Study of ER, PR and Ki-67 Expression in Different Histopathological Pattern of Endometrial Hyperplasia

*Fardousi F,[1]Sultana SS,[2] Kaizer N,[3] Dewan RK,[4] Jinnah MS,[5] Jeba R,[6] Haque WS,[7] Alam SM.[8] Hussain M[9]

Endometrial hyperplasia is one of the major gynaecological problem in peri and postmenopausal women worldwide. It deserves special attention because of its relationship with endometrial carcinoma. To study the histopathological pattern of endometrial hyperplasia in peri and postmenopausal women and their association with ER, PR and Ki-67 expression by immunohistochemistry is essential for early diagnosis with effective treatment. Out of 70 endometrial hyperplasia cases, 53 cases were simple endometrial hyperplasia without atypia, 8 cases were simple endometrial hyperplasia with atypia, 6 cases were complex endometrial hyperplasia without atypia and 3 cases were complex endometrial hyperplasia with atypia. ER expression in majority (35) of the cases of endometrial hyperplasia was between 51-100%. All the cases (3) of complex endometrial hyperplasia with atypia express ER less than 10%. The PR expression in majority of the cases (37) of endometrial hyperplasia was between 51-100%. All the cases (3) of complex endometrial hyperplasia with atypia expressed PR less than 40%. Ki-67 expression in majority of the cases of all types of endometrial hyperplasia 32 (78%) was less than 35%. All the cases (24) of simple endometrial hyperplasia without atypia had less than 35% Ki-67 expression. Only 6 cases had Ki-67 expression of more than 35%. In this study, 5 cases had negative (0%) Ki-67 expression. It can be inferred that after evaluating the ER, PR and Ki-67 expression, conservative treatment with progestogen and GnRH-agonists can be effective in selected cases of simple endometrial hyperplasia without or with atypia and complex endometrial hyperplasia without atypia.

[Journal of Histopathology and Cytopathology, 2018 Jul; 2 (2):74-84]

Key words: Endometrial hyperplasia, Gynecological problem, Peri and postmenopausal women, Histopathological pattern, Immunohistochemistry, ER, PR and Ki-67 expression

1. **Dr. Farzana Fardousi, Lecturer, Department of Cytopathology, National Institute of Cancer Research and Hospital (NICRH), Mohakhali, Dhaka. farzanafardousi@yahoo.com.*
2. *Dr. Sk Salowa Sultana, Assistant Professor, Department of Pathology, Ad Din Women's Medical College. Dhaka.*
3. *Dr. Nahid Kaizer, Associate Professor (CC), Department of Pathology, Shahabuddin Medical College.*
4. *Professor Dr. Rezaul Karim Dewan, Head of the Department of Pathology, Dhaka Medical College.*
5. *Dr. Mohammed Shahed Ali Jinnah, Associate Professor, Department of Pathology, Dhaka Medical College.*
6. *Dr. Ruksana Jeba, Associate Professor, Department of Pathology, Dhaka Medical College.*
7. *Lt Col (Dr) Wasim Selimul Haque. Classified spl in Pathology, Armed Forces Institute of Pathology.*
8. *Prof Brig Gen Dr. SM. Mahbubul Alam, Ex Deputy Commandment, Armed Forces Institute of Pathology, Senior consultant- Histopathology, Apollo Hospitals Dhaka.*
9. *Professor Dr. Maleeha Hussain, Ex Head of the Department of Pathology, Dhaka Medical College.*

**For corespondence*

Introduction

Endometrial hyperplasia has a high risk for malignant transformation into endometrial carcinoma in peri and postmenopausal women.[1,2] Eendometrial hyperplasia usually develops due to the continuous estrogen stimulation unopposed by progesterone. In the years before menopause, women may have numerous cycles without ovulation (anovulatory) during which there is sustained and unopposed estrogen activity. It is also likely that hormone replacement therapy (HRT) consisting of estrogen without progesterone may lead to endometrial hyperplasia.[3] The postmenopausal endometrium which despite being atrophic, retain a weak proliferative pattern for many years probably as a response to continuous low level of estrogenic stimulation. These are at a higher risk of progression to endometrial hyperplasia and subsequently to endometrial malignancy. Although the diagnosis of endometrial hyperplasia can be made by histopathological examination; the immunohistochemistry aids for the prognosis, in order to establish the best treatment.[4]

The receptor status for estrogen and progesterone in endometrial hyperplasia can be a prognostic indicator in the treatment. The expression of estrogen and progesterone receptors varies in different types of endometrial hyperplasia. Several studies have found that, all types of hyperplasia present a smaller number of receptors compared to the endometrium in proliferative phase, but higher compared with secretory endometrium and endometrial carcinoma.[5] The proliferative activity of endometrial hyperplasia can be examined by using an antibody to Ki-67 antigen, a non-histone protein, a well established marker of proliferative activity. The expression of the human Ki-67 protein is strictly associated with cell proliferation.

