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The differential expression of oestrogen receptors, progesterone receptors, Bcl-2 and Ki67 in endometrial polyps

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Objective To obtain a greater understanding of the pathogenesis of endometrial polyps and to gain insight into which factors play a pivotal role in their growth.

Design Retrospective analysis of archived paraffin-embedded specimens.

Setting St James's University Hospital.

Sample Thirty secretory phase endometrial samples, 10 secretory phase endometrial polyps, 8 proliferative phase endometrial samples and 10 proliferative phase endometrial polyps.

Methods Immunohistochemistry was used to characterise the expression of oestrogen and progesterone receptors, Bcl-2 and Ki67 in cycling endometrium and phase-matched endometrial polyps. Patterns of expression were compared between the polyps and the endometrium.

Main outcome measure The expression of oestrogen receptors, progesterone receptors, Bcl-2 and Ki67.

Results Three significant differences were found between the endometrium and the polyps. Polyps taken from the proliferative phase of the cycle displayed significantly elevated expression of Bcl-2 and weak or no expression of progesterone receptors. Secretory phase polyps displayed an elevated expression of oestrogen receptors.

Conclusion A localised increase in Bcl-2 expression and consequential decline or cessation of apoptosis is an important mechanism underlying the pathogenesis of endometrial polyps. Elevated Bcl-2 expression results in failure of the polyp tissue from undergoing normal cyclical apoptosis during the late secretory phase. This may mean the polyp is not shed along with the rest of the endometrium during menstruation.

INTRODUCTION

The pathogenesis of endometrial polyps is poorly understood[1]. However, it is thought that they originate as a focal hyperplasia of the basalis and then develop into localised overgrowths which extend upwards through the functionalis to project into the uterine cavity. The usual histological pattern of endometrial polyps consists of irregular proliferative glands, with a fibrotic stroma containing thick-walled blood vessels[2]. They are most easily identified in the secretory phase of the menstrual cycle, when the non-progestational type of glands in the polyp stand out in stark contrast to the normal surrounding secretory endometrium.

Although endometrial polyps are almost always benign, occasionally, a focus of malignancy may be found. Endometrial polyps have been found in around 12–34% of uteri containing endometrial carcinoma[3], and metaplastic changes have been reported in endometrial polyps[2]. Ismail[4] suggested that endometrial polyps represent an intermediate stage in the development of carcinoma and commented on the rarity of polyp-associated cancers in the general population compared with the frequency observed in women taking tamoxifen. Silva *et al.*[5] found that 10 of 13 tamoxifen-related endometrial carcinomas were associated with endometrial polyps.

Few studies have investigated endometrial polyps and their pathogenesis in detail. Maia *et al.*[6] and Mittal *et al.*[1] investigated the expression of oestrogen and progesterone receptors in endometrial polyps. Other studies have concentrated on the clinical observations and incidence of polyps in postmenopausal women.

Oestrogens and progestogens are known modulators of endometrial proliferation and differentiation via their receptors. The relationship between the expression of oestrogen receptors and cell proliferation has already been demonstrated in both normal and malignant endometrium.

The balance between mitotic activity and apoptosis is thought to regulate normal endometrial development during the menstrual cycle[7]. Bcl-2 is a proto-oncogene and a representative member of a family of genes. It has been reported to prolong the survival of cells by specifically inhibiting apoptosis[8]. Bcl-2 expression has been characterised in normal cycling endometrium[9], and recent studies have also observed that Bcl-2 is strongly expressed in

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simple and complex hyperplasia[10]. A recognised indicator of cell mitotic activity is Ki67. An increase in Ki67 expression is indicative of increased cell mitotic activity and proliferation. During the proliferative phase of the menstrual cycle, Ki67 expression is normally elevated.

We tested the hypothesis that polyps are a focus of simple hyperplasia, by examining the hormone receptor status and markers of proliferation (Ki67) and apoptosis (Bcl-2) in the polyp at different stages in the menstrual cycle.

