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LEVEL OF EPITHELIAL AND STROMAL CELL ACTIVITY IN HYPERPLASTIC AND NORMAL ENDOMETRIUM OF POST REPRODUCTIVE FEMALES

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ABSTRACT

Background: HPE, or hyperplastic processes of the endometrium, refers to a collection of altered stromal and endometrial cells that are benign and controlled by a variety of diverse variables.

Aim: To examine the link between BCL-2, p53, and Hif-1 α markers in epithelial and stromal cells in atypical, hyperplastic, and normal endometrium in females of various ages.

Methods: The study examined 92 endometrial tissues from perimenopausal females aged 45-55 years and late reproductive-aged females aged 35-45 years, which were divided into six groups and two control groups for late reproductive and perimenopausal age ranges. 1, 2, 3, 4, 5, and 6 included 16, 18, 11, 15, 19, and 13 females from normal endometrium, females from late reproductive age with hyperplasia without atypia, females from late reproductive age with atypical hyperplasia, perimenopausal females with no proliferative tissue changes, perimenopausal females with no atypical hyperplasia, and perimenopausal females with atypical hyperplasia. All females were submitted to immunohistochemistry analysis.

Results: Increased Hif-1 α levels in endometrial cells with atypical hyperplasia suggest hypoxia of the endometrium, potentially leading to disease. Although not statistically significant, it can cause atypia hyperplasia in females of perimenopausal and late reproductive age, with Hif-1 α levels of 2.07 ± 0.05 and 1.87 ± 0.07 units, respectively. In epithelial cells of perimenopausal and late reproductive-age females, p53 levels were shown to be elevated. Females with atypia in late reproductive and perimenopausal age had higher BCL-2 indicator levels than females without hyperplasia ($p = 0.01$).

Conclusion: The current study suggests that hypoxia, in conjunction with apoptotic marker alterations in endometrium tissues, plays an important role in the development of endometrial hyperplastic processes.

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

INTRODUCTION

HPE, or hyperplastic processes of the endometrium, refers to a collection of altered stromal and endometrial cells that are benign and controlled by a variety of diverse variables. The primary genetic molecular mechanism responsible for the development of endometrial hyperplastic processes is poorly understood and involves complicated mechanisms.

However, it has been associated to genetic, immunochemical, and molecular changes in the endometrium, as well as incorrect progesterone and estrogen levels. In a few HPE instances, these variables can result in malignant changes in humans.¹

Previous literature data focus on the molecular genetic carcinogenesis hypothesis, which focuses on viral tumor origin, radiation, and chemical theory. The emerging tumor contains self-sustaining mutagenic stimuli to some extent, as well as little sensitivity to anti-mitogenesis signals and continuous division. These tumors also exhibit typical features such as tissue alteration, metastasis and tissue invasion, reduced apoptotic ability, and virtually limitless capability for replication.²

The processes and mechanisms that lead to tissue hypoxia are regarded as critical in the extant literature. Hypoxia is thought to have an important part in the growth and development of the malignancy, which was initially described in 1924 by O.Warburg. Many variables are thought to be related with decreasing oxygen levels at the tissue level in response.

Hif-1 α activity increases with lower blood oxygen concentration. This makes it an appropriate signal for tissues to adapt to hypoxia. Previous studies have thoroughly examined Hif-1 α levels in tumor cells. Research is ongoing to analyze the impact of Hif-1 α and develop treatments to address malignancy development.³

Cell proliferation and apoptosis contribute to maintain cell homeostasis in the human body. Previous study has shown that in hyperplastic situations, these mechanisms change, especially in cases of atypical endometrial hyperplasia. However, other groups of researchers have been unable to replicate similar findings. Furthermore, it has been stated that all patients with endometrial hyperplasia proceed to cancer.⁴

Assessing DNA damage generally results in a mutation of the TP53 gene, which can lead to the production of the non-functional p53 proteins. p53 protein is routinely found in almost half of malignant cells, and mutations in the p53 gene are frequently the first step in carcinogenesis. It is critical to recognize that p53 mutation alone cannot cause carcinoma development; additional variables heavily influence the existence and features of the malignancies.⁵ Cell division is a complicated process that involves a number of systems that aid in managing cell development and maintaining genetic integrity. A few mutations necessitate sufficient regulation of the genetic condition.