The endometrium of reproductive-aged women undergoes cyclic developmental changes in response to the steroids - estrogen and progesterone. The highest score of estrogen and progesterone receptors are observed in the epithelial and stromal cells of the normal uterine endometrium at the early proliferative phase; then, throughout the secretory phase, the ER and PR scores decline. Again, the highest score of estrogen receptors and progesterone receptors are observed in non-atypical hyperplasia and lowest score of estrogen receptors and progesterone receptors are observed in atypical hyperplasia.[6]

Ferrandina et al. (2005)[7] found that, ER and PR positive cases of endometrial hyperplasias had a statistically significant association with the clinicopathological parameters, which correlates with a more favorable prognosis. In addition, the hormone receptor status appears to correlate with the treatment response to the progesterone therapy. This finding may be of particular clinical importance, since almost all endometrial hyperplasia cases contain estrogen/ progesterone receptors and progesterone therapy could be beneficial in this cases.[8]

The expression of the human Ki-67 protein is strictly associated with cell proliferation. During interphase, the antigen can be exclusively detected within the nucleus, whereas in mitosis most of the protein is relocated to the surface of the chromosomes. The fact that the Ki-67 protein is present during all active phases of the cell cycle (G(1), S, G(2), and mitosis), but is absent from resting cells G(0), makes it an excellent marker for determining the so-called growth fraction of a given cell population. The highest score of Ki-67 expression is observed in atypical hyperplasia and lowest score of Ki-67 expression is observed in non-atypical hyperplasia. Scholzen and Gerdes, (2009)[9] in

their study showed that, cell proliferation is highly correlated with Ki67 expression.

Perez-Medina et al have observed that, ER, PR and Ki-67 expression rate, along with the histopathological features of endometrial hyperplasia are important prognostic factors.[10] After evaluating the ER, PR and Ki-67 expression rate, they have shown that, for all cases of endometrial hyperplasia, the general treatment protocol i.e. hysterectomy is not rationally justified. Patients who want to complete their family or who have heart disease or surgical or anaesthetic risk history, if they have positive ER, PR expression and low Ki-67 expression; they can be selected for conservative treatment with progestogens and GnRH-agonists. These patients should be followed for 5 years by biopsies every 6 months.

Hysterectomy is usually recommended for cases with endometrial hyperplasia.[2] Several studies have revealed that less than 5 % of simple and complex non-atypical hyperplasia cases undergo malignant transformation in the long term (20 years), whereas this percentage increases to 30 % when there is an atypical hyperplasia.[11] So, the rationality of hysterectomy in the treatment of endometrial hyperplasia in general needs to be evaluated.

The evaluation of receptor status by markers for hormone receptors (estrogen and progesterone receptors) and proliferative activity by proliferative marker (Ki-67) in patients with endometrial hyperplasia can predict the treatment option in selected cases.[12 -16]

Methods

This is a descriptive cross sectional study which was carried out at the Department of Pathology, Dhaka Medical College, and Dhaka during the period of January 2013 to December 2014. A total of 110 peri and postmenopausal women with dysfunctional uterine bleeding or postmenopausal bleeding who underwent D&C or hysterectomy were screened. A total of seventy histopathologically diagnosed cases of endometrial hyperplasia who met the enrollment criteria (inclusion & exclusion criteria) were included in this study. Among 70 cases, endometrial curettage biopsy specimens were 45 and hysterectomy specimens were 25. Routine Hematoxylin and Eosin staining was done on all 70 samples. Out of these 70 cases, ER, PR and Ki-67 immunostaining was done on 41 cases. Ethical clearance was taken for this study from institutional ethical committee of Dhaka Medical College. Each patient was interviewed before collection of the specimen and relevant information was recorded in a prescribed clinical proforma. Detail history with particular attention to age, clinical features, age at menarche, parity, obesity, history of contraceptives, history of hormone replacement therapy, history of diabetes, history of hypertension, history of estrogen producing ovarian tumor, age at menopause were taken.