METHODS

Ethical approval for this study was granted by our local ethics committee. The endometrium and endometrial polyps used in this retrospective study were retrieved using a pathology database, by entering the keywords 'proliferative endometrium', 'secretory endometrium' and 'endometrial polyps'. Proliferative endometrium ranged from days 6 to 13 and secretory endometrium from days 18 to 24 of the menstrual cycle. The last menstrual period was known in each case and the histology confirmed the day of the menstrual cycle. Samples were excluded if they were associated with fibroids, abnormal cytology or other pathologies such as hyperplasia. The samples of histologically normal endometrium were obtained by routine biopsies from women who had dysfunctional uterine bleeding but in whom histological examination of the endometrium and hysteroscopy had revealed no abnormality. In the secretory phase, 30 samples of endometrium and 10 polyps were retrieved. In the proliferative phase, 8 endometrial samples and 10 polyps were retrieved.

Positive control tissue sections of tonsil (Bcl-2 and Ki67) and breast carcinoma (oestrogen and progesterone receptors) were used throughout. These tissues are known to contain the antigen and always stained positive, thus providing a means to monitor any loss of sensitivity in detection of the antibody. Sections of 5 μm were cut from each sample and were secured on glass microscope slides (Superplus, BDH, Poole, UK).

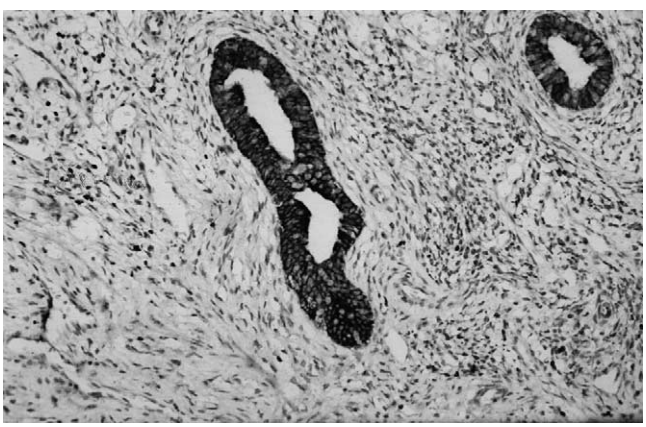

Fig. 1. Photograph (×40) of an endometrial gland demonstrating typical brown cytoplasmic staining, which is characteristic of Bcl-2 positivity. Note the blue, negatively stained nucleus.

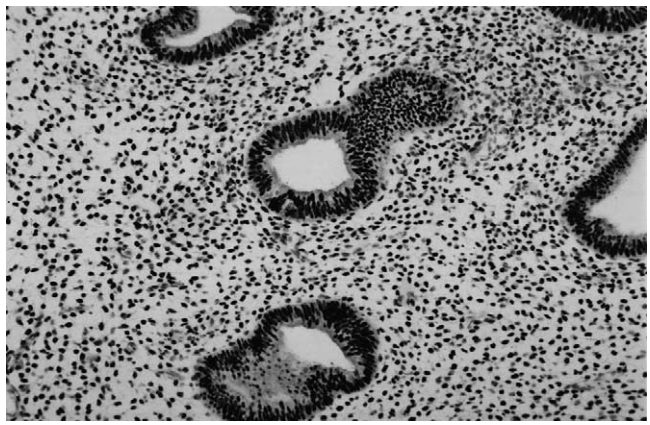

Fig. 2. Photograph (×40) illustrating brown/black nuclear staining in both the glandular epithelium and stroma. This photograph is a good example of positive nuclear expression, in this case + + + +, for oestrogen receptor.

The formalin-fixed, paraffin-embedded sections were dewaxed using xylene and then rehydrated in ethanol. Endogenous peroxidase activity within the tissue was quenched by flooding the sections with hydrogen peroxide solution (30% H_2O_2 in 100% methanol). Heated antigen retrieval was achieved by immersing the sections in hot citrate buffer (0.1 M solution, pH 6) and microwaving them (750 W) for 10 minutes on full power. Sections designated for the detection of oestrogen receptors were microwaved for 15 minutes.

