ORIGINAL RESEARCH

Assessing the level of epithelial and stromal cell activity in hyperplastic and normal endometrium of perimenopausal and late reproductive females

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ABSTRACT

Background: In the hyperplasia cases of the endometrium, benign stromal and endometrial cells are seen in groups depicting the change in their growth patterns. **Aim:** The present study aimed to assess the role of BCL-2 and p53 (apoptosis markers) and hypoxia as shown by Hif-1α in developing the hyperplastic processes of the endometrium. **Methods:** The study assessed 92 tissues from the endometrium of perimenopausal age range females aged 45-55 years and late reproductive-aged females in the age range of 35-45 years that were arranged in 6 groups along with two control groups for late reproductive and perimenopausal age range. 1, 2, 3, 4, 5, and 6 included 16, 18, 11, 15, 19, and 13 females respectively from normal endometrium, females from late reproductive age having hyperplasia without atypia, females from late reproductive age having atypical hyperplasia, perimenopausal females with no proliferative tissue changes, perimenopausal females with no atypical hyperplasia, and perimenopausal females with atypical hyperplasia. All females were subjected to immunohistochemical assessment. **Results:** Increased Hif-1α levels in endometrial cells having atypical hyperplasia depicting hypoxia of endometrium which can lead to pathology. However, it was statistically non-significant, it can lead to atypia hyperplasia in females of perimenopausal and late reproductive age with Hif-1α of 2.07±0.05 and 1.87±0.07 units respectively. In perimenopausal and late reproductive-aged females, in epithelial cells, raised p53 levels were seen. In females with atypia in late reproductive and perimenopausal age, BCL-2 indicator levels were high compared to females without hyperplasia (p=0.01). **Conclusion:** The present study concludes that in the development of hyperplastic processes of the endometrium, hypoxia plays a vital role along with the apoptotic marker changes seen in the endometrium tissues.

Keywords: Apoptosis, perimenopausal females, reproductive age, BCL-2, p53

INTRODUCTION

HPE or hyperplastic processes of endometrium depict a group of altered stromal and endometrial cells that are benign and governed by different heterogeneous factors. The principle genetic molecular mechanism causing the development of hyperplastic processes of the endometrium is poorly understood and comprises complex processes. However, it is reported that it is linked to genetic, immunochemical, and molecular alterations in the endometrium, inappropriate progesterone, and imbalanced estrogen levels. In a few HPE cases, these factors can cause malignant alterations in humans.[1]

Previous literature data focus on the molecular genetic carcinogenesis theory which is focused on viral tumor origin, radiation, and chemical theory. The tumor arising has self-sustaining mutagenic stimuli to some extent along with no sensitivity to the anti-mitogenesis signals and constant division. These tumors also have typical characteristics of tissue change, the tendency of metastasis and tissue invasion, decreased apoptosis ability, and unlimited potential for replication.[2]

Processes and mechanisms that lead to tissue hypoxia are considered vital in the existing literature data. Hypoxia is considered to play a vital role in the progression and development of the malignancy which was first reported in 1924 by O. Warburg. Many factors are considered to be associated with decreased oxygen levels at the tissue-level response. One of these factors is Hif-1α, the activity of which is accelerated with decreased blood oxygen concentration. This makes it a suitable marker for tissues to adapt to hypoxia. Previous literature has extensively assessed the Hif-1α levels in the tumor cells. Research is still in progress to assess the effect of Hif-1α and to attain therapeutics to work on the process of malignancy development.[3]

Cell proliferation and apoptosis help maintain cell homeostasis in the human body. Previous literature research reported that in hyperplastic conditions, these processes undergo alterations, particularly in cases of atypical endometrial hyperplasia. However, other groups of researchers have not been able to confirm these findings. Also, it has been reported that all subjects with endometrial hyperplasia progress to malignancy.[4]