Histopathological examination

The gross examination of specimens, routine tissue processing and Hematoxylin & Eosin staining were done at the Department of Pathology, Dhaka Medical College.

Microscopic analysis

According to WHO classification endometrial hyperplasia is classified into simple endometrial hyperplasia without atypia, Simple endometrial hyperplasia with atypia, Complex endometrial hyperplasia without atypia, Complex endometrial hyperplasia with atypia.[17]

Immunohistochemical examination

Immunostaining for ER, PR and Ki-67 was done at AFIP (Armed Forces Institute of

Pathology, Dhaka). A total of 41 cases were selected for immunohistochemical examination for ER, PR and Ki-67 expression. For immunostain, 3cases of proliferative phase of endometrium, 3 cases of secretory phase of endometrium and 3 cases of well differentiated endometrial carcinoma were enrolled as control.

All the data were recorded in data sheet along with patient's clinical findings. The main objectives of this study were to observe the histopathological pattern of endometrial hyperplasia in peri and postmenopausal women and their association with ER, PR and Ki-67 expression by immunohistochemical method.

1) Primary antibody –Mouse monoclonal Anti-Human Estrogen Receptor, clone 1D5, code M7047(1:60 dilution), Mouse monoclonal Anti-Human Progesterone Receptor, clone PgR636(1:50 dilution) and Mouse monoclonal antibody against ki67 antigen(1:100 dilution) were used as primary antibody.
2) Secondary antibody – Envision (ready to use, Dako), was used as secondary antibody.
3) Positive control –
i. High grade breast carcinoma was taken as positive control for ER and PR.
ii. Follicular hyperplasia of lymph node was taken as positive control for Ki-67.

Immunohistochemical analysis
For ER and PR expression in endometrial tissue: [18]

Score for proportion staining		Score for staining intensity	
1.	0-25 % nuclei	1.	Absent or weak staining
2.	26-75 % nuclei	2.	Strong staining
3.	≥ 76 % nuclei	3.	Very strong staining

Cat – I	Total score 2	Immuno negative
Cat – II	Total score 3-4	Immuno reactive
Cat – III	Total score 5-6	Immuno reactive

For Ki-67 expression in endometrial tissue:
The patients are divided into two groups:
1. Low Ki-67 expression (≤ 35 % cells are Ki-67 positive)
2. High Ki-67 expression (> 35 % cells are Ki-67 positive)

(Nuclear staining in endometrial glandular epithelial cells are evaluated)

Statistical analysis
Statistical analyses of the results were obtained by using window based computer software devised with Statistical Packages for Social Sciences (SPSS-16). Percentages were calculated to find out the proportion of the findings. The results are presented in Tables and Figures.

Results
A total of 70 histopathologically diagnosed endometrial hyperplasia cases were included in this study. Among 70 cases endometrial curettage biopsy specimens were 45 and hysterectomy specimens were 25. Out of all endometrial hyperplasia cases, 53 cases were simple endometrial hyperplasia without atypia, 8 cases were simple endometrial hyperplasia with atypia, 6 cases were complex endometrial hyperplasia without atypia and 3

cases were complex endometrial hyperplasia with atypia. Out of these all the 8 cases of simple endometrial hyperplasia with atypia, 6 cases of complex endometrial hyperplasia without atypia, 3 cases of complex endometrial hyperplasia with atypia and 24 cases of simple endometrial hyperplasia without atypia.

Table I: Distribution of the study patients by immunohistochemistry findings (ER %) (n=50)

Immunohisto-chemistry findings (ER %)	PP (n=3)	SP (n=3)	SEH without atypia (n=24)	SEH with atypia (n=8)	CEH without atypia (n=6)	CEH with atypia (n=3)	End. Ca. grade 1 (n=3)	Total (n=50)	
								n	%
≤10	0	1	0	0	0	3	1	5	10.0
11-20	0	0	0	0	0	0	1	1	2.0
21-30	0	0	0	0	0	0	1	1	2.0
31-40	0	1	0	0	0	0	0	1	2.0
41-50	0	0	3	0	0	0	0	3	6.0
51-60	0	0	2	2	0	0	0	4	8.0
61-70	0	1	2	0	1	0	0	4	8.0
71-80	0	0	10	0	0	0	0	10	20.0
81-90	2	0	5	5	4	0	0	16	32.0
91-100	1	0	2	1	1	0	0	5	10.0
Total	3	3	24	8	6	3	3	50	100