Proliferation is temporarily halted in the presence of changes, whereas irreparable damage results in apoptosis or programmed cell death. This process is primarily directed by the p53 protein, although apoptosis antagonists also play an important role.⁶ The cell survival factor, Bcl-2 gene product, and BCL-2 protein protect cells from apoptosis. According to the literature, high levels of BCL-2 protein can indicate oncogenesis and are found in atypical endometrial hyperplasia. Previous research has demonstrated that BCL-2 expression is lower in endometrial cancer and atypical hyperplasia. This highlights the need for more research to better understand the function and processes of BCL-2 in future therapeutic therapies and oncogenesis.⁷

Endometrial hyperplastic processes indicate a risk of developing oncological disease, with around 35-70% of atypia patients progressing to cancer within 1-3 years. Furthermore, new research has revealed a relationship between hyperplastic endometrial processes in females of late reproductive age and the development of disorders such as PCOS (polycystic ovarian syndrome), obesity, and hypomenstrual syndrome in their kids.⁸

This study examined the link between BCL-2, p53, and Hif-1 α markers in epithelial and stromal cells in atypical, hyperplastic, and normal endometrium in females of various ages.

MATERIALS AND METHODS

This study examined the link between BCL-2, p53, and Hif-1 α markers in epithelial and stromal cells in atypical, hyperplastic, and normal endometrium in females of various ages. The research participants were recruited from the Institute's Department of Obstetrics and Gynecology. The study comprised 92 female individuals who met the study's inclusion criteria. Endometrial tissue samples were taken from all of the included female individuals. Females were separated into two groups: perimenopausal females aged 45 to 55 and late reproductive age females aged 35 to 45.

The study also included two control groups, one for perimenopausal females, known as Group 4, and the other for the reproductive age group. Group 1 created comparison criteria. Group 1 included 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 the 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 perimenopause.

All endometrial samples underwent immunohistochemistry analysis. Scraping was used to collect the samples, which were subsequently fixed in 10% formalin that was neutrally buffered before being embedded in paraffin blocks. Primary specific antibodies were generated against p53 and BCL-2. The nuclei labeled per 100 cells percentage in the gland's stroma and epithelium were used to evaluate and express p53.

The intensity of the brown hue was used to measure BCL-2 expression, with 0, +, ++, and +++ indicating no staining, mild staining, moderate staining, and strong staining, respectively. The intensity of the color was measured in points and the H-score was determined. Hif-1 α expression was assessed at the mRNA level using cDNA PCR (polymerase chain reaction).

The collected data were statistically analyzed using SPSS software version 21.0 (IBM Corp., Armonk, NY, USA) and MS Excel. The criterion of significance was set at $p < 0.05$.

RESULTS

This study examined the link between BCL-2, p53, and Hif-1 α markers in epithelial and stromal cells in atypical, hyperplastic, and normal endometrium in females of various ages. The study included 92 females, separated into two groups: perimenopausal females aged 45-55 and late reproductive age females aged 35-45.

The study also included two control groups, one for perimenopausal females (Group 4) and another for the reproductive age group (Group 1), which served as comparison criteria. The study found no significant outcomes for Hif-1 α levels. A reduction in oxygen content in endometrial tissues was identified as a key factor for Hif-1 α expression. This was critical given the abnormal processed presence. Hif-1 α levels were 2.07 ± 0.05 in perimenopausal females and 1.87 ± 0.07 c.u. in late reproductive age groups. These changes can have an impact on the development of clinical symptoms at the tissue level, especially when hyperproliferation occurs.

