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Bcl-2 and Ki67 expression in endometrial polyps in postmenopausal women and their association with obesity

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Abstract

Background: Obesity is a global problem and is associated with numerous diseases, including endometrial disorders in postmenopausal women, such as adenocarcinoma, hyperplasias and endometrial polyps, and with risk of malignant transformation of these structures. We aimed to evaluate the influence of body mass index (BMI) on the cell proliferation markers (Bcl-2 and Ki67) on the endometrial polyps in postmenopause women. Methods: Prospective cross-sectional study using immunohistochemical analysis of the expression of the cell proliferation marker (Ki67) and an anti-apoptotic gene (Bcl-2) in endometrial polyps, in postmenopausal women. The polyps were identified by hysteroscopy and all were removed. The patients were divided into two groups related to BMI: a) BMI <29.9 kg/m2 (normal weight); b) BMI >30 kg/m2 (obese). We analysed the expression of these markers in relation to polyp size, histological type and time since menopause. The interpretation of the Ki67 and Bcl-2 expression data in the endometrial polyps accounted for the percentage of positive cells: score 1 (mild: less than 5% of cells showed expression); score 2 (moderate: between 5 and 50% of cells showed expression); score 3 (severe: more than 50% of cells showed expression). Statistical analysis was performed using the SPSS Statistics program for Windows v23, and the non-parametric Mann-Whitney test was used to analyse the Bcl-2 and Ki67 expression in the glandular and stromal components of the polyps. A significance level of $p<0.05$ was adopted for rejecting the null hypothesis. Results: There was greater glandular expression of Ki67 in hyperplastic polyps ($p=0.04$) and greater expression of Bcl-2 in the stroma of polyps larger than 2 cm ($p=0.04$). No difference in the Ki67 and Bcl-2 expression was found in the glands and stroma of the polyps when compared obese and non-obese postmenopause patients. Conclusion: Our findings suggest that the BMI may not have influence on the proliferation markers (Ki67 and Bcl-2) in the polyps of postmenopausal women.

Background

Obesity is a global problem and is associated with numerous diseases, including endometrial disorders in postmenopausal women, such as adenocarcinoma, hyperplasias and endometrial polyps, and with risk of malignant transformation of these structures.[1,2,3]

The modernisation of medical imaging tests in recent years has popularised the use of ultrasonography, which is a simple and practical method for studying the endometrium of postmenopausal women, mainly in cases of bleeding. Consequently, the prevalence of endometrial polyps in the general population, suggested by transvaginal ultrasound, has increased and ranges from 7.8 to 35%.[4]

The pathogenesis of endometrial polyps is not yet clear, due to both the wide variety of tumour types and their presence across different age groups. These polyps are considered risk factors for endometrial cancer, but malignancies are uncommon in histopathological studies. There is no consensus regarding the systematic removal of all identified polyps.[3]

Microscopic analysis of endometrial polyps identify glands and stroma. Although the endometrial tissue of some polyps has a functional cyclic reaction similar to that of the surrounding endometrium, in others an immature type of endometrium is observed, with irregular glands that are relatively unresponsive to hormones.[5]

However, in postmenopausal patients, Pinheiro A et al. (2014) found high expression of progesterone receptors (PRs), in both the glands and stroma of polyps from obese patients. It is possible that oestrogen activity in obese (known hyper-oestrogenic) and postmenopausal patients causes the appearance of PRs in both: glands and stroma.[6]

Polyps contain stroma and blood vessels with thick walls, which are commonly found in the endometrial basal layer. They can be classified as atrophic, hyperplastic or malignant.[7]

Bcl-2 is a gene that regulates the permeability of the outer mitochondrial membrane and has, mainly, an anti-apoptotic function. This gene prevents cell death and therefore leads to uncontrolled division, with accumulation of abnormal cells. Therefore, Bcl-2 is a cancer-promoting factor (facilitates the action of other oncogenes). Ki67 is a gene associated with cell proliferation via ribosomal synthesis of ribonucleic acid. Ki67 antigens are present during the active phase of the cellular cycle (G1, S, G2 and mitosis) and absent during the other phase (G0). Ki67 is an excellent marker of both cell proliferation and tumour aggressiveness.[8]

Obesity is a risk factor for the development of endometrial pathologies.[3] However, the effect of body weight on the pathogenesis of endometrial polyps in both apoptosis and cell proliferation is still unclear.