Assessing the DNA damage usually corresponds to a mutation of the TP53 gene which can further cause the formation of the p53 protein which is usually non-functional. p53 protein is normally seen in nearly half of the malignant cells and the mutations in the p53 gene are usually the early process in carcinogenesis. It is vital to acknowledge that p53 mutation alone cannot independently lead to carcinoma formation, and, other factors largely govern the presence and characteristics of the tumors.[5]

The process of cell division is a complex phenomenon comprising various mechanisms that help in controlling the cell growth and maintenance of genetic stability. In a few mutations, it is necessary to have adequate control of the genetic state. In cases of alterations, proliferation is temporarily barred and in cases of irreversible damage, apoptosis or programmed cell death can be seen. This process is largely governed by p53 protein; however, apoptosis antagonists also play a vital role.[6]

The cells are protected from the apoptosis processes by the cell survival factor, Bcl-2 gene product, and BCL-2 protein. Literature data has depicted that high BCL-2 protein levels can show the properties of oncogenesis and are seen in atypical endometrial hyperplasia. Other previous studies have shown reduced BCL-2 expression in endometrial adenocarcinoma and atypical hyperplasia. This warrants the need for further studies for a better understanding of the role and mechanisms of BCL-2 in further potential therapeutic interventions and oncogenesis.[7]

Hyperplastic processes of endometrium depict the risk of oncological pathology development with nearly 35-70% of atypia cases showing the further progression of carcinoma in 1-3 years. Also, recent literature research suggested the possible link in hyperplastic processes of endometrium in females of late reproductive age and leads to the development of conditions like PCOS (polycystic ovarian syndrome), obesity, and hypo menstrual syndrome in their offspring.[8]

The present study aimed to assess, compare, and determine the correlation between BCL-2, p53, and Hif-1α markers in the epithelial and stromal cells in atypical, hyperplastic, and normal endometrium in females of different age groups.

MATERIALS AND METHODS

The present study aimed to assess, compare, and determine the correlation between BCL-2, p53, and Hif-1α markers in the epithelial and stromal cells in atypical, hyperplastic, and normal endometrium in females of different age groups. The study was done at Department of Obstetrics and Gynecology, JNU Institute of Medical Science and Research Center, Jaipur, Rajasthan after the clearance was taken from the concerned Institutional Ethical committee. The study subjects were recruited from the Department of Obstetrics and Gynecology of the Institute.

The study included 92 female subjects who fulfilled the inclusion criteria for the study. In all the included female subjects, the endometrial tissue samples were collected. The females were divided into two groups comprised of perimenopausal females in the age range of 45-55 years and late reproductive age females in the age range of 35-45 years. The study also included two control groups one for the perimenopausal females termed Group 4 and the other for the reproductive age group made Group 1 made comparison criteria.

Group 1 comprised 16 females with no pathological changes in the endometrium, group 2 included 18 females from the late reproductive age group having hyperplastic changes without atypia, group 3 included 11 subjects from late reproductive age having atypical endometrial hyperplasia, group 4 had 15 females from perimenopausal age with no proliferative tissue changes, group 5 had 19 perimenopausal females with no atypical hyperplasia, and group 6 had 13 females of perimenopausal age having atypical hyperplasia.

All the endometrial samples were subjected to immunohistochemical analysis. The samples were collected by scraping which was then fixed in 10% formalin which was neutral buffered and the samples were then fixed in paraffin blocks. Primary specific antibodies were made by antibodies to p53 and BCL-2. Evaluation and expression of p53 was done by evaluation of the nuclei stained per 100 cells percentage in the stroma and the epithelium of the glands.

The BCL-2 expression was assessed by evaluating the intensity of the brown color where the intensity of staining was assessed based on the following scale where 0, +, ++, and +++ denoted no staining, weak staining, moderate staining, and strong staining

respectively. The intensity of the color was assessed in points and the H-score was calculated. cDNA PCR (polymerase chain reaction) was used at the mRNA level to assess Hif-1α expression.

The data gathered were assessed statistically using SPSS software version 21.0 (IBM Corp., Armonk, NY, USA) using MS Excel. The level of significance was taken at $p<0.05$.