(PP- Proliferative phase of endometrium, SP- Secretory phase of endometrium, SEH without atypia- Simple endometrial hyperplasia without atypia, SEH with atypia- Simple endometrial hyperplasia with atypia, CEH without atypia - Complex endometrial hyperplasia without atypia, CEH with atypia- Complex endometrial hyperplasia with atypia and End. Ca. grade 1- Endometrial carcinoma Grade-I)

Distributions of the patients with PR expression (%). PR expression in majority of the cases (37) of endometrial hyperplasia is between 51-100%. All the cases (3) of complex endometrial hyperplasia with atypia express PR less than 40% (Table II).

Table II: Distribution of the study patients by immunohistochemistry findings (PR %) (n=50)

Immunohist-ochemistry findings (PR %)	PP (n=3)	SP (n=3)	SEH without atypia (n=24)	SEH with atypia (n=8)	CEH without atypia (n=6)	CEH with atypia (n=3)	End. Ca. grade 1 (n=3)	Total (n=50)	
								n	%
≤10	1	0	0	0	0	0	0	1	2.0
11-20	0	0	0	0	0	2	1	3	6.0
21-30	0	0	0	0	0	0	0	0	0.0
31-40	0	0	1	0	0	1	0	2	4.0
41-50	0	0	0	0	0	0	0	0	0.0
51-60	0	0	3	0	0	0	0	3	6.0
61-70	0	0	2	0	0	0	1	3	6.0
71-80	0	0	6	0	0	0	0	6	12.0
81-90	2.0	1	6	5	1	0	1	16	32.0
91-100	0	2	6	3	5	0	0	16	32.0
Total	3	3	24	8	6	3	3	50	100

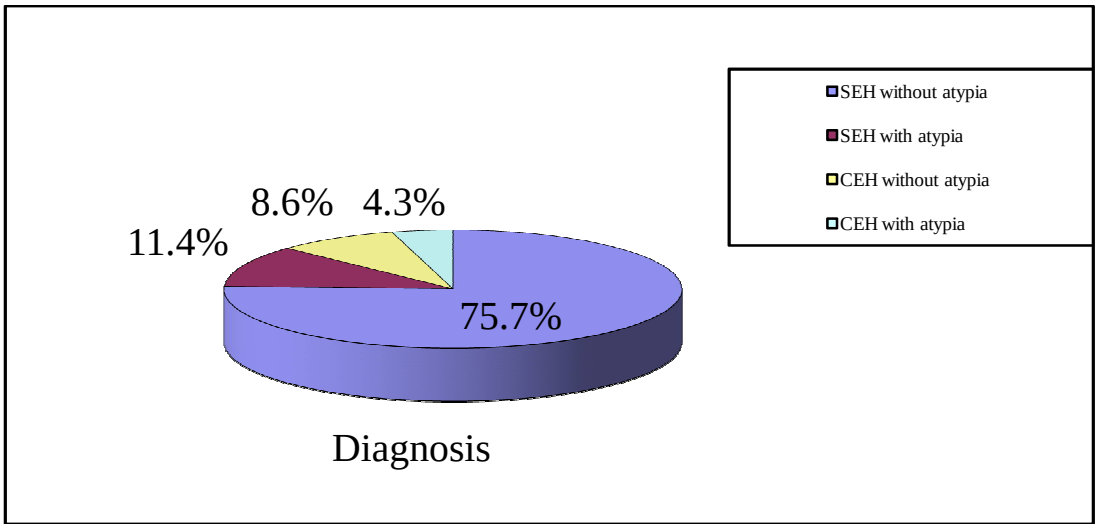
(PP- Proliferative phase of endometrium, SP- Secretory phase of endometrium, SEH without atypia- Simple endometrial hyperplasia without atypia, SEH with atypia- Simple endometrial hyperplasia with atypia, CEH without atypia - Complex endometrial hyperplasia without atypia, CEH with atypia- Complex endometrial hyperplasia with atypia and End. Ca. grade 1- Endometrial carcinoma Grade-I)

Table-III shows distributions of the patients with Ki-67 expression (%). Ki-67 expression in majority of the cases of all types of endometrial hyperplasia 32(78%) is less than 35%.