The current study's findings show that a low concentration of oxygen in endometrial tissues can be the primary cause of atypia and hyperproliferation in endometrial cells, which can alter the levels of BCL-2, a protein that aids in cell apoptosis regulation, and p53, a protein critical in cell proliferation regulation. When p53 indicators were measured in the stromal and epithelial cells of endometrial tissues, significant differences were seen in p53 expression in h females of late reproductive age with normal endometrium.

However, no expression factors were detected in stromal cells, indicating that these alterations may be due to dormant mechanisms. Furthermore, the time of tissue collection and processing is critical for prognostic value evaluation and study, since this might alter the accuracy of the results. The study found that epithelial cells expressed p53 at 2.14% and 2.07 ± 0.05 , but stromal cells did not. In the perimenopausal age range, negligible signs showed similar alterations. The current study's findings revealed a substantial association between the possibility and dependency of endometrial tissue growth and p53 protein expression.

In atypical hyperplasia, p53 expression was significantly higher in endometrial tissue stroma cells (19.86% and 6.35 ± 2.24) and in the late reproductive period (35.54% and 7.97 ± 0.56), compared to similar indicators of hyperplasia cases of endometrium and unchanged proliferative processes. Similar findings were seen in perimenopausal females with atypical endometrial hyperplasia in stromal and epithelial cells, with 18.65% and 5.55 ± 2.45 and 43.46% and 6.35 ± 3.69 .

These findings revealed that endometrial tissue proliferation is influenced by endometrial hyperplasia and can be observed alongside specific modifications that might possibly lead to cancer when other contributing variables are present. However, this mechanism is not necessarily linked to other elements that might either block or stimulate the system.

BCL-2 expression in age-related alterations in endometrial tissues showed distinct abnormalities in stromal and epithelial cells. The presence of markers in stromal and epithelial cells was shown to be statistically significant. Correction in stromal cells prevented proliferative changes and progression in epithelial cells ($p < 0.05$). These alterations indicate immunohistochemistry problems in stromal and epithelial cells, yet these pathological changes can be repaired by stromal cells.

BCL-2 expression study revealed alterations in both stromal and epithelial cells. However, epithelial cell modifications were more dramatic than stromal cell alterations, which showed more major or minor oscillations in the expression of BCL-2 markers. These rectifications are critical in perimenopausal females who are evaluated for systemic analysis, which causes metabolic and hormonal alterations in the female body that might impact the possible malignancy and overall health of the females.

As the proliferation process progresses, the BCL-2 apoptosis inhibitor indicator increases, which is regarded unusual and statistically insignificant. When comparing the BCL-2 indication evaluation displaying +++, it was discovered that this occurs less frequently in Group 3 than in Group 1 ($p = 0.01$). Similar findings were seen in the endometrial tissues of females from Groups 6 and 1, with $p = 0.03$ for both when compared to the control group.

Assessing changes in the indicator's expression yields cumulative outcomes; hence, it is critical to assess the indicator in complicated ways when determining the likelihood of malignancy in endometrial tissue cells. The current study found a difference in BCL-2 expression in cells with endometrial disturbance, such as atypical hyperplasia and hyperplasia of the endometrium. Alterations in endometrial epithelial cells in atypical and glandular hyperplasia revealed a remarkable reduction in the process of apoptosis inhibition, particularly in cases of atypical hyperplasia, where it was 15.38% in late reproductive females and 7.69% in perimenopausal females.

DISCUSSION

The study found no significant outcomes for Hif-1 α levels. A reduction in oxygen content in endometrial tissues was identified as a key factor for Hif-1 α expression. This was critical given the abnormal processed presence. Hif-1 α levels were 2.07 ± 0.05 in perimenopausal females and 1.87 ± 0.07 c.u. in late reproductive age groups. These changes can have an impact on the development of clinical symptoms at the tissue level, especially when hyperproliferation occurs.

It was also discovered that the current study shows that a low concentration of oxygen in endometrial tissues can be the primary cause of atypia and hyperproliferation in endometrial cells, which can alter the levels of BCL-2, a protein that aids in cell apoptosis regulation, and p53, a protein critical in cell proliferation regulation. Previous investigations by Artyomenko VV et al⁹ in 2021 and Artyomenko V et al¹⁰ in 2022 found that females of late reproductive age have greater levels of Hif-1 α and lower oxygen levels.