We decided to study the expression of tissue proliferation (Ki67) and apoptosis (Bcl-2) markers in endometrial polyps of postmenopausal women with body mass index (BMI) greater than or equal to 30 (obese) and to compare them with those of postmenopausal women with a BMI less than 29,9 (non-obese).

Methods

Patients and Methods

This was a prospective cross-sectional study with histological and immunohistochemical analysis of endometrial polyps excised from obese and non-obese postmenopausal women, between January 2015 and July 2018. Women without menstrual flow for at least one year were included. Obesity was defined when body mass index (BMI) was greater than 30 kg/m^2 and normal weight when her BMI was<29.9 kg/m^2.

The patients underwent video hysteroscopy under general anaesthesia and polyp excision with a bipolar electrode and Bettocchi system (2.9mm Karl Storz® rodlens system). Saline was used as a distension

medium. The mean procedure time was 25 minutes, and no anaesthetic or surgical complication was identified.

Patients with complex endometrial hyperplasia, endometrial cancer and hormone therapy users were excluded from the study. Fifty-nine patients were initially identified and 35 patients remained. Twenty-one patients were of normal weight and 14 in the obese group.

Age, time since menopause (years), BMI, histopathology and endometrial polyp size (≥2 cm or <2 cm) were analyzed

Analysis of Ki67 expression was performed by counting in the field of greatest expression ("hot spot") at 400x magnification. Bcl-2 analysis was performed with a panoramic count at 10x and 100x magnifications. Primary antibodies for Ki67 (clone MIB-1; Dako; code: IR626, lot: 20024778 expiration date of 05/2019) and for Bcl-2 (clone 124; Dako; code: IR614, lot: 20021755, expiration date of 02/2019) were used. The antibodies were prediluted and ready to use (RTU) mouse monoclonal antibodies. The Envision-Flex® Dako detection and visualisation system code SM802, lot 10101615, with expiration date of 05/2019 was used.

Three-micron thick histological sections were obtained with a manual microtome. Deparaffinisation and antigen retrieval were then performed in a single step with high pH buffer, Pt-link equipment (Dako®). There was a positive and negative control on all slides, with a palatine tonsil section with lymphoid hyperplasia as the positive control.

The interpretation of the Ki67 and Bcl-2 expression data in the endometrial polyps accounted for the percentage of positive cells: score 1 (mild: less than 5% of cells showed expression); score 2 (moderate: between 5 and 50% of cells showed expression); score 3 (severe: more than 50% of cells showed expression) (Figure 1).

The polyps were classified as atrophic (covered by low columnar to cuboidal glandular epithelium) and hyperplastic (response to oestrogen was similar to that of diffuse endometrial hyperplasia).

Statistical analysis was performed using the SPSS Statistics program for Windows v23 (IBM Corp., Armonk, NY), and the non-parametric Mann-Whitney test was used to analyse the Bcl-2 and Ki67 expression in the glandular and stromal components of the polyps. A significance level of $p<0.05$ was adopted for rejecting the null hypothesis.

The study was approved by the Research Ethics Committee of Gaffrée e Guinle University Hospital under number 1,610,750.

Results

Histological analysis of the endometrial polyps revealed that 28% (10/35) of the polyps were hyperplastic and 72% (25/35) were atrophic. Obese patients represents 22.8% (8/35) of study group, and 77.2%

(27/35) were of normal weight.

Analysis of Ki67 expression in the endometrial polyps (< 2cm) showed no difference between obese and non-obese women. Similarly, the Bcl-2 expression was not significantly different between groups (Table 1). Ki67 and Bcl-2 gene expression analyses were performed both in the glands and stroma (Figure 1).

In polyps greater then 2cm, we found hyperexpression of Bcl-2 gene in the stroma (p=0,04). Furthermore, the endometrial polyps were classified by histological type into atrophic and hyperplastic polyps for immunohistochemical analysis. In hypertrophic polyps, the Ki67 expression in glandular tissue was greater than that in atrophic polyps (p=0.04) (Table 2).

