UNIVERSIDADE ESTADUAL DE CAMPINAS

Faculdade de Ciências Médicas

EXPRESSÃO DE RECEPTORES DE ESTRÓGENO E PROGESTERONA, Ki-67, Bcl-2 E CICLO-OXIGENASE-2 EM PÓLIPOS ENDOMETRIAIS DE MULHERES NA PÓS-MENOPAUSA

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Tese de Doutorado apresentada ao Programa de Pós-Graduação em Tocoginecologia da Faculdade de Ciências Médicas da Universidade Estadual de Campinas - UNICAMP para obtenção do Título de Doutor em Ciências da Saúde, área de concentração, Fisiopatologia Ginecológica, sob orientação da Profª. Drª. Lúcia Helena Simões da Costa Paiva.

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Dedico este trabalho...

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Sumário

Símbolos, Siglas e Abreviaturas

Antígeno Ki- 67	Marcador do ciclo celular e crescimento tumoral
Bcl-2	*B cell-lymphoma/leukemia-2 Gene*
CAISM	Centro de Atenção Integral à Saúde da Mulher
COX-2	Ciclo-oxigenase-2
DM	Diabetes Mellitus
DP	Desvio padrão
et al.	e colaboradores
FAPESP	Fundação de Amparo à Pesquisa do Estado de São Paulo
FCM	Faculdade de Ciências Médicas
FIV	Fertilização "*in vitro*"
HAS	Hipertensão Arterial Sistêmica
H&E	Coloração hematoxilina-eosina
IHQ	Imuno-histoquímica
IC	Intervalo de Confiança
IMC	Índice de Massa Corpórea
n	Número de casos
OR	Odds Ratio
p-valor	Probabilidade de Significância Estatística

RE	Receptor de estrógeno
RP	Receptor de progesterona
TMA	Microarranjo de tecidos (*tissue microarray*)
UNICAMP	Universidade Estadual de Campinas
vs	versus

Resumo

Introdução: Os pólipos endometriais são achados frequentes em mulheres na pós-menopausa e têm sido raramente associados a lesões precursoras e neoplasia endometrial. O desconhecimento de sua patogênese e potencial de malignidade tem levado a polipectomia de rotina o que pode estar expondo muitas mulheres a um risco cirúrgico desnecessário. **Objetivo**: Avaliar a prevalência de malignidade e a expressão de receptores de estrógeno e progesterona, marcadores tumorais e COX-2 em pólipos endometriais malignos e benignos em mulheres na pós-menopausa. **Sujeitos e métodos**: Realizou-se um estudo de corte transversal com mulheres submetidas à polipectomia no período de janeiro de 1998 a dezembro de 2008 no Hospital da Mulher "Prof. Dr. José Aristodemo Pinotti"-CAISM-UNICAMP. Foram incluídas 390 mulheres na pós-menopausa que não eram usuárias de terapia hormonal e tamoxifeno. Foram avaliadas as características clínicas como idade, sangramento pós-menopausa, paridade, presença de hipertensão arterial, diabetes *mellitus*, obesidade e diagnóstico histológico dos pólipos. Foi avaliada a expressão imuno-histoquímica de RE e RP e dos marcadores Bcl-2, Ki-67 e COX-2 em microarranjo de amostras teciduais (TMA). Foi comparada a expressão desses receptores e marcadores entre pólipos benignos e pré-malignos/malignos. **Análise estatística**: Para análise estatística os pólipos foram agrupados em benignos e pré-malignos/malignos. As características

clínicas entre os grupos benignos/pré-malignos e malignos foram comparadas utilizando-se os testes qui-quadrado, exato de Fisher ou não paramétrico de Mann-Whitney. Para a comparação do escore final dos receptores e marcadores foram utilizados os testes exato de Fisher, qui-quadrado ou teste de Mann-Whitney. **Resultados**: A prevalência de malignidade nos pólipos endometriais em estudo foi de 7,1% e esteve associada ao sangramento pós-menopausa e à baixa expressão de RE no estroma. O escore final da expressão do RE no estroma dos pólipos foi maior nos benignos em relação aos pré-malignos/malignos, sendo esta diferença significativa. Não houve diferença de expressão do RP, Ki-67 e Bcl-2 entre os pólipos benignos e pré-malignos/malignos. O escore final da expressão da COX-2 foi significativamente maior nos pólipos pré-malignos/malignos em relação aos benignos no epitélio glandular e no estroma. **Conclusões:** Os pólipos na pós-menopausa apresentam uma alta expressão dos RE no estroma e no epitélio glandular. Esta expressão foi menor nos pólipos pré-malignos/malignos em relação aos benignos. Observou-se alta expressão da COX- 2, sendo maior nos pólipos pré-malignos/malignos em relação aos benignos.

Summary

Introduction: Endometrial polyps are common findings in postmenopausal women that are rarely associated with precursor lesions and endometrial neoplasm. Lack of understating of the pathogenesis and oncogenic potential of polyps has led to the routine performance of polypectomy which may expose many women to unnecessary surgical risks. **Objective**: To evaluate the prevalence of malignancy and expression of estrogen/progesterone receptors, tumor markers and COX-2 in malignant and benign endometrial polyps in postmenopausal women. **Subjects and Methods**: A cross-sectional study was conducted with women undergoing polypectomy from January 1998 to December 2008 in the "Prof. Dr. José Aristodemo Pinotti" Women's Hospital-CAISM-UNICAMP. Included in the study were 390 postmenopausal women who were non-users of hormone therapy and tamoxifen. Clinical characteristics such as age, postmenopausal bleeding, parity, presence of arterial hypertension, diabetes mellitus, obesity and histologic diagnosis of polyps were assessed. Immunohistochemical expression of ER/PR, Bcl-2, Ki-67 and COX-2 markers in tissue microarray (TMA) samples was evaluated. A comparison of these receptors and markers was made between benign and premalignant/malignant polyps. **Statistical analysis**: For statistical analysis, polyps were grouped into benign and premalignant/malignant. Clinical

characteristics between the benign/premalignant group and the malignant group were compared using the chi-square, Fisher exact or Mann-Whitney nonparametric test. To compare the final score of receptors and biomarkers, the Fisher exact tests, chi-square or Mann-Whitney test were used. **Results**: The prevalence of malignancy in endometrial polyps in the study was 7.1% and was associated with postmenopausal bleeding and low ER expression in the stroma. The final score of ER expression in the stromal component of the polyp was higher in benign polyps than in premalignant/malignant and this was a significant difference. There was no difference in PR, Ki-67 and Bcl-2 expression between benign and premalignant/malignant polyps. The final score of COX-2 expression was significantly higher in premalignant/malignant polyps in comparison to benign polyps in the glandular epithelium and stroma. **Conclusions:** Polyps in the postmenopause show a high ER expression in the stroma and glandular epithelium. Expression was lower in premalignant/malignant polyps than in benign polyps. Elevated COX-2 expression was observed that was higher in premalignant/malignant polyps than in benign polyps.

1.Introdução

Os pólipos endometriais constituem projeções localizadas do tecido endometrial, sésseis ou pediculadas, únicas ou múltiplas, nas quais se observa uma irregular distribuição das glândulas, estroma hipercelular e vasos sanguíneos com paredes espessadas, recobertas por epitélio pseudoestratificado ativo ou, na pós-menopausa, por epitélio plano e inativo (1). O diâmetro dos pólipos endometriais varia de poucos milímetros a vários centímetros (2). Eles podem ser de difícil diagnóstico quando o material encaminhado para avaliação histológica é obtido mediante biópsia ou curetagem (3). Nestes casos, apenas fragmentos do pólipo são obtidos, e a diferenciação com hiperplasia endometrial é feita histologicamente examinando o estroma. O estroma do pólipo endometrial é composto de células fusiformes, contendo abundante tecido conectivo extracelular e vasos com paredes espessadas. As glândulas dos pólipos usualmente mostram alguma dilatação cística (4). Kim et al. (2004), ao compararem características histológicas de pólipos endometriais obtidos de peças cirúrgicas de histerectomia e de polipectomia, constataram que, quando o maior eixo das glândulas endometriais está disposto paralelamente à superfície do epitélio, o diagnóstico histológico dos pólipos endometriais é facilitado (5).

As glândulas endometriais dos pólipos frequentemente não ciclam normalmente; nelas, as alterações secretórias podem ser fracas ou ausentes, em contraste com o endométrio adjacente. Hiperplasia, adenocarcinoma e carcinossarcoma podem envolver ou estar totalmente confinados ao pólipo endometrial (1). Pólipos endometriais são lesões morfologicamente diversas, difíceis de serem subclassificadas. Ainda assim, os pólipos podem ser categorizados em: pólipos da mucosa endometrial quando exibem padrão glandular normal de fase secretória ou estroma decidualizado de fase secretória final; pólipos hiperplásicos quando apresentam glândulas ativamente proliferativas com estroma hipercelular e mitoticamente ativo; pólipos fibrosos quando apresentam um estroma denso por acúmulo de colágeno, circundando as glândulas revestidas por uma única camada de epitélio plano; pólipos carcinomatosos e pólipos mistos quando exibem combinações histológicas dos demais (5).

A prevalência dos pólipos endometriais varia de 7,8% a 34%, dependendo das características da população estudada e do método diagnóstico utilizado (6, 7). Os pólipos são diagnosticados em até 12% de mulheres assintomáticas durante a realização de exame ginecológico de rotina, incluindo a ultrassonografia transvaginal. Eles são comumente observados na perimenopausa e na pós-menopausa, podendo levar a sangramento uterino anormal, mas também podem ser assintomáticos (8).

A etiologia dos pólipos endometriais não está bem esclarecida (9,10). Eles são frequentemente benignos, mas podem apresentar alterações tissulares pré-malignas e malignas. A associação entre pólipos endometriais e lesões pré-

malignas e malignas não é ainda bem determinada (11). Dados epidemiológicos sugerem que os pólipos endometriais devam ser vistos como fator de risco para adenocarcinoma endometrial (12-15).

A taxa de malignidade associada aos pólipos endometriais é baixa. Estudo anterior, que avaliou risco de malignidade em pólipos de mulheres pós-menopausa atendidas no CAISM/Unicamp, mostrou prevalência de 3,8%, associada, principalmente, à idade acima de 60 anos e à presença de sangramento na pós-menopausa (16). A atual prevalência de malignidade nos pólipos endometriais sintomáticos encontra-se entre 1,8% e 13% em diferentes estudos na literatura (17). Uma revisão sistemática conduzida por Lieng et al. (2010) mostrou que os pólipos malignos estiveram presentes entre 0 e 12,9% das mulheres, e as lesões pré-malignas de 0,2 a 23,8% dos pólipos endometriais (9).