Using a Sequensa staining system (Shandon, Runcorn, UK), the sections were flooded with a blocking antibody (150 μl of 0.15% normal horse serum solution, Vecta, Burlingame, California). Primary monoclonal antibodies (Novocastra, Newcastle-upon-Tyne, UK) were diluted as follows: for oestrogen and progesterone receptors, 1 in 60 μl, Bcl-2 1 in 80 μl and Ki67 1 in 150 μl. Each primary antibody was applied (100 μl) to the appropriate section, and left to incubate for 1 hour. Normal horse serum was applied to the negative control sections. Following a wash and a second block with normal horse serum, biotinylated horse anti-mouse antibody (Vecta) was then applied and allowed to incubate for 30 minutes. The avidin–biotin peroxidase (ABC) complex was prepared according to the manufacturer's instructions (Vecta), applied to all the sections and left to bind for 30 minutes. Antibody staining was visualised with DAB (Sigma, Poole, UK), counterstained with Meyer's haematoxylin (0.1% solution) and Scott's tap water substitute (20% magnesium sulphate, 7% sodium bicarbonate). Finally, the sections were dehydrated, cleared in xylene and mounted using DePex (BDH).

Each section was independently scored (% positive staining) by two research fellows (LT, JR), who did not know the phase of the menstrual cycle. Any discrepancies regarding the scoring were independently assessed by a third party, a histopathologist (AR), who was also blind to the phase of the menstrual cycle. Using a magnification objective of ×40,

the percentage of positive staining throughout the whole tissue section was assessed semi-quantitatively. Positive cells expressing oestrogen receptor, progesterone receptor and Ki67 were identified by a brown precipitate in the nucleus (illustrated in Fig. 1). Bcl-2 demonstrated brown cytoplasmic staining as demonstrated in Fig. 2. In accordance with previously published protocols[11,12], the expression was graded as negative (indicating that there was no positive staining), or positive, which was classed as: + (less than 25%), ++ (25–50%), +++ (50–75%) and ++++ (75–100%). The glandular epithelium and stromal compartments were scored separately. The intensity of staining in the tissue was not assessed.

Non-parametric statistical analysis was performed using Biostat100 software. A Mann–Whitney *U* test was used to find any significant differences between groups. Statistical significance was taken as $P < 0.05$.

RESULTS

Proliferative phase endometrium demonstrated predominately strong positive expression (+++ or ++++) in the glandular epithelium and stroma for both oestrogen receptor and progesterone receptors. The expression of Bcl-2 ranged from negative to ++++ in the glandular epithelium; however, in the stroma, expression of Bcl-2 was absent in all the samples. Ki67 expression ranged from negative to +++ in the glandular epithelium, but was mainly negative in the stroma.

Table 1. The expression of oestrogen receptor (ER), progesterone receptor (PR), Bcl-2 and Ki67 in proliferative phase premenopausal endometrial polyps (P), compared with proliferative phase endometrium (E).

Receptor	Positive expression	Glandular epithelium		Stroma	
		P	E	P	E
PR	neg	8*	1	8*	1
	+	1	0	1	0
	++	0	0	0	0
	+++	1	1	1	2
	++++	0	6	0	5
Bcl-2	neg	0	2	2*	8
	+	0	0	2	0
	++	1	2	4	0
	+++	1	2	1	0
	++++	8*	2	1	0
ER	neg	0	0	0	0
	+	0	0	0	0
	++	0	0	0	0
	+++	1	0	4	2
	++++	9	8	6	6
Ki67	neg	3	3	7	7
	+	2	3	1	1
	++	3	1	2	0
	+++	2	1	0	0
	++++	0	0	0	0

* $P < 0.05$ (Mann–Whitney *U* test).

Table 2. The expression of oestrogen receptor (ER), progesterone receptor (PR), Bcl-2 and Ki67 in secretory phase premenopausal endometrial polyps (P), compared with secretory phase endometrium (E).