RESULTS

The present study aimed to assess, compare, and determine the correlation between BCL-2, p53, and Hif-1α markers in the epithelial and stromal cells in atypical, hyperplastic, and normal endometrium in females of different age groups.

The study included 92 females who were divided into two groups comprised of perimenopausal females in the age range of 45-55 years and late reproductive age females in the age range of 35-45 years. The study also included two control groups one for the perimenopausal females termed Group 4 and the other for the reproductive age group made Group 1 made comparison criteria.

The study results showed that non-significant results were seen for Hif-1α levels and decreased concentration of oxygen in the endometrial tissues was taken as a vital characteristic for the expression of Hif-1α. This was vital concerning the atypical processed presence. In the females of the perimenopausal age range, the Hif-1α level was 2.07±0.05, and in the late reproductive age group, it was 1.87±0.07 c.u. These variations can affect the pathological manifestations development at the tissue level, particularly in cases where hyperproliferation is seen.

The results of the present study depict that reduced concentration of oxygen in the tissues of the endometrium can be the main cause of atypia and hyperproliferation in the cells of the endometrium which can further change the levels of BCL-2 which is a protein that helps in cell apoptosis regulation and p53 levels which is a protein vital in cell proliferation regulation.

On assessing the levels of p53 markers in the stromal and epithelial cells of the tissues from the endometrium, statistical alterations were seen in p53 expression in h females of late reproductive age having normal endometrium. However, in the stromal cells, no expression factors were seen depicting that these changes might be attributed to inactive processes. Also, the tissue collection and processing timings are vital during the assessment of prognostic values and research conduction as this can affect the result accuracy. In the present study, p53 expression in epithelial cells was 2.14% and 2.07±0.05 which was not seen in the stromal cells. In the perimenopausal age range, insignificant indicators depicted these changes. The present study results showed a significant correlation between the possibility and dependence of endometrium tissue proliferation and expression of p53 protein. This was seen in atypical hyperplasia where increased p53 expression in endometrial tissue stroma cells was 19.86% and 6.35±2.24 and in the late reproductive period was 35.54% and 7.97±0.56 which was significantly more predominant than similar indicators of hyperplasia cases of the endometrium and of unchanged proliferative processes. Similar results were seen in atypical endometrial hyperplasia of perimenopausal females in stromal and epithelial cells with 18.65% and 5.55±2.45 and 43.46% and 6.35±3.69.

These results showed that endometrial tissue proliferation is affected by endometrial hyperplasia and can be seen along with specific changes which can be seen along with specific changes which can potentially cause malignancy when other contributing factors are present. However, this process is not always attributed to other factors that can inhibit or promote the system.

Concerning BCL-2 expression in age-related changes in tissues of the endometrium, peculiar disturbances were seen in stromal and epithelial cells. These alterations were statistically significant concerning marker presence in stromal and epithelial cells where they get corrected in stromal cells preventing proliferative changes and progress in epithelial cells which was significant with $p<0.05$. These changes depict immunohistochemical disorders in stromal and epithelial cells, however, these pathological changes can be corrected by the stromal cells.

On BCL-2 expression analysis, it was seen that changes are seen in both stromal and epithelial cells. However, epithelial cell alterations were more significant than the alterations in the stromal cells where more significant changes are seen or minor fluctuations are seen in the expression of BCL-2 markers. These rectifications are vital in the perimenopausal females which are considered for systemic analysis which brings metabolic changes along with hormonal changes in the female body that can affect the potential malignancy and general condition of the females.

With the proliferation process strengthening, an increase is seen in the BCL-2 apoptosis inhibitor indicator which is considered atypical and is statistically non-significant. On comparison of the BCL-2 indicator assessment depicting +++, it was seen that this is rarely seen in Group 3 compared to Group 1 with $p=0.01$. Similar results were seen in the endometrial tissues of females from Group 6 and Group 1 with $p=0.03$ for both in comparison to the control group.