Alguns fatores são reconhecidos como de risco para o desenvolvimento de câncer endometrial. São os casos da idade avançada, obesidade, hipertensão arterial, histórico de tratamento com estrógeno exógeno para sintomas da pós-menopausa, distúrbios hormonais associados com o Diabetes *Mellitus* e histórico de tratamento com tamoxifeno para câncer de mama (10,11). Muitos estudos demonstraram que o uso de tamoxifeno, um modelador seletivo de receptor estrogênico usado como tratamento coadjuvante do câncer da mama, aumenta o risco de malignidade em pólipos endometriais (18). Martinez et al. (2004) relataram significativo maior risco de malignidade em pólipos de usuárias de tamoxifeno (15).

Em relação à terapia hormonal na perimenopausa, Orvietto et al. (1999) evidenciaram significativa associação com pólipos malignos (19). Esse risco é maior com terapia hormonal em esquema combinado sequencial comparado com o esquema contínuo pela menor proteção endometrial pelo progestágeno; observando-se que a formação de pólipos endometriais é dependente do tipo e dosagem do estrógeno e progestágeno utilizados. Especialmente a progesterona com alta atividade antiestrogênica tem um importante papel preventivo no desenvolvimento dos pólipos endometriais (20). Nenhum estudo mostra associação do uso de contraceptivo oral e malignidade nos pólipos (19).

A obesidade e o diabetes *mellitus* foram avaliados como fatores de risco para malignidade em pólipos endometriais em alguns estudos; tendo sido observada correlação estatisticamente significativa com malignidade no estudo de Gregoriou et al. (2009) (21). Da mesma forma, em alguns estudos, a hipertensão arterial também tem sido identificada como fator de risco independente para malignidade (22,23).

Na pós-menopausa, existe uma relação direta entre o tamanho do pólipo endometrial e a existência de hiperplasia atípica e carcinoma endometrial. Um estudo realizado por Rahimi et al. (2009) determinou que pólipos maiores que 15mm têm 3,6 vezes mais risco de estar associado a lesões malignas quando comparados com pólipos menores (7). Ferrazzi et al. (2009), num estudo multicêntrico, verificaram que pólipo com diâmetro maior ou igual a 18mm foi a única variável independente com associação estatisticamente significativa com malignidade na avaliação histológica de pólipos de mulheres sintomáticas e

assintomáticas na pós-menopausa (24). Wang et al. (2010) observaram que pólipos endometriais maiores que 1,0cm e sangramento uterino anormal estiveram associados a maior risco de malignidade (25).

Apesar de estudos anteriores indicarem que o estudo Doppler da vascularização do pólipo, através de ultrassonografia transvaginal, seria útil em predizer atipia e malignidade do pólipo, o exame histopatológico é ainda necessário para se excluir atipia e malignidade (26,27). O curso natural dos pólipos endometriais não está completamente elucidado, porém, os pequenos pólipos (<1cm) parecem regredir espontaneamente em alguns casos (28,29). A histeroscopia e a polipectomia são considerados padrão ouro no diagnóstico do pólipo endometrial (30,31). A histeroscopia diagnóstica tem uma alta acurácia no diagnóstico dos pólipos endometriais, todavia, a estimativa de lesões hiperplásicas e pré-malignas é baixa mediante esta técnica. A especificidade do diagnóstico visual para se detectar câncer endometrial em pólipos intrauterinos por meio da histeroscopia é baixa, e, mesmo realizando-se biópsias, nem todas as lesões malignas endometriais associadas aos pólipos serão detectadas. Por conta dessa limitação, seria mais prudente que, ao se encontrar um pólipo endometrial durante a realização de uma histeroscopia diagnóstica, se resseque toda a estrutura encontrada, para, assim, se obter uma avaliação histológica fidedigna (17).

Segundo Kurman (2002), o pedículo e o endométrio adjacente devem estar livres de câncer para se excluir pólipos invadidos por câncer endometrial, porém, desta forma, exclui-se também o câncer primário do pólipo que se propagou para o endométrio adjacente, o que torna ainda mais difícil a

comparação das taxas de malignidade associadas aos pólipos endometriais encontradas nos diferentes estudos na literatura (1). O aspecto do endométrio adjacente, que pode ser classificado como espessado ou atrófico, é útil para se diferenciar duas classes de pacientes: aquelas com pólipos e endométrio espessado, possivelmente associado à hiperplasia, correspondendo a cerca de 10% dos casos (19); e aquelas com endométrio atrófico, que representam a maioria dos casos encontrados em pacientes assintomáticas na pós-menopausa (32,33).

A remoção dos pólipos assintomáticos por histeroscopia na perimenopausa tornou-se uma prática habitual, quer como uma ressecção prudente de lesão potencialmente maligna, cujo potencial é baixo e não muito bem estabelecido; quer pela prevenção de uma possível transformação maligna do pólipo endometrial (34). Parece importante indagar se, de fato, seria necessário submeter todas as mulheres com pólipos endometriais a ressecções sistemáticas, como propõem alguns autores (35). Em vários serviços de ginecologia, devido ao uso rotineiro da ultrassonografia pélvica transvaginal no acompanhamento de mulheres assintomáticas na pós-menopausa, o diagnóstico de pólipo tem sido cada vez mais frequente. Esta conduta de “ver-e-tratar” é questionável, porque é baseada na opinião de especialistas e em poucos dados de publicação (36). Entretanto, esta rotina está causando custos adicionais e eventuais efeitos médicos e psicológicos adversos (24). A histeroscopia cirúrgica é, geralmente, considerada de fácil realização em mãos de ginecologistas treinados, e o risco de complicações é relativamente baixo (37,38). A polipectomia histeroscópica é

comumente realizada sob anestesia geral endovenosa, porém, os pólipos endometriais podem ser ressecados em nível ambulatorial (39). A identificação de fatores de risco fidedignos para se distinguir pólipos endometriais benignos dos malignos ou pré-malignos seria útil para se selecionar mulheres portadoras de pólipos com risco aumentado para desenvolverem câncer endometrial, evitando-se, assim, a intervenção cirúrgica (histeroscopia cirúrgica) em mulheres com baixo risco (17).

Fatores hormonais também parecem estar envolvidos na patogênese dos pólipos endometriais. Uma maior expressão de receptores estrogênicos em pólipos endometriais na pós-menopausa em relação à observada no endométrio atrófico sugere uma participação do estrógeno na patogênese do pólipo endometrial como um processo hiperplásico (40). A detecção por imunohistoquímica de receptores estrogênicos (RE) e receptores progestagênicos (RP) no epitélio glandular dos pólipos é maior do que no endométrio adjacente. No estroma, apenas a detecção de RE é maior do que no endométrio adjacente, o mesmo não se observando com relação à detecção de RP (41). Belisário et al. (2006) avaliaram a expressão imunohistoquímica de RE e RP em pólipos endometriais de 35 mulheres na pós-menopausa que não haviam feito uso de medicações hormonais durante os seis meses anteriores ao estudo, e que foram submetidas a histeroscopia para polipectomia no CAISM-Unicamp. Os resultados deste estudo mostraram que, nos pólipos da pós-menopausa, ocorre uma maior expressão de RE, o que levaria a uma maior proliferação do tecido endometrial; sugerindo uma participação dos RE na patogênese dos pólipos (42).

Além dos fatores hormonais, o processo de desenvolvimento normal do endométrio parece ter dois componentes integrados, a proliferação e a apoptose celular, e estes devem estar em desequilíbrio nos pólipos endometriais. Estudos experimentais mostram que o crescimento dos pólipos endometriais pode ser causado pela perda do mecanismo pró-apoptose devido a um aumento da expressão do Bcl-2 tanto em pacientes na pré como na pós-menopausa. Esta perda do mecanismo pró-apoptose deve estar relacionada ao hiperestrogenismo, porque a expressão do Bcl-2 aumenta em resposta ao aumento dos níveis de estrógenos (43). Dados na literatura mostram que o hiperestrogenismo pode levar a um aumento anormal de alguns fatores de crescimento, como o fator de crescimento de fibroblastos, o fator de crescimento epitelial e o fator de crescimento de receptores no endométrio, o que estimularia o crescimento dos pólipos endometriais (44). Em pólipos endometriais de mulheres na pós-menopausa, observa-se uma maior expressão do marcador de apoptose celular Bcl-2 comparada ao tecido endometrial atrófico adjacente, o que sugere que a inibição da apoptose celular seja um mecanismo importante no desenvolvimento destas lesões. A expressão do marcador Bcl-2 é conhecida por demonstrar indiretamente o aumento da longevidade celular, inibindo a apoptose (45).

O Ki-67 está presente nas células endometriais em fase proliferativa, sendo, por este motivo, um excelente marcador de proliferação celular, com alta expressão nos carcinomas endometriais (46). Existem dados conflitantes sobre a expressão do Ki-67 no endométrio da pós-menopausa (47, 48), mas Hachisuga et al. (1999) encontraram níveis baixos de Ki-67 no endométrio da pós-menopausa

(48). Outros estudos têm mostrado uma expressão muito baixa do Ki-67 também nos pólipos endometriais (46,49).

Outros estudos têm investigado se a enzima ciclo-oxigenase-2 (COX-2) está presente nos pólipos endometriais durante a menopausa e como o uso prévio de hormônio poderia afetar esta expressão. As enzimas ciclo-oxigenase existem em duas formas (tipos 1 e 2), e diferentes mecanismos controlam suas expressões nos vários tecidos. Estas enzimas levam à produção de prostaglandinas. A COX-1 está presente na maioria dos tecidos e atua mantendo a homeostase celular. A COX-2 quase não é produzida sob condições normais, sendo, sua expressão, induzida durante os processos de inflamação, de proliferação e de diferenciação celular (50). Essa enzima parece ter participação no desenvolvimento dos pólipos nas mucosas colo-retal e gástrica. A COX-2 possui propriedades de estimular o crescimento das neoplasias colo-retais, e sua expressão está presente em 50% dos adenomas de cólon e em 90% dos carcinomas colo-retais (50,51).

É bem conhecido que a enzima COX-2 está envolvida em diferentes etapas do processo de carcinogênese (52,53). No carcinoma endometrial, estudos mostram um aumento de sua expressão principalmente nos tumores de grau histológico mais avançado (54,55). A expressão de COX-2 também tem sido detectada em hiperplasias e em pólipos endometriais, sugerindo, deste modo, uma possível participação das prostaglandinas na sua patogênese, apesar de não estar ainda completamente esclarecida (51). No endométrio normal, a expressão da COX-2 sofre variações durante o ciclo menstrual em resposta à flutuação dos

níveis estrogênicos e progestagênicos. Os estrógenos são potentes estimuladores da expressão da COX-2, enquanto que os progestágenos exercem efeito oposto, diminuindo a expressão da COX-2 no epitélio glandular durante a fase lútea (56, 57). Os estrógenos estimulam a COX-2 no endométrio, criando uma retroalimentação focal que leva ao aumento dos níveis de estradiol e de prostaglandina, que não estão presentes no endométrio normal (50). Nos pólipos endometriais, ainda não está estabelecido se existe participação da atividade COX-2. Poucos estudos mostram que pólipos endometriais na pós-menopausa, que se desenvolveram espontaneamente ou após uso de terapia hormonal ou de tamoxifeno, apresentam diferentes níveis de expressão de COX-2 no tecido glandular, sugerindo que a COX-2 deve ter participação no seu desenvolvimento semelhante àquele observado na mucosa intestinal (52).

