands**			
	Deficient secretory state with co-ordinated delay	11	9	81.81
	Deficient secretory state with dissociated delay	9	0	0.0
	Irregular shedding	7	7	100.0

Table-7: Stromal changes in ovulatory disturbances

S. No	Characteristic	N	Frequency	Percentage (%)
1	**Predecidual changes were decreased**			
	Deficient secretory state with dissociated delay	9	9	100.0
	Deficient secretory state with co-ordinated delay	11	6	54.55
	Irregular shedding	7	6	85.75
2	**Stromal mitosis absent**			
	Deficient Secretory state with dissociated delay	11	0	0.0
	Deficient Secretory state with dissociated delay	9	0	0.0
	Irregular shedding	7	0	0.0
3	**Stromal oedema**			
	Deficient secretory state with co-ordinated delay	11	1	9.05
	Deficient secretory state with diassociated delay	9	6	66.65
4	**Stromal haemorrhage seen**			
	Deficient secretory with coordinated delay	11	4	36.35
	Deficient secretory state dissociated delay	9	3	33.35
	Irregular shedding	7	1	14.28
5	**Endometrial granulocyte**			
	Deficient secretory with coordinated delay	11	7	63.65
	Deficient secretory state dissociated delay	9	2	22.22
	Irregular shedding	7	5	71.42
6	**Spiral arteriole appeared**			
	Deficient secretory with coordinated delay	11	9	81.85
	Deficient secretory state dissociated delay	9	2	22.2
	Irregular shedding	7	5	71.42
7	**Inflammatory cell presence**			
	Deficient secretory with coordinated delay	11	2	18.15
	Deficient secretory state dissociated delay	9	1	9.09
	Irregular shedding	7	1	14.28
8	**Reticulin stain**			
	Deficient secretory with coordinated delay	11	5	45.45
	Deficient secretory state dissociated delay	9	6	66.65
	Irregular shedding	7	7	100.0
9	**PAS stain positivity**			
	Late secretory phase	6	6	100.0
	Deficient secretory with coordinated delay	11	9	81.81
	Deficient secretory state dissociated delay	9	9	100.0
	Irregular shedding	7	7	100.0

DISCUSSION

Histomorphological analysis of endometrial biopsy is the simple and effective mode of evaluation in Abnormal Uterine Bleeding (AUB). Light microscopic examination of the endometrial biopsy is the principle technique used to evaluate the endometrium.

There is a need for morphological evaluation of the endometrium, even in the presence of normal hormonal levels, in order to observe for the presence of irregular shedding or refractoriness with receptor deficiency. This is essential as it determines the choice of management of AUB. Histomorphological analysis significantly helps in categorization of AUB, with the exception of receptor deficiency and estrogen withdrawal bleeding.

In our study, all the AUB case showed significant morphological findings. Only six cases showed classical late secretory feature which corresponds to the day menstrual cycle on which, the endometrial biopsy was done. In cases of luteinized unruptured follicle syndrome the endometrium showed normal secretory phase In our study, about 25% of the participants' endometrial findings did not correspond to their menstrual cycle. In addition, there was a deficient secretory state with co-ordinated delay in 9.40% and irregular shedding in 7.30% of the participants. Moreover, the atrophic endometrium showed sparse glands, arranged in loosely packed spindle cell stroma and glands lined by low cubical epithelium. However, patients with hypomenorrhoea occasionally have normal endometrium. In our study, 25.0% cases showed normal proliferative at the 24th day of menstrual cycle.

In the ovulatory disturbances the glandular epithelium often shows delay in maturation and contains decreased secretory material. In our study, the hormonal influence on the glandular epithelium in anovulatory and ovulatory disturbances showed changes in 90.60% cases. The glandular dilatation in anovulatory and ovulatory disturbances is brought about by constriction of narrowed neck part, because of the abnormally proliferating epithelium protruding into the neck and blocking it. It is the predominant feature in irregular proliferation as well as in deficient secretory state. In our study, the glandular dilatation in anovulatory and ovulatory disturbances was seen in 39.60% cases.

Though mitosis is rare in deficient proliferation, the glandular mitosis clearly denotes adequate estrogenic influence in anovulatory disturbances and in ovulatory disturbances its presence indicates deficiency of progesterone influence. If mitosis still persists in late secretory phase, it is due to either hyperestrogenism or hyperprogesteronism. In our study the glandular mitosis was seen in 58.35% cases, which appeared to be statistically highly significant.

The glandular secretory activity is clearly, the feature of glandular epithelial maturation. It is often found to be decreased or absent in insufficient hormonal levels. In our study, the glandular secretory activity was seen only in 19.80% cases ($p<0.05$). Variation in developmental state of glands is the predominant feature in irregular proliferation and deficient secretion. In our study, variation in developmental state of gland was found in 29.15% cases.