Assessing the alterations in the expression of the indicator shows cumulative results, hence, it is vital to assess the indicator in complex ways when assessing the malignancy probability in the tissue cells of the endometrium. The present study reported a difference in BCL-2 expression in cells having endometrial disruption like atypical hyperplasia and hyperplasia cases of the endometrium. Alterations in epithelial

cells of endometrium in atypical and glandular hyperplasia seen a peculiar reduction in the process of apoptosis inhibition, especially in cases of atypical hyperplasia where it was 15.38% in late reproductive females and was 7.69% in perimenopausal females.

Table 1: p53 expression in the stromal and epithelial cells of the endometrial tissues

Groups	Stromal cells		Epithelial cells	
	Mean ± S. D	Percentage (%)	Mean ± S. D	Percentage (%)
1	-	-	2.07±0.05	2.14
2	2.04±0.04	1.63	2.56±0.07	7.06
3	6.35±2.24	19.86	7.97±0.56	35.54
4	2.06±0.04	0.92	2.06±0.05	2.35
5	2.03±0.08	1.54	6.76±1.05	9.76
6	5.55±2.45	18.65	6.35±3.69	43.46

Table 2: BCL-2 expression in the stromal and epithelial cells of the endometrial tissues

Groups	Stromal cells		Epithelial cells	
	Mean ± S. D	Percentage (%)	Mean ± S. D	Percentage (%)
1	2.2±0.05	2.02	10.22±16.94	10.26
2	2.2±0.4	1.73	12.31±14.02	12.35
3	2.6±0.05	2.05	10.40±11.95	10.44
4	3.2±0.8	3.85	11.03±14.01	11.99
5	4.2±0.05	4.82	9.37±11.04	11.03
6	3.2±0.4	3.94	13.96±16.03	9.37

Table 3: BCL-2 expression in the epithelial cells of the endometrial tissues

Markers	Group 1 n=16 (%)	Group 2 n=18 (%)	Group 3 n=11 (%)	Group 4 n=15 (%)	Group 5 n=19 (%)	Group 6 n=13 (%)
BCL-2 (0)	0	0	1 (9.09)	0	1 (5.26)	1 (7.69)
BCL-2 (+)	1 (6.25)	3 (16.66)	4 (36.36)	1 (25)	2 (10.52)	5 (38.46)
BCL-2 (++)	8 (50)	10 (55.55)	5 (45.45)	8 (53.33)	11 (57.89)	2 (15.38)
BCL-2 (+++)	7 (43.75)	5 (27.77)	1 (9.09)	6 (40)	5 (26.31)	1 (7.69)

DISCUSSION

The study results showed that non-significant results were seen for Hif-1α levels and decreased concentration of oxygen in the endometrial tissues was taken as a vital characteristic for the expression of Hif-1α. This was vital concerning the atypical processed presence. In the females of the perimenopausal age range, the Hif-1α level was 2.07±0.05, and in the late reproductive age group, it was 1.87±0.07 c.u. These variations can affect the pathological manifestations development at the tissue level, particularly in cases where hyperproliferation is seen. It was also seen that the present study depicts that reduced concentration of oxygen in the tissues of the endometrium can be the main cause of atypia and hyperproliferation in the cells of the endometrium which can further change the levels of BCL-2 which is a protein that helps in cell apoptosis regulation and p53 levels which is a protein vital in cell proliferation regulation. These results were consistent with the studies of Artyomenko VV et al[9] in 2021 and Artyomenko V et al[10] in 2022 where authors reported that Hif-1α and decreased oxygen levels were reported to be higher in females of late reproductive age.