Os pólipos endometriais são um problema comum em mulheres na pós-manopausa e a polipectomia é um procedimento cirúrgico amplamente realizado. É importante salientar que, apesar do baixo risco de malignidade, a falta de conhecimento da etiopatogenia dessas lesões polipoides ainda não nos permite uma conduta expectante mesmo na ausência de fatores de risco conhecidos. Esses fatos ressaltam a necessidade de um maior entendimento dos fatores envolvidos na carcinogênese destas lesões. Estudar a expressão dos receptores hormonais (RE e RP), dos marcadores Bcl-2, Ki67 e da enzima COX-2 poderá contribuir para um melhor conhecimento do processo de malignização de lesões polipoides do endométrio de mulheres na pós-menopausa.

2. Objetivos

2.1 Objetivo Geral

Avaliar a prevalência de malignidade e a expressão de receptores de estrógeno e progesterona, Ki-67, Bcl-2, ciclo-oxigenase-2 em pólipos endometriais de mulheres na pós-menopausa.

2.2 Objetivos Específicos

– Avaliar a prevalência e fatores associados à malignidade em pólipos endometriais de mulheres na pós-menopausa;

– Comparar a expressão de receptores hormonais para estrógeno e progesterona entre pólipos endometriais benignos e malignos de mulheres na pós-menopausa;

– Comparar a expressão de marcadores de proliferação (Ki-67), apoptose celular (Bcl-2), ciclo-oxigenase-2 (COX-2) entre pólipos endometriais benignos e malignos de mulheres na pós-menopausa.

3. Publicações

3.1. Artigo 1

Immunohistochemical expression of estrogen and progesterone receptors in endometrial polyps: comparison between benign and malignant polyps in the postmenopause

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Abstract

Objectives: To evaluate ER and PR expression in the glandular epithelium and stroma of benign and malignant endometrial polyps in the postmenopause. **Material and methods:** A total of 1050 women underwent surgical hysteroscopy in the Prof. Dr. Aristodemo Pinotti Women's Hospital–CAISM/UNICAMP from January 1998 to December 2008. Of the total number, 390 postmenopausal women with endometrial polyps were included in the study. Polypoid lesions were histologically classified as benign (endometrial polyps, polyps with non-atypical simple hyperplasia or non-atypical complex hyperplasia) and premalignant and malignant lesions (polyps with atypical simple hyperplasia or atypical complex hyperplasia and carcinomatous polyps). ER and PR expression was evaluated by immunohistochemistry according to stained cells, intensity of nuclear staining and final score. The final score of receptor expression was compared between benign and premalignant/malignant polyps. **Results:** The prevalence of malignancy in endometrial polyps was 7.1% and was associated with postmenopausal bleeding. Only the final score of ER expression in the stroma of endometrial polyps was higher in the benign group than in the premalignant/malignant group and this difference was significant. There was no difference in PR expression. The risk of malignancy in endometrial polyps was significantly higher when both ER and PR receptor expressions were negative in the stromal component of the polyp ($p<0.01$). **Conclusions:** Malignancy of endometrial polyps was associated with a low expression of stromal estrogen receptor. PR expression did not show any association with the risk of malignancy.

Keywords: endometrial polyp, postmenopause, estrogen receptor, progesterone receptor

Introduction

Endometrial polyps are localized overgrowths of the endometrium with histologic features composed of irregular proliferation of glands and stroma, containing thick-walled blood vessels, lined by pseudostratified or flat epithelium (1).

The prevalence of polyps ranges from 7.8% to 34.9%, depending on the method used for diagnosis and the study population (2). Prevalence increases with age and is higher in the postmenopause than in the premenopause (3).

Malignancy rate associated with endometrial polyps is low. In a recent meta-analysis on the oncogenic potential of polyps, it was observed that the malignancy rate of endometrial polyps ranged from 0.8 to 8% in different studies analyzed (4). In a previous study, we observed a higher occurrence of premalignant and malignant polyps in postmenopausal women aged over 60 years who had vaginal bleeding (5). Other studies showed an association between malignancy and risk factors such as obesity, arterial hypertension, diabetes mellitus and tamoxifen use (6, 7).

Hormonal factors seem to be present in the pathogenesis of endometrial polyps. Estrogen and progesterone are known modulators of endometrial proliferation and differentiation by means of steroid receptors. Furthermore, the development of polyps may be related to a higher receptor expression in the glandular epithelium, leading to focal hyperplasia of the endometrium (8). Few studies with a limited number of tissue samples have assessed the expression of these receptors in endometrial polyps (9-11). In the glandular epithelium of endometrial polyps, immunohistochemical expression of estrogen (ER) and progesterone (PR) receptors is higher than in the adjacent endometrium. In the

stromal component of endometrial polyps, only ER expression is higher than in the adjacent endometrium. The same is not observed with PR (9).

Despite a low prevalence of malignancy in endometrial polyps, the role of ER and PR expression in the mechanisms of carcinogenesis is still unknown. There are no data in the literature evaluating these receptors in malignant polyps. We hypothesized that there might be a difference in receptor expression between malignant and benign polyps.

The aim of this study was to evaluate ER and PR expression in the glandular epithelium and stroma of malignant and benign polyps in postmenopausal women.

Methods

This study was conducted in the "Prof. Dr. José Aristodemo Pinotti" Women's Hospital (CAISM) at the Universidade Estadual de Campinas (UNICAMP). Approval was obtained from the Research Ethics Committee of the UNICAMP School of Medicine under number 769/2009. According to information stored in the computerized database of this institution, 6018 surgical hysteroscopies were performed in this service from January 1998 to December 2008 for the diagnosis and treatment of diverse uterine conditions. Of the women examined, 1050 underwent surgical treatment of endometrial polyps and 508 were postmenopausal. Excluded were women in whom there was no histologic confirmation of endometrial polyp and also users of hormonal therapy and tamoxifen. A total of 390 postmenopausal women, aged 39 to 86 years, diagnosed with endometrial polyp by ultrasound or diagnostic hysteroscopy were included in

this study. Menopause was defined as amenorrhea that had lasted for more than twelve months.

Clinical, histopathologic and hysteroscopic data was retrieved from patient medical records. The following clinical characteristics were observed: age, postmenopausal bleeding, time since menopause, parity, presence of arterial hypertension, obesity, diabetes mellitus and history of breast cancer.

Diagnostic hysteroscopy was performed by using a 2.8-mm optical system (Karl Storz, Tuttlingen, Germany). For distension of the uterine cavity, CO_2 and saline infusion were used. Surgical hysteroscopy was performed by a gynecologist with the patient under spinal anesthesia. A 10-mm resectoscope with a loop electrode was used for the surgical procedure (Karl Storz). Distension of the uterine cavity was obtained by administration of 1.5% glycine solution. The endocervical channel and endometrial cavity were evaluated. Resection of endometrial polyps was performed by electrocautery using the monopolar mode of energy.

Pathologists from the Department of Pathological Anatomy of the UNICAMP Medical School analyzed the endometrial samples obtained, using hematoxylin and eosin (H&E) staining. Polyps were classified as benign, non-atypical simple hyperplasia, non-atypical complex hyperplasia, atypical simple hyperplasia, atypical complex hyperplasia, and malignant.

Construction of TMA (tissue microarray)

Initially, a pathologist from the Department of Pathological Anatomy of the UNICAMP School of Medicine studied the slides representative of endometrial polyps stained with H&E. Two regions that best represented the stroma and

glandular epithelium for the construction of tissue microarray (TMA) were selected following a technique validated for the endometrium (12). Subsequently, the selected regions were identified in archival paraffin blocks (donor blocks). These marked donor blocks were sent to the Laboratory of Immunohistochemistry of the Division of Pathologic Anatomy of the Cancer Hospital-São Paulo for the construction of receptor blocks using the TMA technique. A Tissue Microarrayer (Beecher Instruments, Silver Springs, USA) available at the Department of Pathologic Anatomy in the A C Camargo Women's Hospital (São Paulo) was used. Cylinder cores measuring 1.0 mm from the region of interest obtained by the equipment were transferred to a new block with a two-dimensional layout and 0.2 spacing between the cores, determined and recorded by the equipment. From this new block, named recipient TMA block, histologic sections were obtained with a manual microtome and were transferred in adhesive tape to special adhesive-coated slides (Instrumentics Inc., Hackensack, NJ, EUA). The adhesive tape was removed under exposure to ultraviolet light. Sections were stored, paraffin-embedded, vacuum-packed and frozen at -20°C.

Immunohistochemistry

Estrogen and progesterone receptor expression was performed in the Laboratory of Immunohistochemistry of the Department of Pathology of the Cancer Hospital-São Paulo. TMA sections were 5µm thick. Deparaffinization was performed for 24 hours at 60^{o}C in an incubator. Subsequently, the sections were rinsed in xylene at 60^{o}C for 20 minutes at room temperature for 20 minutes/ 100% ethanol for 30 seconds/ 85% ethanol for 30 seconds/ 70% ethanol for 30 seconds. Sections were washed under distilled running water.

A 10 mM citrate buffer solution (pH 6.0) was heated to a boil in a pressure cooker without sealing the lid (Eterna®, Nigro). The slides were immersed and the cooker lid was sealed with the safety valve in open position. After release of saturated vapor, the safety valve was lowered until total pressurization. Timing was started when the pressure indicator valve reached the maximum (about 4 minutes). The pressure cooker remained closed under running water until total depressurization. We opened the lid of the cooker containing the slides and washed them in distilled running water.

Endogeneous peroxidase was blocked with 3% H_2O_2, (hydrogen peroxide-10 vol) with 3 changes of 10 minutes each. Sections were washed in distilled running water and using 10mM phosphate buffered saline (PBS) (pH 7.4) for 5 minutes.

Slides were incubated with primary antibody diluted in a predefined titer, in PBS buffer containing 1% bovine albumin (BSA) (Sigma, A9647, EUA) and 0.1% sodium azide (NaN_3) for 18 hours in a humidity chamber at 4°C. The procedure used primary monoclonal antibodies for ER (Dako® code M7047, clone 1D5, dilution 1:250), RP (Dako® code M3569, clone PgR 636, dilution 1:500).