Receptor	Positive expression	Glandular epithelium		Stroma	
		P	E	P	E
PR	neg	4	20	4	8
	+	3	2	0	3
	++	3	0	2	5
	+++	0	0	1	8
	++++	0	8	3	6
Bcl-2	neg	2	12	6	21
	+	1	10	3	7
	++	5	4	1	2
	+++	2	4	0	0
	++++	0	0	0	0
ER	neg	1	3	1	0
	+	0	6	1	2
	++	0	4	1	10
	+++	0	7	3	17
	++++	9*	10	4	1
Ki67	neg	7	21	9	23
	+	2	3	0	3
	++	0	3	1	2
	+++	1	0	4	2
	++++	0	3	0	0

* $P < 0.05$ (Mann–Whitney *U* test).

Secretory phase endometrium revealed a range of glandular expression of oestrogen receptors, which ranged from negative, +++ or ++++ in over half the samples. Stromal oestrogen receptor expression was moderate, with most samples being ++ or +++. Glandular expression of progesterone receptors was predominately negative, with stromal expression ranging from negative to ++++. The expression of Bcl-2 in the glandular epithelium was mainly negative or +, with stromal expression demonstrating a similar pattern. The expression of Ki67 in the glandular epithelium was predominately negative or +, but with some samples demonstrating +++ expression. Stromal expression showed a similar pattern of expression, being mainly negative or low (+ or ++).

Proliferative phase endometrial polyps demonstrated significantly greater expression of Bcl-2 in both the glandular epithelium and the stroma, compared with proliferative endometrium (Table 1). Proliferative phase endometrial polyps also demonstrated significantly lower expression of progesterone receptors in both the glandular epithelium and stroma, compared with progesterone receptor expression in the proliferative endometrium. There were no significant differences in the expression of oestrogen receptors or Ki67 in the proliferative phase polyps.

Endometrial polyps taken from the secretory phase demonstrated a significantly elevated expression of oestrogen receptors but only in the glandular epithelium (Table 2). No other significant differences were found for the expression of progesterone receptors, Bcl-2 or Ki67 in secretory phase endometrial polyps.

DISCUSSION

Few studies[1,6] have investigated the expression and distribution of oestrogen and progesterone receptors in endometrial polyps. No study cited in Medline has investigated the expression of Bcl-2 or Ki67 in endometrial polyps.

The findings of this study revealed significant differences in the expression of progesterone and oestrogen receptor and Bcl-2 in endometrial polyps compared with histologically normal endometrium from the same menstrual phase.

The patterns of expression of oestrogen receptors, progesterone receptors, Bcl-2 and Ki67 in the normal cycling endometrium agree with previous studies[9,13–15], and demonstrate similar positive staining patterns and phase specificity. Coppens *et al.*[14] noted that staining for progesterone receptors and oestrogen receptors was strong in the proliferative phase of the menstrual cycle, with expression most prominent in the glandular epithelium. As the secretory phase progresses, progesterone receptor staining gradually decreases with oestrogen receptor expression remaining at very low levels. The expression of Bcl-2 in the endometrium was investigated by Gompel *et al.*[9], who noted prominent glandular staining, with a slight peak at the end of the proliferative phase. Similar staining was demonstrated in this study. Ki67 expression occurs predominantly in the proliferative phase of the menstrual cycle, with glandular epithelium showing the strongest expression. Stromal staining of Ki67 is more apparent in the secretory phase but is still lower than that of the endometrial glands in the proliferative phase.

Mittal *et al.*[1] found no significant differences in the glandular epithelial expression of oestrogen and progesterone receptors in polyps compared with normal cycling endometrium. Fewer stromal cells expressed oestrogen and progesterone receptors in polyps than in the normal endometrium. The authors postulate that endometrial polyps may result from a decrease in oestrogen and progesterone receptors in the stromal cells[1]. However, the phase of the cycle in which the polyps were excised was not reported.