Time since menopause (greater or less than 10 years) was also not a relevant factor in the expression of cell proliferation (Ki67) and anti-apoptotic (Bcl-2) markers. We found greater Bcl-2 expression in the stroma of polyps larger than 2cm and greater glandular Ki67 expression in hyperplastic polyps than in atrophic ones.

Discussion

Our study showed no significant difference in Bcl-2 and Ki67 expression in the endometrial polyps of obese and non-obese patients, except in polyps greater than 2cm, with overexpression of Bcl-2 in stroma.

Doubt persists regarding the origin of endometrial polyps. The polyps were thought to originate in an area with intense proliferation of endometrial basal cells. Vinatier et al. (1996) postulated that the epithelial cells of the endometrial basal layer did not undergo apoptosis due to Bcl-2 overexpression.[9]

Inceboz US et al. (2006) found Bcl-2 overexpression in the glandular compartment of polyps in both pre- and postmenopausal obese women, suggesting that apoptosis blockage is more important than the proliferative action of Ki67.[10]

Arends (1999) verified overexpression of Bcl-2, which has anti-apoptotic functions, in the endometrial basal layer throughout the menstrual cycle. This finding explains the renewal capacity of the endometrium after menstruation, which allows basal cells to remain intact.[11]

Mittal et al. (1996) suggest that oestrogen receptor (OER) overexpression and PR underexpression are possible causes of the formation of endometrial polyps.[12] Taylor (2003) also found a significant increase in OER in polyps in the secretory phase of the menstrual cycle. Under normal conditions, OER synthesis is inhibited by the activity of circulating progesterone during this phase of the menstrual cycle.[5]

Taylor (2003) analysed the Bcl-2 and Ki67 expression in polyps during both the proliferative and secretory phases of the menstrual cycle. The researchers found Bcl-2 overexpression in the gland and stroma compared to the surrounding endometrium during the proliferative phase. There was no difference in

Ki67 expression at this stage of the cycle. During the secretory phase, there was no difference in expression of these genes relative to that in the surrounding endometrium.[5]

Gompel et al. (1994) found Bcl-2 and Ki67 overexpression in the glandular tissue of polyps in the proliferative phase. In the secretory phase, Ki67 expression was higher but still lower than the level observed in the proliferative phase. According to these authors, both Bcl-2 and Ki67 play a role in the formation of polyps.[13]

In postmenopausal women, however, Pinheiro (2014) found PR overexpression in both the gland and stroma of endometrial polyps in obese patients.[6] Gul (2010) found PR overexpression in the stroma of polyps, with high circulating oestrogen levels.[14] The same findings were reported by Belisario (2006).[15]

The hyperoestrogenism of obese menopause women explains these findings. Siiteri's (1973) showed peripheral conversion of androgen to oestrogen in adipose tissue (aromatase activity). This activity is stronger in obesity, especially after menopause. At this stage of life, there is no opposite progesterone for oestrogenic activity, thus explaining its possible role in the origin of polyps and the PR overexpression secondary to oestrogenic activity.[16] Oguz (2005) agree with this hypothesis.[17]

We did not find Bcl-2 overexpression in polyps of obese postmenopausal patients, except in polyps greater than 2cm, and in stroma. It is possible that the small number of patients in our sample affected the result. The inclusion of more patients may have produced results similar to those of the authors mentioned above.

Pinheiro et al. (2014) found higher Bcl-2 expression in the glandular tissue of the polyps of obese postmenopausal patients than in non-obese patients ($p<0.0001$). In the stroma, however, there was no significant difference in Bcl-2 expression ($p=0.1975$). Regarding Ki67 expression, the authors found no difference between obese and non-obese postmenopausal patients.[6]

Villavicencio et al. (2010) found 9.9-fold higher Ki67 expression on endometria in obese patients compared to non-obese patients. Obese patients showed up to 12.6-fold higher proliferation than the normal-weight group.[18]

As we known, Bcl-2 is an oncogene involved in apoptosis and its loss of expression was related to more aggressive endometrial cancer (higher grade, advanced stage and lymph node invasion). Stanescu et al., (2014) didn´t find a correlation between expression of bcl-2 and grade or stage of the endometrial cancer.[19]

Troncon et al., (2017) evaluate the expression of genetic markers in endometrial polyps of patients with and without postmenopausal bleeding (Bcl-2 and others). The authors didn´t found statistical differences between the two groups concerning the expression of the studied endometrial cancer risk factor genes, or with regard to the clinical aspects evaluated.[20]

We didn´t find overexpression of Bcl-2 or Ki67 in the polyps of obese postmenopausal patients. The results, however, were not what we expected. The same findings were published by the cited authors, in part or in full.