In the current investigation, the levels of p53 indicators in the stromal and epithelial cells of endometrial tissues, statistical variations in p53 expression were detected in females of late reproductive age with normal endometrium. However, no expression factors were detected in stromal cells, indicating that these alterations may be due to dormant mechanisms. Furthermore, the time of tissue collection and processing is critical for prognostic value evaluation and study, since this might alter the accuracy of the results. The study found that epithelial cells expressed p53 at 2.14% and 2.07 ± 0.05 , but stromal cells did not. In the perimenopausal age range, negligible markers represented these alterations.

The current study's findings revealed a substantial association between the possibility and dependency of endometrial tissue growth and p53 protein expression. In atypical hyperplasia, p53 expression was significantly higher in endometrial tissue stroma cells (19.86% and 6.35 ± 2.24) and in the late reproductive period (35.54% and 7.97 ± 0.56), compared to similar indicators of endometrial hyperplasia and unchanged proliferative processes. Similar findings were seen in perimenopausal females with atypical endometrial hyperplasia in stromal and epithelial cells, with 18.65% and 5.55 ± 2.45 and 43.46% and 6.35 ± 3.69 .

These findings were consistent with those of Hutt S et al¹¹ in 2019 and Zhu C et al¹² in 2019, who found that p53 expressions are considerably higher in epithelial endometrial cells of perimenopausal and late reproductive-aged females.

BCL-2 expression in age-related alterations in endometrial tissues showed distinct abnormalities in stromal and epithelial cells. The presence of markers in stromal and epithelial cells was shown to be statistically significant. Correction in stromal cells prevented proliferative changes and progression in epithelial cells ($p < 0.05$). These alterations represent immunohistochemistry diseases in stromal and epithelial cells, but these pathological changes can be repaired by the stromal cells.

. BCL-2 expression study revealed alterations in both stromal and epithelial cells. However, epithelial cell modifications were more dramatic than stromal cell alterations, which showed more major or minor oscillations in BCL-2 marker expression. These rectifications are critical in perimenopausal females who are evaluated for systemic analysis, which causes metabolic and hormonal alterations in the female body that might impact the possible malignancy and overall health of the females. These findings were consistent with those published by Levakov SA et al¹³ in 2018 and Lee SH et al¹⁴ in 2021, who found elevated BCL-2 expression in endometrial epithelial and stromal cells of late reproductive and perimenopausal females with hyperplastic alterations.

Along with the proliferation process strengthening, there is a rise in the BCL-2 apoptosis inhibitor indication, which is regarded unusual and statistically non-significant. When comparing the BCL-2 indication evaluation displaying +++, it was discovered that this occurs less frequently in Group 3 than in Group 1 ($p = 0.01$). Similar findings were seen in the endometrial tissues of females from Groups 6 and 1, with $p = 0.03$ for both when compared to the control group. These findings were consistent with Zinovkin DA et al. 15 in 2019 and Bily OM et al. 16 in 2016, who showed that a rise in the proliferation process is associated with an increase in the BCL-2 apoptosis inhibitor.

Alterations in indicator expression have cumulative effects, hence it is critical to examine indicator in complicated ways when assessing malignancy likelihood in endometrial tissue cells. The current study found a variation in BCL-2 expression in cells with endometrial disturbance, such as atypical hyperplasia and endometrial hyperplasia instances.

Alterations in endometrial epithelial cells in atypical and glandular hyperplasia revealed a remarkable reduction in the process of apoptosis inhibition, particularly in cases of atypical hyperplasia, where it was 15.38% in late reproductive

females and 7.69% in perimenopausal females. These findings were consistent with those published by Artyomenko VV et al. 17 in 2020 and Zaporozhan VN et al. 18 in 2012, who found expression of BCL-2 in cells with endometrial disturbance, such as 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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TABLES

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

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

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

Table 2: BCL-2 expression in the stromal and 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)

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