The stromal cell in anovulatory disturbances is often spindle shaped, consisting of white, scanty cytoplasm and elongated nuclei with dense chromatin. In ovulatory disturbances the stromal cell will not mature with normal secretion. In our study the stromal cells were small, spindle shaped with scanty cytoplasm and elongated nucleus with dense chromatin and showed little maturation with less glycogen content, even in the ovulatory phases. These features of decreased hormonal influence were seen in 93.75% cases and were statistically highly significant ($p<0.05$).

The stromal mitosis clearly indicates proliferation though mitosis in stromal cells extent up to the early secretory phase. With progesterone influence the mitotic activity cease to appear In our study, the mitotic activity was seen in 37.50% cases and was statistically significant. Stromal oedema is a dominant feature of estrogenic influence In our study the presence of oedema in anovulatory disturbance was very minimal in amount and in ovulatory disturbances it was observed in deficient secretory state with dissociated delay. In total, stromal oedema was seen in 53.1% cases.

Stromal hemorrhage denotes the dissolution of blood vessels, which may be brought about by contraction of smooth muscle cells cells in arterioles and hyaline degeneration with loss of elastic fibers In our study the stromal haemorrhage was found, only in 13.55% cases, which is statistically significant with reference to the absence of stromal haemorrhage in other cases.

Endometrial granulocytes thought to secrete, proteolytic hormone- relaxin and dissolves the reticulin network. In our study, the endometrial granulocyte was found 62% of cases with ovulatory disturbances.

Spiral arteriole development is mainly due to the influence progesterone. In progesterone deficient status, the spiral arterioles will be under developed. In our study, under developed spiral arteriole was seen in 71.8% of cases. The reticulin network shows evidence of hormonal influence as the maturation takes place under the control of progesterone. The fibers grow thicker and denser with increasing progesterone levels. As long as the corpus luteum continues to produce progesterone, the reticulin network remains intact. Its dissolution denotes decreased progesterone level. In our study, the dissolution of dense network corresponding to the hormonal status in different categories was found in 59.40% which, appeared to be statistically significant.

The PAS stain, highlight the glycogen granules in the glandular as well as stromal cells and also stain the ground substances which contain mucopolysccharides In anovulatory and ovulatory disturbances the decreased secretion or disparity in distribution of secretion indicates functional disturbances. In cases of atrophy the glycogen and glycoprotein will not be found. In our study the PAS stain positivity was present in 46.58% which was statically significant ($p<0.05$).

CONCLUSION

Histomorphological analysis of the cases on our study highlighted certain important diagnostic features in the interpretation of endometrial biopsy. An accurate histomorphological diagnosis and an interpretation of hormonal status from the endometrial pattern, definitely gives an edge to clinician in the management of DUB. The glandular epithelial lining, glandular epithelial mitosis, secretory activity of epithelial cells, stromal cell morphology, stromal cell mitosis and pattern of reticulin network were found to be, very significant in the classification of endometrium in AUB. Other features like stromal hemorrhage, Stromal oedema, endometrial granulocytes and variation in the development of glands were found to be less reliable features for interpretation of endometrial biopsy.

In the modern era of minimally invasive surgical and diagnostic procedures, there is a growing need for the histopatholosgist to develop acumen for diagnosis of AUB. This study has extensively highlighted the variations in the endometrial presentation for an in depth analysis of AUB.

Declaration
Conflict of interest -Nil
Funding -Nil
Ethical approval -Nil

REFERENCES

1. Strauss, J. F., & Barbieri, R. L. (2013). *Yen & Jaffe's Reproductive Endocrinology E-Book: Physiology, Pathophysiology, and Clinical Management*. Elsevier Health Sciences.
2. Gompel, C., & Silverberg, S. G. (1994). *Pathology in gynecology and obstetrics*. Lippincott Williams & Wilkins.
3. Mills, S. E. (2009). Sternberg's Diagnostic surgical pathology Text Book, (5th edi), Lippincot Williams and Wilkins, London.
4. Nucci, M. R., & Oliva, E. (2009). *Gynecologic Pathology: A Volume in the Series: Foundations in Diagnostic Pathology*. Elsevier Health Sciences.
5. Dallenbach-Hellweg, G. (2003). Functional Disturbances of Endometrium - text book (5th edi), Springer, Berlin.
6. Bancroft, J. D., & Gamble, M. (Eds.). (2008). Staining technique PAS Reaction McManus Method-text book, Bancroft-Theory and Practice of Histological Technique, (6th edi), Churchill Livingstone, London.
7. Gamble, M., & Wilson, I. (2008). The hematoxylins and eosin. *Theory and practice of histological techniques*, *6*, 121-34.
8. Munro, M. G., Critchley, H. O., Broder, M. S., & Fraser, I. S. (2011). FIGO classification system (PALM-COEIN) for causes of abnormal uterine bleeding in nongravid women of reproductive age. *International Journal of Gynecology & Obstetrics*, *113*(1), 3-13.