There is no significant difference in Bcl-2 and Ki67 expression in the endometrial polyps of obese and non-obese patients in menopause. Questions still exist regarding both the formation and treatment of endometrial polyps. Which patients should be monitored and which should be subjected to the systematic excision of these polyps due to the risk of malignancy? Further studies are needed to better answer these questions.

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Declarations

Ethics approval and consent to participate

The study was approved by the Research Ethics Committee of Gaffrée e Guinle University Hospital, Federal University of Sate of Rio de Janeiro - Brazil, under number 1,610,750.

All women agreed to participate in the study and signed the free and informed consent form.

Consent for publication

“Not applicable”

Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request. All data generated or analysed during this study are included in this published article.

Competing interests

"The authors declare that they have no competing interests"

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Disclosure statement

The authors report no conflicts of interest.

Authors' contributions

MVG, LAG e HLS performed clinical history, physical examination and hysteroscopy. RKAF, MGG and JMSJ helped in the preparation of the article and statistical analysis. All authors contribution in writing the manuscript, read and approved the final manuscript.

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Tables

Table 1. Expression of Bcl-2 and Ki67 in endometrial polyps (stroma and gland), stratified for body mass index (BMI)

	BMI < 29,9	BMI ≥ 30	P value*
Bcl-2 G	2,7 ± 0,60	2,7 ± 0,46	0,97
Bcl-2 S	2,6 ± 0,48	2,6 ± 0,74	0,84
Ki67 G	1,4 ± 0,50	1,7 ± 0,46	0,09
Ki67 S	1,0 ± 0,26	1,1 ± 0,35	0,68

*BMI: body mass index; Bcl-2 G: Bcl-2 glandular; Bcl-2 S: Bcl-2 stromal; Ki67 G: Ki67 glandular; Ki67 S: Ki67 stromal. Expression of Bcl-2 and Ki67 in endometrial polyps were classified as 1 (weak), 2 (moderate) and 3 (intense). *Mann-Whitney test*

Tabela 2. Bcl-2 and Ki-67 expression in endometrial polyps.

	Polyp size			Hystologic type (polyp)			Years since menopause		
	< 2cm	≥ 2cm	P value*	A	H	P value*	< 9y	≥ 10y	P value*
Bcl-2 G	2,8 ± 0,4	2,6 ± 0,6	0,61	2,6 ± 0,6	2,8 ± 0,3	0,41	2,6 ± 0,6	2,7 ± 0,5	0,41
Bcl-2 S	2,3 ± 0,6	2,7 ± 0,4	0,04	2,7 ± 0,4	2,5 ± 0,6	0,30	2,5 ± 0,6	2,7 ± 0,4	0,58
Ki67 G	1,3 ± 0,5	1,5 ± 0,5	0,24	1,3 ± 0,4	1,7 ± 0,4	0,04	1,5 ± 0,5	1,5 ± 0,5	0,87
Ki67 S	1,0 ± 0,3	1,0 ± 0,2	0,97	1,0 ± 0,2	1,1 ± 0,3	0,23	1,0 ± 0,2	1,1 ± 0,3	0,60

*Bcl-2 G: Bcl-2 glandular; Bcl-2 S: Bcl-2 stromal; Ki67 G: Ki67 glandular; Ki67 S: Ki67 stromal; A: atrophic; H: hyperplasic. <9y: nine years since menopause or less; ≥ 10y: tem years since menopause or more. Expression of Bcl-2 and Ki67 in endometrial polyps were classified as 1 (weak), 2 (moderate) and 3 (intense). *Mann-Whitney test*