The slides were washed in PBS buffer with 3 changes of 3 minutes each and incubated for 30 min at 37° C with Advance™ HRP Link (Dako code# K4068, Carpinteria, CA, EUA). Then they were washed with PBS buffer with 3 changes of 3 min each. Incubation was performed with Advance™ HRP-Enzyme for 30 min at 37° C. Slides were washed with PBS buffer with 3 changes of 3 minutes each and incubated in substrate solution: 100mg de 3,3' Diaminobenzidine-Tetrahydrochloride (DAB; code D-5637, Sigma, St Louis, MO, EUA); 1mL Dimethyl

sulfoxide (DMSO); 1mL 6% H_2O_2 (hydrogen peroxide-20 vol); 100mL PBS; for 5 minutes at 37ºC, protected from light. The slides were washed in distilled running water for 3 minutes and counterstained with Harris' Hematoxylin for 1 minute and then washed thoroughly in distilled running water. Slides were immersed twice in ammoniacal water (0.5% ammonium hydroxide solution), then washed in distilled running water. Sections were dehydrated in: 80% Ethanol, 30 seconds/ Ethanol 95%, 30 seconds/ 100% Ethanol twice, 30 seconds each/ Xylene 4 times, 30 seconds each. The slides were mounted on Entellan-neu (code 1.07961, Merck, Darmstadt, Germany). On microscopy, we observed that a final reaction product appeared as a golden brown precipitate, varying according to the type of marker.

Reading immunohistochemical slides

Hormonal receptors (ER and PR)

TMA slides were read manually by only one pathologist using conventional light microscopy. ER and PR expression was evaluated in the stroma and glandular epithelium in polyp tissues. Receptor expression was evaluated by using a semiquantitative method of nuclear reaction through analysis of the percentage of stained cells, intensity of nuclear staining and final scoring (13). The percentage of stained cells was visually estimated and categorized as: grade 0: no stained cell; grade 1: < 1% stained cells; grade 2: from 1 to 10% stained cells; grade 3: from 11 to 33% stained cells; grade 4: from 34 to 66% stained cells; grade 5: > 66% stained cells. Regarding the intensity of nuclear staining, categories were as follows: grade 0: negative; grade 1: weak reaction; grade 2: moderate reaction and grade 3:

intense reaction (13). The sum of positivity and intensity resulted in a final score, ranging from 0 to 8 (excluding value 1). **Figure 1**

Statistical analysis

For statistical analysis**,** polyps were grouped into benign (including polyps of the endometrial mucosa, polyps with non-atypical simple hyperplasia or non-atypical complex hyperplasia) and premalignant/malignant (including polyps with atypical simple hyperplasia or atypical complex hyperplasia and carcinomatous polyps). Clinical characteristics between groups of benign and malignant polyps were compared using the chi-square, Fisher's exact or Mann-Whitney nonparametric tests. To compare the final scores of ER and PR in the glandular epithelium and stroma of polyps, a final score of up to 2 was considered a negative reaction. A final score of 3 or higher was considered a positive reaction. This comparison was made by using Fisher's exact test and the chi-square test. A combination of ER/PR receptor expression in the glandular epithelium and stroma of endometrial polyps in comparison to malignant and benign lesions was calculated using Fisher's exact test. The SAS program (Statistical Analysis System), version 9.2, was used for these calculations. Significance level was set at $P < 0.05$.

Results

Table 1 shows the histologic diagnosis of resected lesions. Three hundred and sixty-two (362) benign lesions were diagnosed (92.8%), including 313 endometrial polyps (80.2%), 41 polyps with non-atypical simple hyperplasia (10.5%) and 8 polyps with non-atypical complex hyperplasia (2.05%). Premalignant lesions consisted of 5 polyps with atypical simple hyperplasia (1.28%) and 3 polyps with

atypical complex hyperplasia (0.76%). Twenty (20) malignant polyps (5.11%) were diagnosed. Among the malignant polyps, endometrioid adenocarcinoma was the histologic type of the majority. There was one with less-differentiated endometrial carcinoma and one with serous endometrial cancer.

The mean age of women with benign polyps was 61.7 years (SD±7.8) and 64.4 years (SD±10.4) for those with malignant polyps. There was no difference in mean age at menopause in both groups (48.9±7.5 vs 50.4±4.8) (p=0.236). Table 2 shows a comparison of clinical characteristics in patients studied. There was no difference related to the presence of comorbid disorders such as arterial hypertension, diabetes mellitus, breast cancer, obesity and parity among women with benign and premalignant/malignant polyps. The presence of postmenopausal bleeding was significantly greater in women with premalignant/malignant polyps (p= 0.0015) (Table 2).

Comparing the final ER and PR score between benign and premalignant/malignant polyps, we observed that only the final score of ER expression in the stroma of endometrial polyps was higher in benign polyps than in premalignant and malignant polyps, and this was a significant difference (Table 3). Comparing a combination of ER/PR expression, we observed that the risk of malignancy in polyps was significantly higher when expression of both receptors was negative (ER-/PR-) in the stroma of endometrial polyps (OR 6.5 95%CI 2.05-20.29). There was no significant difference in the glandular epithelium (Table 4).

Discussion:

This study was conducted to evaluate ER and PR expression in malignant and benign endometrial polyps in the postmenopause. It indicated that malignant polyps had a lower glandular and stromal ER expression. PR expression was not associated with malignancy.

To the best of our knowledge, this study concerning ER and PR receptor expression in the postmenopause is the largest case study to evaluate an association with malignancy. The prevalence of malignancy in the sample studied was 7.1% and postmenopausal bleeding was the only clinical parameter associated with the risk of malignancy in endometrial polyps. A previous study conducted by our group showed a prevalence of 4.1% (14). These prevalence rates are in agreement with those of other authors who have shown a prevalence of malignancy ranging from 0.8 to 8.0 % (15, 16).

Concerning hormone receptor expression, ER and PR are specific nuclear receptors that belong to the steroid receptor family. The activity of estrogen receptors is based on specific regions of the gene (17). Furthermore, the formation and concentration of new receptors seem to be self-regulated and dependent on hormonal factors (18). For progesterone, tissue expression is not related to the hormonal status found in the postmenopause, in which progestational activity is not observed (19, 20). The induction of progesterone receptor formation in the endometrium is mainly a consequence of estrogen stimulation (19).

We observed that in both benign and premalignant/malignant polyps, ER and PR have a higher expression in glandular cells than in stromal cells. This higher glandular expression of ER and PR receptors has also been observed in

postmenopausal endometrial polyps compared to the atrophic endometrium (10) or adjacent endometrium (21-24). Other studies demonstrated that polyps in postmenopausal women have increased ER expression in both the stroma and gland compared to polyps in premenopausal women (11, 25). However, PR is higher only in the glandular epithelium, with no difference in relation to the stroma (25).

There are few studies investigating the pathogenesis of endometrial polyps in detail, but this was the first to compare receptor expression between benign and malignant cases. In the present study, benign polyps also showed a higher ER expression in the glandular epithelium and stroma. However, there was no difference regarding PR when compared to premalignant/malignant polyps. This seems to suggest that benign polyps in the postmenopause may respond to an increased number of receptors, consequent to low estrogen levels in the menopause. A high expression in the glandular epithelia suggests a higher sensitivity of these structures to steroid hormones, which may be responsible for the development of benign polyps in the presence of low serum estrogen levels, while malignant polyps appear to be developed by a different etiology.

In contrast to the high ER and PR receptor expression observed in benign endometrial polyps, some studies have demonstrated that the loss of steroid receptors is an early event in endometrial carcinogenesis, and endometrial carcinoma usually has a lower level of ER and PR receptors than the normal endometrium or in endometrial hyperplasia (26, 27). These findings suggest that the development of benign polyps and carcinomatous polyps may follow distinct pathways. In contrast, the majority of studies show that estrogens promote

endometrial carcinogenesis directly by stimulating the rapid proliferation of epithelial cells and a high estrogen receptor expression is observed in hyperplasia and carcinoma in populations of stromal and epithelial cells (28-30).

According to the literature, ER and PR expression may be lower in more advanced tumors and less-differentiated tumors, and is a factor of worse prognosis (29, 30). In this study, the presence of serous carcinoma and a less-differentiated carcinoma may have contributed to a decrease in ER expression in the group of premalignant/malignant polyps. In the present study, we also observed a malignancy risk that was six times higher when expression was negative in both receptors (ER/PR). Other studies have demonstrated that steroid receptor expression is not an independent prognostic factor for endometrial cancer, and there are doubts as to the usefulness of determining receptor expression in patients with endometrial neoplasm (31,32). We did not find any study in the literature evaluating these receptors in malignant polyps for the comparison of results. However, it can be inferred from these differences that pathways of carcinogenesis in endometrial polyps may be different from those observed in endometrial cancer or may be closer to neoplasms of worse prognosis.

Discrepancies regarding the results of our study and the different studies in the literature may in part be explained by variations in methodology, especially differences in antibody specificity and dilutions used. The lack of consensus in the criteria defined for positivity and the semiquantitative nature of the method may have also contributed to the different results between studies, according to the criteria used. Another possible limitation was the small number of premalignant/malignant polyps analyzed in the case study, which may have

interfered with the capacity of statistical tests to identify significant differences between groups. Nevertheless, it is important to highlight that the number of premalignant/malignant polyps may be high, in view of the low prevalence of malignancy associated with polyps.

Conclusion

In conclusion, our findings have shown that polyps in the postmenopause have a high estrogen receptor expression in the stroma and gland. This expression is lower in premalignant/malignant polyps than in benign polyps. This data suggests that a lower estrogen receptor expression may be one more risk factor for the malignancy potential of polyps in postmenopausal women. Polypectomy has been routinely indicated to stop bleeding and to exclude malignancy. No tool is currently available to make predictions of malignancy in these lesions and histologic evaluation of the resected polyp continues to be the only form of diagnosing malignant cases. The usefulness of measuring receptors in polyps is still questionable. The real etiology of polyps and their mechanisms of carcinogenesis seem to occur by different mechanisms that are still unclear, but necessary for adequate management of endometrial polyps.