In the present study, the levels of p53 markers in the stromal and epithelial cells of the tissues from the endometrium, statistical alterations were seen in p53 expression in the females of the late reproductive age having normal endometrium. However, in the stromal cells, no expression factors were seen depicting that these changes might be attributed to inactive processes. Also, the tissue collection and processing timings are vital during the assessment of prognostic values and research conduction as this can affect the result accuracy. In the present study, p53 expression in epithelial cells was 2.14% and 2.07±0.05 which was not seen in the stromal cells. In the perimenopausal age range, insignificant indicators depicted these changes. The present study results showed a significant correlation between the possibility and dependence of endometrium tissue proliferation and expression of p53 protein. This was seen in atypical hyperplasia where increased p53 expression in endometrial tissue stroma cells was 19.86% and 6.35±2.24 and in the late reproductive period was 35.54% and 7.97±0.56 which was significantly more predominant than similar indicators of hyperplasia cases of the endometrium and of unchanged proliferative processes. Similar results were seen in atypical endometrial hyperplasia of perimenopausal females in stromal and epithelial cells with 18.65% and 5.55±2.45 and 43.46% and 6.35±3.69. These findings were in agreement with the findings of Hutt S

et al[11] in 2019 and Zhu C et al[12] in 2019 where authors reported that p53 expressions are significantly seen in epithelial endometrial cells of perimenopausal and late reproductive-aged females.

For BCL-2 expression in age-related changes in tissues of the endometrium, peculiar disturbances were seen in stromal and epithelial cells. These alterations were statistically significant concerning marker presence in stromal and epithelial cells where they get corrected in stromal cells preventing proliferative changes and progress in epithelial cells which was significant with $p<0.05$. These changes depict immunohistochemical disorders in stromal and epithelial cells, however, these pathological changes can be corrected by the stromal cells. On BCL-2 expression analysis, it was seen that changes are seen in both stromal and epithelial cells. However, epithelial cell alterations were more significant than the alterations in the stromal cells where more significant changes are seen or minor fluctuations are seen in the expression of BCL-2 markers. These rectifications are vital in the perimenopausal females which are considered for systemic analysis which brings metabolic changes along with hormonal changes in the female body that can affect the potential malignancy and general condition of the females. These results correlated with the studies by Levakov SA et al[13] in 2018 and Lee SH et al[14] in 2021 where authors reported increased BCL-2 expression in epithelial and stromal cells of the endometrium of females of late reproductive and perimenopausal females with hyperplastic changes.

Along with the proliferation process strengthening, an increase is seen in the BCL-2 apoptosis inhibitor indicator which is considered atypical and is statistically non-significant. On comparison of the BCL-2 indicator assessment depicting +++, it was seen that this is rarely seen in Group 3 compared to Group 1 with $p=0.01$. Similar results were seen in the endometrial tissues of females from Group 6 and Group 1 with $p=0.03$ for both in comparison to the control group. These findings were in line with Zinovkin DA et al.[15] in 2019 and Bily OM et al.[16] in 2016 where authors suggested that along with an increase in the proliferation process, an increase is seen in the BCL-2 apoptosis inhibitor.

The alterations in the expression of the indicator show cumulative results, hence, it is vital to assess the indicator in complex ways when assessing the malignancy probability in the tissue cells of the endometrium. The present study reported a difference in BCL-2 expression in cells having endometrial disruption like atypical hyperplasia and hyperplasia cases of the endometrium. Alterations in epithelial cells of endometrium in atypical and glandular hyperplasia seen a peculiar reduction in the process of apoptosis inhibition, especially in cases of atypical hyperplasia where it was 15.38% in late reproductive females and was 7.69% in perimenopausal females. These findings correlated with Artyomenko VV et al.[17] in 2020 and Zaporozhan VN et al.[18] in 2012 where authors reported expression of BCL-2 expression in cells having endometrial disruption like atypical hyperplasia and hyperplasia endometrium.

CONCLUSION

Considering its limitations, the present study concludes that in the development of hyperplastic processes of the endometrium, hypoxia plays a vital role along with the apoptotic marker changes seen in the endometrium tissues. The immunochemical and genetic markers play a vital role in assessing the efficacy of treating subjects with carcinoma by providing comprehensive knowledge about the severity and progression of the disease.

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