Figures

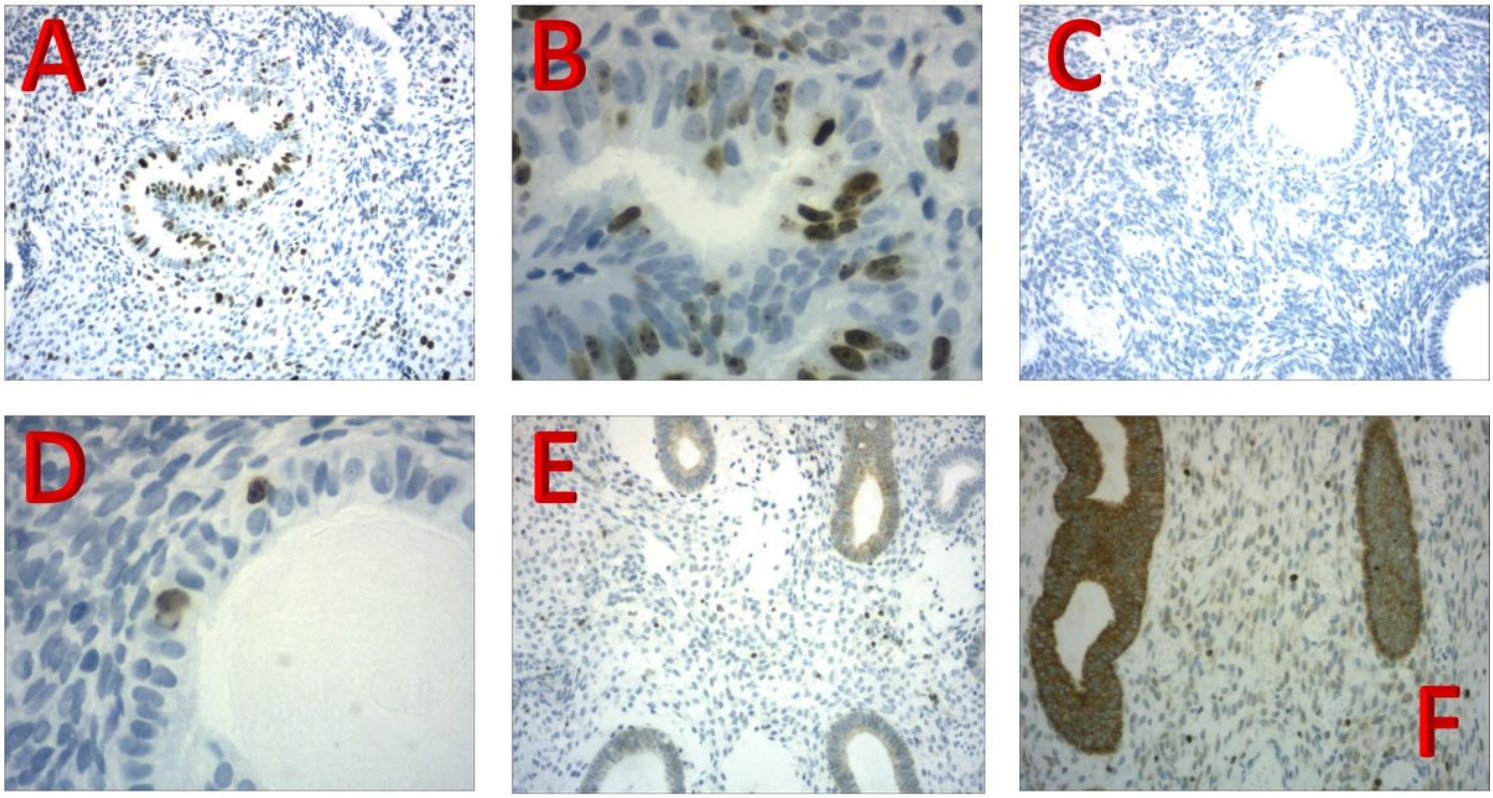


Figure 1

Immunohistochemistry of endometrial polyps. A: moderate expression of Ki67 in the gland and stroma (100x magnification). B: same as A, at 400x magnification. C: weak expression of Ki67 in the gland and stroma (100x magnification). D: same as C at 400x magnification. E: weak expression of Bcl-2 in the stroma (100x magnification). F: strong expression of Bcl-2 in the gland and stroma (400x magnification).