Maia *et al.*[6] reported strongly positive staining for oestrogen receptors in all the endometrial polyps they investigated, with little expression of progesterone receptors in both the glandular epithelium and the stroma. These observations are similar to our findings. Neither of the above studies[1,6] states in which phase of the menstrual cycle the polyps were studied.

No study has investigated the expression of Ki67 or Bcl-2 in endometrial polyps. We have found a marked increase in the expression of Bcl-2 in the proliferative phase polyps in both the glandular epithelium and the stroma compared with the proliferative endometrium. However, this increase was not noted in any of the polyps in the secretory phase.

Quite why the difference in expression of Bcl-2 in endometrial polyps is so marked in the proliferative phase is interesting. In the proliferative phase, low levels of apoptosis are still required to maintain the endometrial cell function and population, although the endometrium is proliferating and thickening. This 'housekeeping' apoptosis is demonstrated by a range of Bcl-2 expression in proliferative endometrium, ranging from negative to ++++. The lack of apoptosis in the proliferative phase polyp and the significantly elevated expression of Bcl-2 are striking, even against this background of low apoptosis in the proliferative phase endometrium. This may indicate a difference in physiology between the polyp and the endometrium, as regards the level of apoptosis. It seems that the polyp lacks the ability to perform 'housekeeping' apoptosis during the proliferative phase.

As the endometrium moves into the secretory phase, Bcl-2 expression within the polyps is still elevated compared with the endometrium. During the early to mid secretory phase, the endometrium reaches its maximum thickness, and even past ovulation proliferation is still occurring. Apoptosis levels decline further, bringing Bcl-2 expression in line with that in the polyp. This is perhaps why the difference between the polyp and the surrounding endometrium is less marked during the secretory phase.

This study has revealed some of the possible mechanisms underlying the pathogenesis of endometrial polyps. It does not appear that endometrial polyps arise as a result of a focus of intense proliferation, as was originally thought. Vinatier *et al.*[8] postulate that in the normal endometrium, the epithelial cells residing in the basalis escaped apoptosis due to a persistently elevated level of Bcl-2. Almost no apoptosis is evident in the basalis throughout the menstrual cycle[16]. This serves to allow the cells to escape death and contribute to the repair of the endometrium after menstruation. Similar theories have been proposed by Niemann *et al.*[17], who noted that in the cycling endometrium, the basal portions of the glands retained Bcl-2 expression. The same study related this to diminished hormonal response that is typical of the basal endometrium.

The polyps investigated in this study also displayed a significantly reduced expression of progesterone receptors. Does the distinct lack of progesterone receptors in the polyp point to insensitivity to progesterone? Mittal *et al.*[1] linked an overexpression of oestrogen receptors and an underexpression of progesterone receptors in the polyp as possible causative factors. Our study also found a significant increase in the expression of oestrogen receptors in the secretory phase polyps. In normal circumstances, autoinduction of oestrogen receptors is inhibited by progesterone in the secretory phase endometrium. After ovulation has occurred, levels of oestrogen receptor decline due to the increased levels of circulating progesterone. It could be that the insensitivity of the polyp to progesterone prevents the normal decline of oestrogen receptor expression in the secretory phase polyps.

The observations reported by Niemann *et al.*[17] and Vinatier *et al.*[8] could explain the formation of endometrial polyps. There may be a prolonged overexpression of Bcl-2

in one particular area of the endometrium, allowing the epithelial cells to escape the normal programme of cell death, allowing clonal expansion, without the expected increase in proliferation index or cell turnover. This cluster of apoptosis-resistant epithelial cells must also develop in such a way that they become insensitive to progesterone due to their lack of progesterone receptors.

However, some apoptosis must occur within the polyp. Bcl-2 expression, although still elevated compared with the surrounding secretory endometrium, decreased to an average of ++ (25–50%) expression, suggesting that some apoptotic activity must be occurring with the polyp. Whether this apoptosis and shedding occurs with the rest of the surrounding endometrium is debatable. Curiously, endometrial polyps often manifest themselves as irregular intermenstrual bleeding. If the polyp tissue is only partially in tune with the menstrual cycle, it may well shed its endometrium at any time.