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Table 1 - Histologic Diagnosis of Endometrial Polyps in the Postmenopause

Histologic Diagnosis	n	%
Benign		
Endometrial Polyp	313	80.25
Polyp without Atypical Simple Hyperplasia	41	10.51
Polyp without Atypical Complex Hyperplasia	8	2.05
Subtotal	362	**92.82**
Premalignant / Malignant		
Polyp with Atypical Simple Hyperplasia	5	1.28
Polyp with Atypical Complex Hyperplasia	3	0.76
Polyp with Endometrioid Adenocarcinoma	18	4.61
Polyp with Less-differentiated Endometrial Carcinoma	1	0.25
Polyp with Serous Endometrial Cancer	1	0.25
Subtotal	28	**7.17**
Total	390	**100**

Table 2 – Clinical characteristics of women with benign and malignant endometrial polyps in the postmenopause and prevalence of malignancy (n=390)

	Benign		*Premalignant/ Malignant*		*p-value*
	n	*%*	*N*	*%*	
Age					0.855*
<40	1	100	0	0	
40-59	153	93.3	11	6.7	
≥ 60	207	92.4	17	7.6	
Postmenopausal bleeding					**0.001**
Yes	143	**87.7**	20	**12.3**	
No	210	**96.3**	8	**3.7**	
SAH					0.185
Yes	254	91.7	23	8.3	
No	107	95.5	5	4.5	
DM					0,232
Yes	103	90.4	11	9.6	
No	257	93.8	17	6.2	
Breast Cancer					0.666*
Yes	20	90.9	2	9.1	
No	341	92.9	26	7.1	
BMI					0.072
<30	150	95.5	7	4.5	
≥30	204	90.7	21	9.3	
Parity					0.709*
Nulliparous	28	96.6	1	3.4	
Multiparous	331	92.5	27	7.5	

Chi-square test/*Fisher's exact test

Table 3- Final ER/PR score in benign and malignant polyps in postmenopausal women (n=390)

Final score	*Benign (n=362) %*	*Premalignant/ Malignant (n=28) %*	*p-value*	*OR (95%CI)*
ER gland (n=381)			0.5721	
Positive	85.6	81.5		1.0
Negative	14.4	18.5		1.4 (0.49-3.73)
ER estroma (n=384)			**0.0024**	
Positive	82.9	59.3		1.0
Negative	17.1	40.7		**3.3** (1.48-7.54)
PR gland (n=379)			0.7089*	
Positive	93.4	92.9		1.0
Negative	6.6	7.1		1.1 (0.24 -4.91)
PR stroma (n=381)			0.1004*	
Positive	89.8	77.8		1.0
Negative	10.2	22.2		1.4 (0.56-3.30)

Fisher's Exact test/*chi-square test

Table 4- Comparison of the combination of ER/PR Final Score of benign and malignant polyps in postmenopausal women (n=390)

RE/RP Final Score	*Benign (n=362) %*	*Premalignant/ Malignant (n=28) %*	*p-value*	*OR (95%CI)*
ER /PR gland (n=372)			0.2269	
ER+/PR+	96.3	90.9		1.0
ER+/PR-	3.7	9.1		2.6 (0.54-12.58)
ER-/PR+	76.6	100.0		2.0 (0.70-5.64)
ER-/PR-	23.4	0,0		0.6 (0.03-10.72)
ER/PR stroma (n=375)			**0.0055**	
ER+/PR+	93.1	93.3		1.0
ER+/PR-	6.9	6.7		1.0 (0.12-7.74)
ER-/PR+	74.1	54.5		2,7 (0.98-7.41)
ER-/PR-	25.9	45.5		**6.5** (2.05-20.29)

Fisher's Exact test

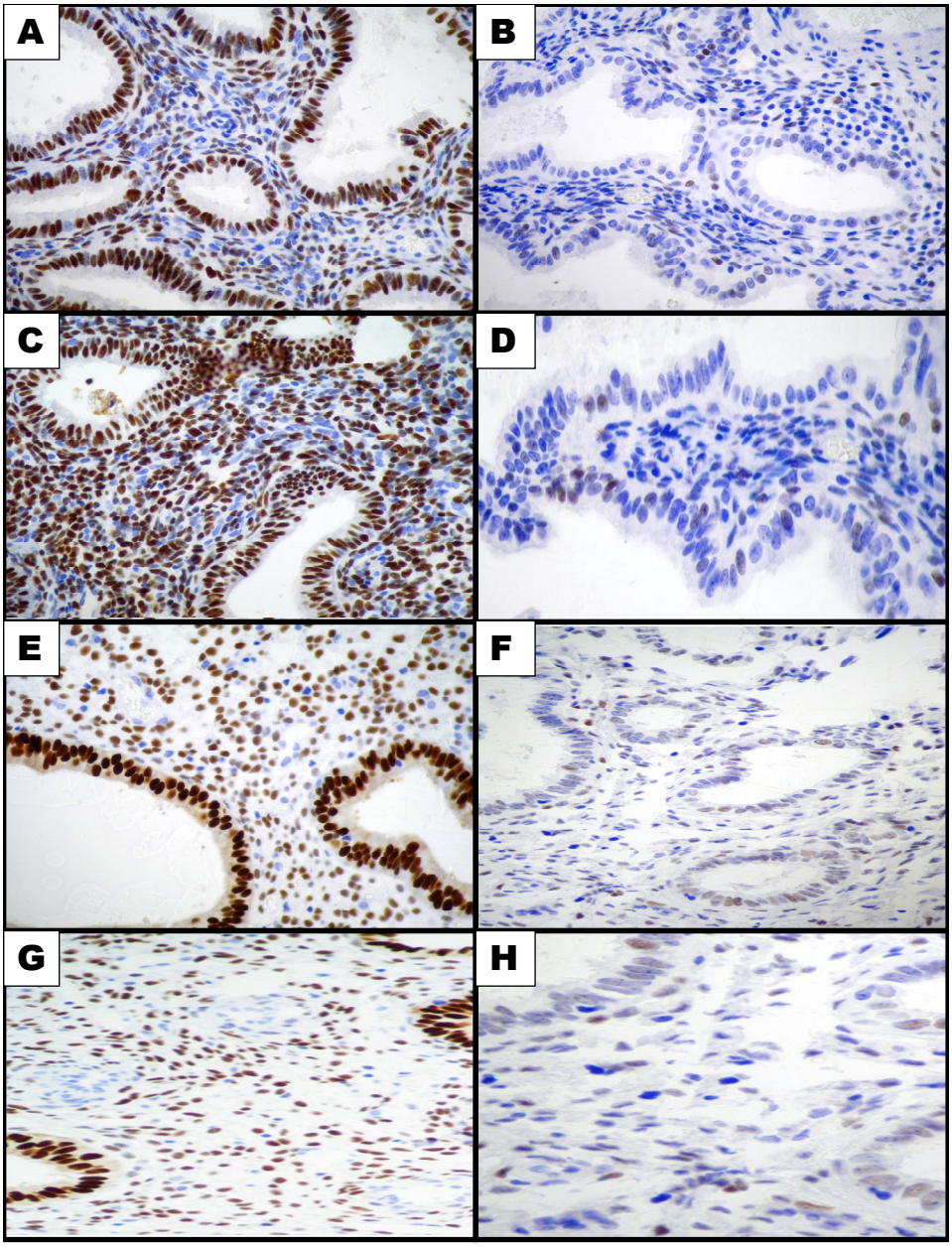

Figure 1. Representative immunohistochemical nuclear staining of RE and RP. (A) ER+ and (B) ER− in the glandular epithelium. (C) ER+ and (D) ER− in the stroma. (E) PR+ and (F) PR− in the glandular epithelium. (G) PR+ and (H) PR−in the stroma.

3.2. Artigo 2

Is the immunohistochemical expression of proliferation (Ki-67) and apoptosis (Bcl-2) markers, and cyclooxigenase-2 (COX-2) related to carcinogenesis in postmenopausal endometrial polyps?

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Abstract

Objectives: To evaluate the pattern of Ki-67, Bcl-2 and COX-2 expression in the glandular epithelium and stroma of malignant and benign endometrial polyps in the postmenopause. **Material and methods:** A total of 1050 women underwent surgical hysteroscopy in the "Prof. Dr. Aristodemo Pinotti" Women's Hospital– CAISM/UNICAMP from January 1998 to December 2008. Of the total number, 390 postmenopausal women with endometrial polyps were included. Polypoid lesions were histologically classified as benign (endometrial polyps, polyps with simple or complex hyperplasia without atypia) and premalignant and malignant (polyps with simple or complex hyperplasia with atypia and carcinomatous polyps) lesions. Ki-67, Bcl-2 and COX-2 expression was evaluated by immunohistochemistry according to percentage of stained cells, staining intensity and final score. **Results:** The prevalence of malignancy in endometrial polyps was 7.1% and was associated with postmenopausal bleeding. The final score showed that only mean COX-2 expression was higher in malignant polyps both in the glandular epithelium (6.1 ± 2.5) ($p<0.001$) and stroma (2.4 ± 3.0) ($p<0.01$). There was a higher Bcl-2 expression, especially in the glandular epithelium, with no differences between benign polyps and premalignant/malignant polyps. Ki-67 expression was low in both benign polyps and premalignant/malignant polyps. **Conclusion:** Polyps in the postmenopause have a high COX-2 expression that is higher in malignant polyps than in benign polyps. There was no difference in Ki-67 and Bcl-2 expression between malignant polyps and premalignant/malignant polyps.

Keywords: endometrial polyp, postmenopause, immunohistochemistry, Ki-67, Bcl-2, COX-2.

Introduction

The diagnosis of endometrial polyp has increased in the last decades due to a more frequent use of transvaginal ultrasound and diagnostic hysteroscopy (1,2). Endometrial polyps are detected in the premenopause and postmenopause and their prevalence rates range from 7.8% to 34.9%, depending on the study population and diagnostic method used (3).

In a systematic review, Lee et al. (2010) observed that among women with endometrial polyps, menopausal status and the presence of vaginal bleeding showed an association with a higher risk of malignancy (4). Another systematic review showed that the prevalence rate of malignancy in endometrial polyps was 0 to 12.9% in the studies included (5).

Despite the low prevalence of malignancy in endometrial polyps, the mechanisms involved in endometrial carcinogenesis remain unclear. The participation of biological markers in endometrial carcinogenesis has been investigated in cases of endometrial carcinoma and appear to influence the early detection and adequate treatment of these lesions, allowing the establishment of correlations with clinical parameters and prognosis (6,7). However, little is known about the participation of these biomarkers in the carcinogenesis of endometrial polyps.

In endometrial polyps, there seems to be an imbalance between cell proliferation and apoptosis. Studies show that the growth of endometrial polyps may be caused by loss of the pro-apoptotic mechanism, due to increased Bcl-2 expression (8,9). This loss of the pro-apoptotic mechanism must be related to

hyperestrogenism, since Bcl-2 expression increases in response to increased estrogen levels (8). The expression of Bcl-2 marker is known for indirectly demonstrating an increase in cell longevity, inhibiting apoptosis (9).

Ki-67 is present in endometrial cells only in the proliferative phase. That is why it is an excellent marker of cell proliferation, with a high expression in endometrial carcinomas (10). There is conflicting data on Ki-67 expression in the postmenopausal endometrium (11,12), but Hachisuga et al. (1999) found low levels of Ki-67 expression in the postmenopausal endometrium (12). Other studies have also demonstrated a very low Ki-67 expression in endometrial polyps (10,13).