Table III: Distribution of the study patients by immunohistochemistry findings (Ki-67%) (n=50)

Immunohisto -chemistry findings (KI-67%)	PP (n=3)	SP (n=3)	SEH without atypia (n=24)	SEH with atypia (n=8)	CEH without atypia (n=6)	CEH with atypia (n=3)	End. Ca. grade 1 (n=3)	Total (n=50)	
								n	%
0	0	0	4	0	1	0	0	5	10.0
1-10	0	2	10	1	1	0	0	14	28.0
11-20	0	1	1	2	2	0	0	6	12.0
21-30	0	0	3	0	1	0	0	4	8.0
31-35	0	0	6	3	0	0	1	12	24.0
36-50	0	0	0	0	1	2	0	1	2.0
51-60	1	0	0	2	0	1	1	5	10.0
61-70	0	0	0	0	0	0	0	0	0.0
71-80	1	0	0	0	0	0	0	1	2.0
81-90	1	0	0	0	0	0	0	1	2.0
91-100	0	0	0	0	0	0	1	1	2.0
Total	3	3	24	8	6	3	3	50	100

(PP- Proliferative phase of endometrium, SP- Secretory phase of endometrium, SEH without atypia- Simple endometrial hyperplasia without atypia, SEH with atypia- Simple endometrial hyperplasia with atypia, CEH without atypia - Complex endometrial hyperplasia without atypia, CEH with atypia- Complex endometrial hyperplasia with atypia and End. Ca. grade 1- Endometrial carcinoma Grade-I)



(SEH without atypia- Simple endometrial hyperplasia without atypia, SEH with atypia- Simple endometrial hyperplasia with atypia, CEH without atypia - Complex endometrial hyperplasia without atypia, CEH with atypia- Complex endometrial hyperplasia with atypia I)

Figure 1. Pie chart showing distribution of the patients by diagnosis (n=70)

Pie chart showing the commonest diagnosis in 70 patients was SEH without atypia (75.7%) followed by SEH with atypia (11.4%) (Fig 1).

Discussion

Endometrial hyperplasia has a significant place in gynecological morbidity in women of reproductive age (10% to 18%).[19] Endometrial hyperplasia is associated with menstrual irregularities and anaemia in women and poses a high risk for malignant transformation into endometrial cancer.[20] World wide endometrial cancer is the most common gynecological cancer in peri and postmenopausal women.[21,22] The incidence of endometrial adenocarcinoma not only has remained high but in recent years has tended to significantly increase in many countries, including Bangladesh.[19,23-32]

In this study, the commonest diagnosed lesion was simple endometrial hyperplasia without atypia which was 53(75.7%) followed by simple endometrial hyperplasia with atypia 8(11.4%), complex endometrial hyperplasia without atypia 6(8.6%) and then complex endometrial hyperplasia with atypia 3(4.3%).

From the present study it was observed that, in all types of endometrial hyperplasia except complex endometrial hyperplasia with atypia, the ER expression was lower than proliferative phase but higher than secretory phase of endometrium. However, the ER expression in complex endometrial hyperplasia with atypia was lower than any other type of endometrial hyperplasia or proliferative phase or secretory phase of endometrium. It was even lower than endometrial carcinoma. Similar study done by Ilie et al. (2011)[33] also found that the ER expression in different types of endometrial hyperplasia and endometrial cancer is lower than proliferative phase but higher than secretory phase of endometrium.[33] Found that, the expression of ER in different types of

endometrial hyperplasia was much lower (41.5%) than the present study (75%) except that of complex endometrial hyperplasia with atypia. This variation may be due to inclusion of menopausal patients in their study.

In this study it was observed that, the mean ER expression was 75±15.1% in simple endometrial hyperplasia without atypia. Similar observations was found by Goncharenko et al. (2013)[20] they have found 75.6% ER expression in simple endometrial hyperplasia without atypia. This may be due to inclusion of perimenopausal women in their study like present study. It was also observed that the mean ER expression was 10±0% in complex endometrial hyperplasia with atypia in the current study. These findings differed from Goncharenko et al. (2013)[20] they found 65.2% ER expression in complex endometrial hyperplasia with atypia. This variation may be due to inclusion of many patients in their study at their reproductive age.