The results of our study suggest that endometrial polyps are tumours of dysregulation, failing to undergo the expected sequence of proliferation, differentiation and shedding during the menstrual phase, rather than a mass of tissue which is simply proliferating uncontrollably. Further studies comparing endometrial polyps with hyperplastic endometrium are needed to explore this hypothesis further.

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References

1. Mittal K, Schwartz L, Goswami S, Demopoulos R. Estrogen and progesterone receptors in the endometrial polyps. *Int J Gynecol Pathol* 1996;**15**:345–348.
2. Nuovo MA, Nuovo GJ, McCaffrey RM, Levine RU, Barron B, Winkler B. Endometrial polyps in postmenopausal patients receiving tamoxifen. *Int J Gynecol Pathol* 1989;**8**:125–131.
3. Dal-Cin P, Vanni R, Marras S. Four cytogenic subgroups can be identified in endometrial polyps. *Cancer Res* 1995;**55**:1565–1568.
4. Ismail SM. Pathology of endometrium treated with tamoxifen. *J Clin Pathol* 1994;**47**:827–833.
5. Silva EG, Tornos CS, Follen-Mitchell M. Malignant neoplasms of the uterine corpus in patients treated for breast carcinoma: the effects of tamoxifen. *Int J Gynecol Pathol* 1994;**13**:248–258.
6. Maia H, Maltez A, Calmon LC, Oliveira M, Marques D, Coutinho EM. Histopathology and steroid receptors in endometrial polyps of postmenopausal patients under hormone replacement therapy. *Gynaecol Endosc* 1998;**7**:267–272.
7. Stewart CJR. Endometrial apoptosis in patients with dysfunctional uterine bleeding. *Histopathology* 1999;**34**:99–105.
8. Vinatier D, Dufour P, Subtil D. Apoptosis: a programmed cell death involved in ovarian and uterine physiology. *Eur J Obstet Gynecol Reprod Biol* 1996;**67**:85–102.
9. Gompel A, Abourin JC, Martin A. Bcl-2 expression in normal endometrium during the menstrual cycle. *Am J Pathol* 1994;**144**:1195–1202.
10. Amezcua CA, Zheng W, Muderspach LI, Felix JC. Down-regulation of Bcl-2 is a potential marker of the efficacy of progestin therapy in the treatment of endometrial hyperplasia. *Gynecol Oncol* 1999;**73**:126–136.
11. Cork BA, Li TC, Warren MA, Laird SM. Interleukin 11 (IL-11) in human endometrium: expression throughout the menstrual cycle and the effects of cytokines on endometrial IL-11 production in vitro. *J Reprod Immunol* 2001;**50**:3–17.
12. Sakuragi N, Salah-Eldin AE, Watari H, et al. Bax, Bcl-2 and P53 expression in endometrial cancer. *Gynecol Oncol* 2002;**86**:288–296.
13. Bouchard PH, Marraoui J, Massai MR. Immunocytochemical localization of oestradiol and progesterone receptors in human endometrium: a tool to assess endometrial maturation. *Bailliere's Clin Obstet Gynaecol* 1991;**5**:107–115.
14. Coppens MT, Dhont MA, De Boever JG. The distribution of oestrogen and progesterone receptors in the human endometrial basal and functional layer during the normal menstrual cycle. *Histochemistry* 1993;**99**:121–126.
15. Ioffe OB, Papadimitriou JC, Drachenberg CB. Correlation of proliferative indices, apoptosis, and related oncogene expression (bcl-2 and c-erbB-2) and p53 in proliferative, hyperplastic and malignant endometrium. *Hum Pathol* 1998;**29**:1150–1159.
16. Arends MJ. Apoptosis in the endometrium. *Histopathology* 1999;**35**:174–178.
17. Niemann TH, Trgovac TL, McGaughy VR, Vaccarello L. Bcl-2 expression in endometrial hyperplasia and carcinoma. *Gynecol Oncol* 1996;**63**:318–322.

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