COX-2 is virtually never produced under normal conditions. Its expression is induced during the processes of inflammation, proliferation and cell differentiation (14). COX-2 expression has also been detected in endometrial hyperplasia, thus suggesting a likely participation of prostaglandins in the pathogenesis of hyperplasia (15). Estrogens are potent stimulants of COX-2 expression, while progestogens exert an opposite effect, decreasing COX-2 expression in the glandular epithelium during the luteal phase of the menstrual cycle (16).

Attention has been focused on the involvement of COX-2 in the initiation and progression of solid tumors (17). In cancerous cells, overexpression of COX-2 is associated with an increased inhibition of apoptosis, metastatic ability and neoangiogenesis (18,19). Its high expression has been related to clinical and pathologic parameters of tumor aggressiveness and poor prognosis (20,21).

In endometrial polyps, it still has not been determined whether there is any participation of COX-2 activity. Few studies have shown that postmenopausal

endometrial polyps have different levels of COX-2 expression in the glandular epithelium (15,22). Maia et al. (2006) observed a higher COX-2 expression in endometrial polyps than in the normal endometrium (15).

The aim of this study was to evaluate the expression of Ki-67, Bcl-2 and COX-2 in the glandular epithelium and stroma of malignant and benign polyps of the postmenopause.

Methods

Data collection

This study was conducted in the Prof. Dr. José Aristodemo Pinotti Women's Hospital, CAISM-University of Campinas (UNICAMP). Protocol approval was obtained from the Research Ethics Committee of the UNICAMP School of Medicine under number 769/2009. According to information retrieved from the computerized database of the institution, 6,018 surgical hysteroscopies were performed in this healthcare service from January 1998 to December 2008 for the diagnosis and treatment of various uterine conditions. Of the women examined, 1,050 underwent surgical treatment of endometrial polyps and 508 were postmenopausal. Women who had not received any histopathological confirmation of endometrial polyp, users of hormone therapy and tamoxifen were excluded from the study. A total of 390 postmenopausal women (aged range: 39-86 years) diagnosed with endometrial polyp by ultrasound or diagnostic hysteroscopy were included. Menopause was defined as amenorrhea that lasted for more than twelve months.

Clinical, histopathological and hysteroscopic data was gathered from patient medical records. The clinical characteristics assessed were age, postmenopausal bleeding, parity, arterial hypertension, obesity, diabetes mellitus and history of breast cancer.

Diagnostic hysteroscopy was performed by using a 2.8-mm optical system (Karl Storz, Tuttlingen, Germany). CO_2 and saline infusion were used to distend the uterine cavity. Surgical hysteroscopy was performed by a gynecologist with the patient under spinal anesthesia. A 10-mm resectoscope with a loop electrode was used for the surgical procedure (Karl Storz®). Distension of the uterine cavity was obtained by use of 1.5% glycine solution. The endocervical channel and endometrial cavity were evaluated. Resection of endometrial polyps was performed by electrocautery using monopolar energy.

Pathologists from the Department of Pathological Anatomy of the UNICAMP Medical School analyzed the endometrial samples collected, using hematoxylin and eosin (H&E) staining. Polyps were classified as benign, simple hyperplasia without atypia, complex hyperplasia without atypia, simple hyperplasia with atypia, complex hyperplasia with atypia, and malignant.

Immunohistochemistry

Construction of TMA (Tissue Microarray)

Initially, a pathologist of the Department of Pathological Anatomy of the UNICAMP School of Medicine studied the section slides that were representative of endometrial polyps stained with H&E. Two regions that best represented the stroma and glandular epithelium for the construction of tissue microarray (TMA) were selected, adopting a technique validated for the endometrium (23). Subsequently, the selected regions were identified in archival paraffin blocks (donor block). These donor blocks were sent to the Laboratory of Immunohistochemistry at the Division of Pathological Anatomy in the Cancer Hospital-São Paulo for the construction of recipient blocks by TMA technique. A Tissue Microarrayer (Beecher Instruments, Silver Springs, USA) available at the Department of Anatomy of the A C Camargo Cancer Hospital-São Paulo was used. Cylinder cores measuring 1.0mm from the region of interest obtained by the equipment were transferred to a new block with a two-dimensional layout and intercore spacing of 0.2 mm, determined and recorded by the equipment. From this new block, named recipient block, histological sections performed with a manual microtome were obtained and transferred in adhesive tape to special adhesive-coated slides (Instrumentics Inc., Hackensack, NJ, USA). The adhesive tape was removed under exposure to ultraviolet light, and the slides were stored and paraffin-embedded, vacuum-packed and frozen in freezer at -20°C.

Immunohistochemistry

The expression of biomarkers of cell proliferation (Ki-67), apoptosis (Bcl-2) and COX-2 enzyme was performed in the Laboratory of Immunohistochemistry in the Department of Pathology at the Cancer Hospital-São Paulo. TMA sections were 5 µm thick. Deparaffinization was performed for 24 hours in an incubator at 60°C. Subsequently, the slides were washed in xylene at 60°C for 20 minutes/ xylene at room temperature for 20 minutes/ 100% ethanol for 30 seconds/ 85% ethanol for 30 seconds/ 70% ethanol for 30 seconds. The slides were washed in distilled tap water.

Citrate buffer solution 10 mM (pH 6.0) was heated to a boil in a pressure cooker (Eterna®, Nigro) without sealing the lid. The slides were immersed into boiling retrieval buffer and the cooker was sealed with the safety valve in the open position. After release of the saturated vapor, the safety valve was lowered until total pressurization. Timing the duration of antigen retrieval was started when total pressurization occurred (about 4 minutes). The cooker remained closed under tap water until total depressurization. The lid of the cooker containing the slides was removed and the slides were washed in distilled tap water.

Endogenous peroxidase blocking was performed with 3% H_2O_2, (hydrogen peroxide-10 vol) with 3 changes of 10 minutes each. The slides were rinsed in distilled tap water and with PBS (phosphate buffered saline) 10mM (pH 7.4) for 5 minutes.

The slides were incubated with primary antibody diluted in a predefined titer in PBS buffer containing bovine albumin (BSA) 1% (Sigma, A9647, USA) and 0.1%

sodium azide (NaN_3) for 18 hours in a humidity chamber at 4°C. Primary monoclonal antibodies for Bcl-2 (Biogen® code M887, dilution 1:200) and Ki-67 (Dako® code M7240, clone MIB 1, dilution 1:1000) were used in the procedure. For COX-2, polyclonal antibody was used (polyclonal from Abcam, dilution 1:200).

The slides were washed in PBS buffer with 3 changes of 3 minutes each and incubated for 30 min at 37° C with Advance™ HRP Link (Dako code# K4068, Carpinteria, CA, USA). The slides were then washed with PBS buffer with 3 changes of 3 min each and incubated with Advance™ HRP-Enzyme for 30 min at 37° C. The slides were washed in PBS buffer with 3 changes of 3 minutes each and incubated in substrate solution: 100mg of 3,3' Diaminobenzidine-Tetrahydrochloride (DAB; code D-5637, Sigma, St Louis, MO, USA); 1mL of Dimethylsulfoxide (DMSO); 1mL of H_2O_2 6% (hydrogen peroxide-20 vol); 100 mL of PBS; for 5 minutes at 37ºC and protected from light. Slides were washed in distilled tap water for 3 minutes. Counterstaining was performed with Harris' Hematoxylin for 1 minute and the slides were rinsed in distilled tap water. The slides were immersed twice in ammonium water (0.5% ammonium hydroxide solution), then rinsed in distilled tap water. Slides were dehydrated in: 80% Ethanol, 30 seconds/ 95% Ethanol, 30 seconds/ 100% Ethanol twice, 30 seconds each/ Xylene 4 times, 30 seconds each. Slides were mounted in Entellan-neu (code 1.07961, Merck, Darmstadt, Germany). On microscopy, we observed a golden brown precipitate as a final reaction product, varying with the type of marker.

Immunohistochemical reading

Markers of proliferation (Ki-67), apoptosis (Bcl-2) and cyclooxygenase-2 (COX-2)

A single pathologist read the TMA slides manually under conventional light microscopy. Bcl-2 and COX-2 expression was evaluated in the stroma and glandular epithelium of polyp tissues. This expression was evaluated by a semiquantitative method of cytoplasmic reaction through analysis of the percentage of stained cells, intensity of nuclear staining and final score (24). The percentage of stained cells was visually estimated and categorized as follows: grade 0: none stained; grade 1: < 1% stained cells; grade 2: 1 to 10% stained cells; grade 3: 11 to 33% stained cells; grade 4: 34 to 66% stained cells; grade 5: > 66% stained cells. Concerning staining intensity, grading was as follows: grade 0: negative; grade 1: weak reaction; grade 2: moderate reaction and grade 3: intense reaction (24). The sum of positivity and intensity resulted in a final score, ranging from 0 to 8 (excluding value 1). Ki-67 expression was analyzed by immunohistochemistry in a scale from 0 to 3+ and categorized as follows: score 0: < 10% cells showing positivity; score 1+: 11 to 50%; score 2+: 51 to 80%; score 3+: > 80%. **Figure 1**

Statistical analysis

For statistical analysis, polyps were grouped into benign (including polyps of the endometrial mucosa, polyps with simple hyperplasia or non-atypical complex hyperplasia) and premalignant/malignant (including polyps with simple hyperplasia or atypical complex hyperplasia and carcinomatous polyps).

The clinical characteristics between the benign polyp group and the premalignant/malignant polyp group were compared using the chi-square test, Fisher's exact test or Mann-Whitney nonparametric test. To compare the mean final scores of Bcl-2 and COX-2 in the glandular epithelium and stroma, the Mann-Whitney nonparametric test was used. Ki-67 expression was evaluated only by positivity of nuclear staining. A negative reaction was considered when the staining nuclear score was 0 and a positive reaction when scores were 1+ to 3+. The SAS program (Statistical Analysis System), version 9.2, was used for these calculations. The significance level was set at $p<0.05$.

Results

The mean age of the women with benign polyps was 61.7 years (SD±7.8) and it was 64.4 years (SD±10.4) for malignant polyps. Table 1 shows the histopathological diagnosis of resected lesions. Three hundred and sixty-two (362) benign lesions were diagnosed (92.8%), including 313 endometrial polyps (80.2%), 41 polyps with non-atypical simple hyperplasia (10.5%) and 8 polyps with non-atypical complex hyperplasia (2.05%). Premalignant lesions consisted of 5 polyps with atypical simple hyperplasia (1.28%) and 3 polyps with atypical complex hyperplasia (0.76%). There was diagnostic confirmation of twenty malignant polyps (5.11%). The histopathological type of the majority of malignant polyps was endometrioid adenocarcinoma. One patient was diagnosed with less-differentiated endometrial carcinoma and one with serous endometrial cancer.

Table 2 shows a comparison of clinical characteristics of the studied patients. There was no difference regarding the presence of comorbid conditions

such as arterial hypertension, diabetes mellitus, breast cancer, obesity and parity among women with benign and malignant polyps. The presence of postmenopausal bleeding was significantly higher in women with malignant polyps ($p< 0.001$). (Table 2).