From the present study it was observed that, the PR expression in all types of endometrial hyperplasia except complex endometrial hyperplasia with atypia, was higher than proliferative phase of normal endometrium and endometrial carcinoma ,but they were lower than secretory phase of normal endometrium. However, the PR expression was lowest in complex endometrial hyperplasia with atypia. In the study done by Ilie et al. (2011)[33] found that, the PR expression in different types of endometrial hyperplasia was lower than proliferative phase but higher than secretory phase of endometrium and endometrial carcinoma. This variation may be due to inclusion of only menopausal women in their study. In this current study it was observed that, the mean PR expression was 80.8±16.4% in simple endometrial hyperplasia without atypia. This finding was nearly similar to the observations found by Goncharenko et al. (2013).[20] They have found 69.3% PR expression in simple endometrial hyperplasia without atypia as they also included perimenopausal patients in their study.

It was observed in our study that the mean PR expression was 25±8.7% in complex endometrial hyperplasia with atypia. These findings differed from Goncharenko et al. (2013)[20] they found 44.3% PR expression in complex endometrial hyperplasia with atypia. This may be due to inclusion of many patients in their study at their reproductive age. This result is in concordance with that of, Uchikawa et al. (2003)[5] in which it was found that, the expression of ER and PR decreased in endometrial hyperplasia compared with normal proliferative endometrium.

In present study, the expression of Ki-67 was compared with proliferative phase (73.3±12.6%) and secretory phase (12.3±5.8 %) of normal endometrium and with endometrial carcinoma, grade I (66.7±30.5%). We found that, the presence of mitotic activity in normal endometrium was observed more in the proliferative phase than the secretory phase. Mitotic activity in neoplastic and hyperplasic endometrium was low compared with proliferative phase of normal endometrium, but more than the endometrium in secretory phase.This findings are similar to the study done by Ilie et al. (2011).[33]

In the present study, the mean Ki-67 expression was 36.3±18.5% in simple endometrial hyperplasia with atypia. This finding are nearly similar to the study done by Goncharenko et al. (2013)[20] who found upto 50% Ki-67 expression in simple endometrial hyperplasia with atypia. This may be due to inclusion of perimenopausal patients like our study. The present study result is in concordance with that of Uchikawa et al. (2003)[5] in which they found that, ki-67

expression was more in atypical hyperplasia (50%) than non-atypical hyperplasia (10%).

In patients with simple endometrial hyperplasia with atypia and complex endometrial hyperplasia without atypia, Ki-67 expression can predict the treatment protocol. The patients with low Ki-67 expression can be selected for conservative treatment with hormone therapy and with high Ki-67 expression should undergo hysterectomy. In complex endometrial hyperplasia with atypia, they should preferably be treated by hysterectomy operation. If, patients who want to complete their family and have low Ki-67 expression, they can be temporarily treated by conservative treatment. After delivery, they must undergo surgical intervention.[16]

The treatment response with hormone therapy depends on expression of ER & PR. So, before hormonal intervention, ER & PR should be evaluated, because in few cases, where expressions are low, have the chance of poor response to treatment. [10]

Limitation of the study

The study population was selected from the department of Pathology of Dhaka Medical College Hospital. But there are many patients with endometrial hyperplasia who are attending other hospitals than DMCH. Therefore, the sample lacks representation of the population. Thus, the study place was selected purposively and the respondents, those are interviewed, were attended a particular department of a specific hospital.

Conclusion

The study revealed that highest ER & PR expression were observed in complex endometrial hyperplasia without atypia and lowest ER & PR expression were observed in complex endometrial hyperplasia with atypia. Again, highest Ki-67 expression was observed in complex endometrial hyperplasia with atypia and lowest Ki-67 expression was observed in simple endometrial hyperplasia without atypia. In complex endometrial hyperplasia with atypia, if patient desires to complete the family, after evaluation of ER, PR and Ki-67 expression, conservative treatment can be given and allowed to conceive. After delivery, if Ki-67 expression remains high, then hysterectomy must be done.

Recommendations

A large follow–up study is recommended for patients of endometrial hyperplasia selected for conservative treatment with progestogen and GnRH-agonists. Monitoring should be done by observing the Ki-67 expression in these patients. If the Ki-67 expression increases, they should be treated by surgical intervention.

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