The final score represented by the sum of positivity scores and the intensity of stained cells showed that the mean COX-2 expression was significantly higher in premalignant/malignant polyps than in benign polyps in both the glandular epithelium (6.1 ± 2.5) ($p<0.001$) and stroma (2.4 ± 3.0) ($p<0.01$). Regarding Bcl-2 expression, there was no significant difference between premalignant/malignant polyps and postmenopausal benign polyps (Table 3). Ki-67 expression was low in the glandular epithelium and stroma, in both benign polyps and premalignant/malignant polyps (Table 4).

Discussion

Few studies have evaluated endometrial polyps and their pathogenesis in detail. This study was carried out to assess the expression of proliferation/apoptosis (ki-67 and Bcl-2) biomarkers and COX-2 in polyps of postmenopausal women and compare this expression between benign and malignant endometrial polyps, and thus confirm the participation of these markers in carcinogenesis.

The malignancy rate in this sample was 7.1% and postmenopausal bleeding was the only clinical parameter associated with malignancy in polyps. A previous study conducted by our group concluded that the prevalence of malignancy was 4.1% (25). These prevalence rates are in agreement with those of

other authors who demonstrated a prevalence of malignancy ranging from 0.8 to 8.0 % (4).

Concerning Bcl-2 biological marker, this study showed a higher Bcl-2 expression in the glandular epithelium with minimum stromal expression. These findings have also been reported by other authors (10,11). Previous studies showed an increased Bcl-2 expression in endometrial polyps compared to the adjacent endometrium (9,26). This high Bcl-2 expression in endometrial polyps in postmenopausal women suggests that the development of these polyps may be due to increased cell longevity by inhibition of apoptosis (10-27).

When we compared the mean final score of Bcl-2 expression between benign and malignant polyps, we did not observe any differences between benign polyps and premalignant/malignant polyps. Amalinei et al. (2011) compared the immunohistochemical expression of Bcl-2 in the glandular epithelium and stroma of normal endometrium, hyperplastic endometrium and endometrial carcinoma, confirming a low expression of this marker that was higher in the glandular component of endometrial carcinomas (28). In contrast, some previous studies confirmed a higher Bcl-2expression in hyperplasia than in endometrial carcinoma (29-31). This suggests that Bcl-2 expression in endometrial glands may have an important role in the early stages of endometrial carcinogenesis (32).

COX-2 is an enzyme that is less frequently expressed under normal conditions. It is produced during processes of inflammation, cell proliferation and differentiation (33). In the normal menstrual cycle, COX-2 expression undergoes fluctuations in response to levels of estrogen and progesterone (16). Estrogen is a

potent stimulant of COX-2 (34) while progesterone exerts the opposite effect (16). There are few studies evaluating COX-2 expression in endometrial polyps. Maia et al (2005) observed that COX-2 was present in the glandular epithelium of postmenopausal polyps, concluding that COX-2 may regulate the growth of polyps (22). Another study showed COX-2 expression in the glandular epithelium and stroma of premenopausal and postmenopausal polyps, with no significant difference in the expression of this enzyme (35). In the present study, the mean final score of COX-2 expression in the glandular epithelium and stroma was significantly higher in malignant polyps than in benign polyps. There are no studies on malignant endometrial polyps to compare with our results. However, these findings are in agreement with those of other studies demonstrating that an increased COX-2 expression in premalignant and malignant lesions (e.g. endometrial hyperplasia and endometrial cancer) was directly correlated with histological grade and level of endometrial tumor invasiveness (36-38).

Ki-67 is an antigen present only in proliferating cells that is not expressed in quiescent cells. It acts as an ideal marker for the determination of cell growth. There are conflicting data on Ki-67 levels in the postmenopausal endometrium, but studies have shown decreased levels in the atrophic endometrium (12). This cellular quiescence might be consistent with the hypoestrogenic status in the postmenopause. In the present study, Ki-67 expression was low in benign and malignant polyps. This data is in agreement with the findings of other authors who also observed a low Ki-67 expression in postmenopausal endometrial polyps (27). These findings reinforce the concept that polyps may develop mostly due to a decrease in apoptosis rather than by increased cell division (26).

Discrepancies in the results from different studies may, in part, be explained by variations in methodology, mainly the difference in antibody specificity and dilutions used. There are diverse scores (24, 39) to analyze the IHC expression of different markers. Some studies show isolated data of staining intensity and the percentage of stained cells, while other studies established a final score through the sum (24) or multiplication (39) of categories attributed to the intensity and percentage of stained cells. The lack of consensus in the criteria defined for positivity of the IHC reaction and semiquantitative nature of the method may have also contributed to the different study results, according to the criteria used. Another possible limitation is the small number of malignant polyps analyzed in the present case study that may have interfered with the capacity of statistical tests to identify significant differences between groups. However, it is important to highlight that the number of malignant polyps in this study may be considered large as opposed to the prevalence of malignancy associated with polyps.

Conclusion

In conclusion, our findings have shown that postmenopausal endometrial polyps have a high COX-2 expression that is higher in malignant polyps. Despite the higher Bcl-2 expression in the glandular epithelium, there was no relationship with malignancy. Ki-67 expression was low in both benign and malignant polyps.

Based on the data from this study, it is possible to formulate a hypothesis that inhibition of apoptosis with increased cell longevity and neoangiogenesis may be involved in the development of endometrial polyps and represent one more risk factor for the malignancy potential of polyps in postmenopausal women. The real

etiology and endometrial carcinogenesis of polyps seems to occur because of complex mechanisms that are still nuclear. Further research studies are necessary to understand the factors that lead to tumor progression of endometrial polyps in the postmenopause.

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Table 1 - Histological Diagnosis of Endometrial Polyps in the Postmenopause

Histological Diagnosis	n	%
Benign		
Endometrial polyp	313	80.25
Polyp without Atypical Simple Hyperplasia	41	10.51
Polyp without Atypical Complex Hyperplasia	8	2.05
Subtotal	362	**92.82**
Premalignant / Malignant		
Polyp with Atypical Simple Hyperplasia	5	1.28
Polyp with Atypical Complex Hyperplasia	3	0.76
Polyp with Endometrioid Adenocarcinoma	18	4.61
Polyp with less-differentiated Endometrial Carcinoma	1	0.25
Polyp with Serous Endometrial Cancer	1	0.25
Subtotal	28	**7.17**
Total	390	**100**

Table 2 – Clinical characteristics of women with benign and malignant endometrial polyps in the postmenopause and prevalence of malignancy (n=390)

	Benign		*Premalignant/ Malignant*		*p-value*
	n	*%*	*N*	*%*	
Age					0.855*
<40	1	100	0	0	
40-59	153	93.3	11	6.7	
≥ 60	207	92.4	17	7.6	
Postmenopausal bleeding					**0.001**
Yes	143	**87.7**	20	**12.3**	
No	210	**96.3**	8	**3.7**	
SAH					0.185
Yes	254	91.7	23	8.3	
No	107	95.5	5	4.5	
DM					0,232
Yes	103	90.4	11	9.6	
No	257	93.8	17	6.2	
Breast Cancer					0.666*
Yes	20	90.9	2	9.1	
No	341	92.9	26	7.1	
BMI					0.072
<30	150	95.5	7	4.5	
≥30	204	90.7	21	9.3	
Parity					0.709*
Nulliparous	28	96.6	1	3.4	
Multiparous	331	92.5	27	7.5	

Chi-square test/*Fisher's exact test

Table 3- Final scores of COX-2 and Bcl-2 expression in benign and malignant polyps in postmenopausal women (n=390)

Final score	*Benign (n=362)* n	Mean	SD	*Premalignant/malignant (n=28)* n	Mean	SD	*p-value*
Glandular COX-2	354	5.3	2.2	28	6.1	2.5	**0.001**
Stromal COX-2	355	1.2	2.1	28	2.4	3.0	**0.010**
Glandular Bcl-2	350	3.7	2.5	28	4.0	2.9	0.645
Stromal Bcl-2	353	1.2	2.0	28	0.6	1.8	0.080

Mann-Whitney test

Table 4- Immunohistochemical expression of Ki-67 in benign and malignant polyps in postmenopausal women (n=390)

Ki-67	*Benign n=362* n	%	*Premalignant/ malignant n=28* n	%	*p-value*
Glandular positivity					0.0842
Negative	280	77.6	17	63.0	
Positive	81	22.4	10	37.0	
Stromal positivity					1.0000*
Negative	353	97.5	27	100.0	
Positive	9	2.5	0	0.0	

Fisher' s exact test/*chi-square test

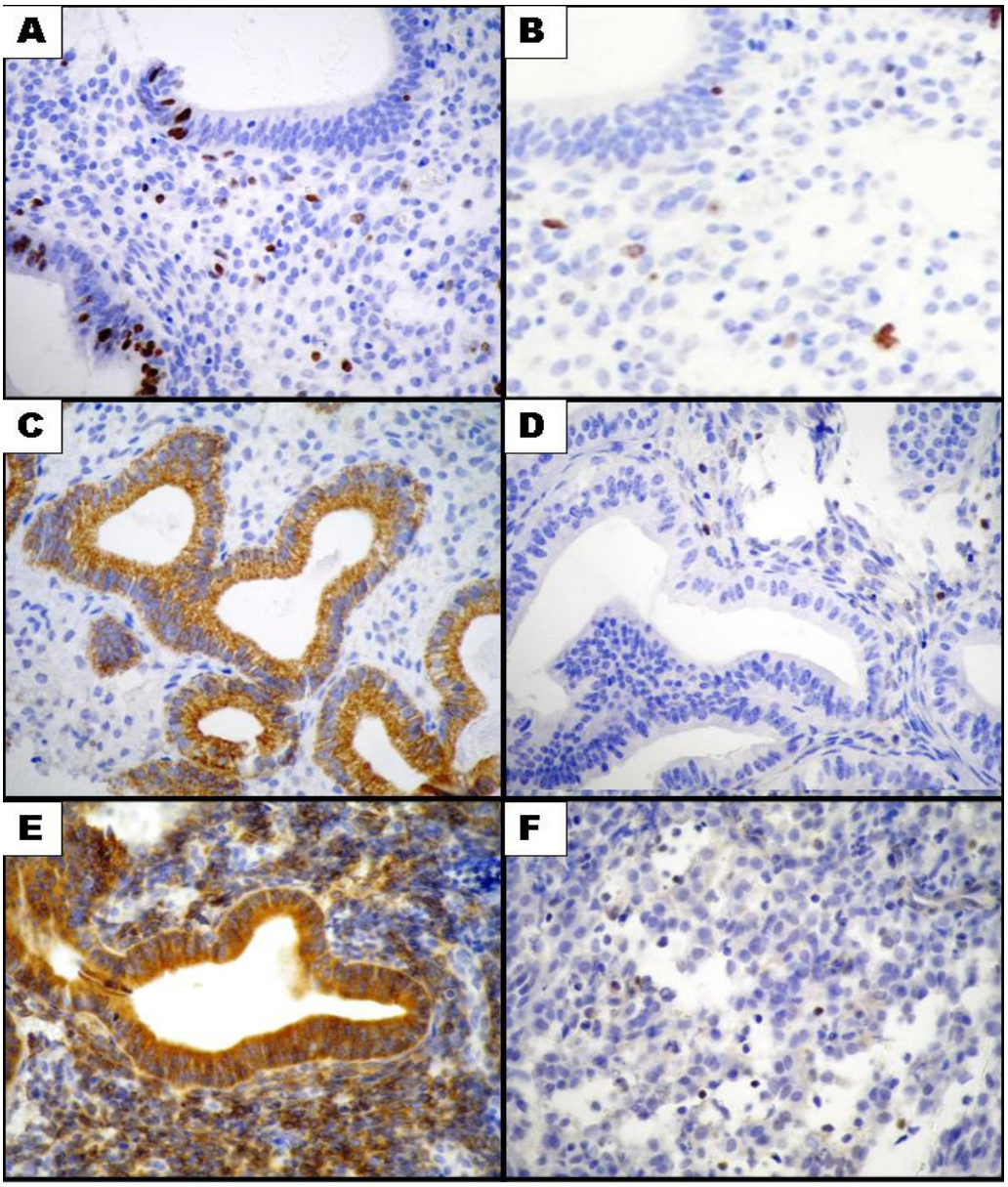

Figure 1. Representative immunohistochemical staining of Ki-67, Bcl-2 and COX-2. (A) weak intensity and (B) negative reaction for Ki-67. (C) positive and (D) negative reactions for Bcl-2. (E) positive and (F) negative reactions for COX-2.

4. Discussão

Os pólipos endometriais têm uma prevalência alta na população feminina e seu diagnóstico tem aumentado nos últimos anos em função da utilização mais frequente da ultrassonografia pélvica transvaginal e da disponibilidade, em grandes centros, da avaliação histeroscópica da cavidade uterina. Diante da alta prevalência de pólipos endometriais tanto na pré como na pós-menopausa, a polipectomia histeroscópica tem sido indicada rotineiramente pelos ginecologistas como tratamento padrão para resolução do quadro de sangramento e para afastar a malignidade possivelmente associada ao pólipo.

Em nosso estudo, observamos que a taxa de malignidade associada aos pólipos endometriais foi baixa. A casuística encontrada na maioria dos estudos é pequena, dificultando a avaliação dos fatores de risco para malignidade nos poucos pólipos. O presente estudo mostra uma das maiores casuísticas encontradas na literatura, e os únicos fatores de risco avaliados que mostraram associação com malignidade foram a idade e a presença de sangramento na pós-menopausa, dados concordantes com a maioria dos estudos (9, 16, 17).

Diante do atual conhecimento dos fatores de risco, acreditamos que muitas das indicações de histeroscopias para polipectomia em mulheres na pós-

menopausa possam ser desnecessárias. Com esta conduta, muitas mulheres estão sendo expostas a um risco cirúrgico, levando-se em consideração que, além da idade avançada, muitas delas apresentam patologias associadas, aumentando ainda mais este risco. Entretanto, ainda não há consenso em relação a quais exatamente devam ser os casos com indicação da ressecção cirúrgica do pólipo, razão pela qual os ginecologistas frequentemente se vêm perdidos diante dos resultados conflitantes disponíveis na literatura.

A amplamente reconhecida necessidade de se produzir um conhecimento mais seguro sobre a patogênese dos pólipos endometriais nas mulheres na pós-menopausa, especialmente as que apresentam maior risco de desenvolver câncer de endométrio, nos levou a estudar a expressão imunohistoquímica de RE e RP, assim como a expressão de marcadores de proliferação/apoptose celular (Ki-67 e Bcl-2) e de COX-2 na glândula e estroma de pólipos benignos e malignos de mulheres na pós-menopausa.

Ao contrário do esperado, observamos uma maior expressão de RE no estroma dos pólipos benignos do que nos malignos, sendo esta diferença significativa. Nosso resultado nos faz pensar que a carcinogênese no pólipo da pós-menopausa seguiria passos diferentes daqueles observados no carcinoma endometrial não associado a pólipo.

A carcinogênese endometrial é complexa e parece envolver a participação de diversos mecanismos, incluindo regulação hormonal, mutação genética, apoptose, desequilíbrio entre metaloproteinases e COX-2 (47, 54, 55). Em relação

à carcinogênese dos pólipos endometriais da pós-menopausa, sabe-se menos ainda.

Em relação à expressão do COX-2, observamos maior expressão no epitélio glandular e no estroma nos pólipos malignos da pós-menopausa do que nos benignos; dado concordante com os estudos anteriores (54, 58, 59). A expressão do Bcl-2 se mostrou aumentada no epitélio glandular dos pólipos, reforçando a ideia da participação da inibição da apoptose celular nos processos proliferativos, entretanto, sua participação na carcinogênese dos pólipos da pós-menopausa ainda necessita de maiores investigações.

A expressão do Ki-67 foi baixa no epitélio glandular e estroma de pólipos benignos e malignos. Estes achados podem sugerir que não existe participação do Ki 67 na carcinogênese dos pólipos, ou que esta participação esteja mais relacionada a tumores endometriais mais agressivos, bem como à invasão miometrial e vascular (47).

Outros estudos deverão ser realizados nesta área da imunohistoquímica, objetivando melhor compreensão da carcinogênese endometrial nos pólipos na pós-menopausa.

5. Conclusões

5.1. A prevalência de malignidade nas lesões polipoides endometriais de mulheres na pós-menopausa foi de 7,1%, e esteve associada ao sangramento na pós-menopausa e à baixa expressão de receptores estrogênicos no estroma;

5.2. Os pólipos endometriais na pós-menopausa apresentam uma alta expressão dos RE no estroma e na glândula. Esta expressão foi menor nos pólipos malignos do que nos benignos. A expressão dos RP não mostrou associação com risco de malignidade;

5.3. Os pólipos na pós-menopausa apresentam expressão de COX-2 maior nos pólipos malignos do que nos benignos. As expressões de Bcl-2 e Ki67 não mostraram diferença entre os pólipos benignos e pré-malignos/malignos.

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7. Anexos

7.1. Anexo 1: Parecer do Comitê de Ética

FACULDADE DE CIÊNCIAS MÉDICAS
COMITÊ DE ÉTICA EM PESQUISA

www.fcm.unicamp.br/pesquisa/etica/index.html

CEP, 22/09/09.
(Grupo III)

PARECER CEP: Nº 847/2009 (Este nº deve ser citado nas correspondências referente a este projeto)
CAAE: 0664.0.146.000-09

I - IDENTIFICAÇÃO:

PROJETO: **"EXPRESSÃO DE RECEPTORES HORMONAIS, CICLO-OXIGENASE-2, AROMATASE, MARCADORES DE PROLIFERAÇÃO E APOPTOSE CELULAR EM PÓLIPOS ENDOMETRIAIS DE MULHERES NA PRÉ E PÓS-MENOPAUSA".**
PESQUISADOR RESPONSÁVEL: Armando Antunes Junior
INSTITUIÇÃO: CAISM/UNICAMP
APRESENTAÇÃO AO CEP: 11/09/2009
APRESENTAR RELATÓRIO EM: 22/09/10 (O formulário encontra-se no *site* acima)

II - OBJETIVOS

Avaliar a prevalência de malignidade e a expressão de receptores hormonais, em pólipos endometriais malignos, benignos e na atrofia endometrial em mulheres na pré e pós-menopausa.

III - SUMÁRIO

Será realizado um estudo de corte transversal com mulheres selecionadas através da revisão da escala cirúrgica do Centro cirúrgico do CAISM/UNICAMP, sendo selecionadas todas aquelas que foram submetidas a histeroscopia cirúrgica para ressecção de pólipos no período de janeiro de 1998 a dezembro de 2007. Estima-se que haja neste período cerca 800 pólipos retirados por histeroscopia. Serão avaliadas as características clínicas e avaliados a expressão dos receptores de estrógeno e progesterona, expressão da aromatase, COX-2, Bcl2 , Ki67 utilizando-se blocos de parafinas arquivados no Departamento de Anatomia Patológica da Faculdade de Ciências Médicas (FCM) da UNICAMP em todos os pólipos selecionados. Serão comparados a expressão desses receptores e marcadores entre pólipos benignos, malignos e mesmo número de amostras de endométrio atrófico de mulheres na pré e pós-menopausa. As reações de imunohistoquimica serão realizadas no Laboratório de Imunohistoquímica do Setor de Anatomia Patológica do Hospital do Câncer através da técnica de arranjo em matriz de amostras teciduais, ou tissue microarray (TMA).

IV - COMENTÁRIOS DOS RELATORES

O projeto apresenta-se redigido com metodologia adequada. Os critérios de inclusão e exclusão estão bem definidos; cálculo do tamanho amostral e análise estatistica muito bem embasados. Os aspectos éticos estão bem discutidos no corpo do projeto e solicita dispensa da aplicação do Termo de Consentimento Livre e Esclarecido. O orçamento é detalhado.

Comitê de Ética em Pesquisa - UNICAMP
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V - PARECER DO CEP

O Comitê de Ética em Pesquisa da Faculdade de Ciências Médicas da UNICAMP, após acatar os pareceres dos membros-relatores previamente designados para o presente caso e atendendo todos os dispositivos das Resoluções 196/96 e complementares, resolve aprovar sem restrições o Protocolo de Pesquisa, a dispensa do Termo do Consentimento Livre e Esclarecido, bem como todos os anexos incluídos na pesquisa supracitada.

O conteúdo e as conclusões aqui apresentados são de responsabilidade exclusiva do CEP/FCM/UNICAMP e não representam a opinião da Universidade Estadual de Campinas nem a comprometem.

VI - INFORMAÇÕES COMPLEMENTARES

O sujeito da pesquisa tem a liberdade de recusar-se a participar ou de retirar seu consentimento em qualquer fase da pesquisa, sem penalização alguma e sem prejuízo ao seu cuidado (Res. CNS 196/96 – Item IV.1.f) e deve receber uma cópia do Termo de Consentimento Livre e Esclarecido, na íntegra, por ele assinado (Item IV.2.d).

Pesquisador deve desenvolver a pesquisa conforme delineada no protocolo aprovado e descontinuar o estudo somente após análise das razões da descontinuidade pelo CEP que o aprovou (Res. CNS Item III.1.z), exceto quando perceber risco ou dano não previsto ao sujeito participante ou quando constatar a superioridade do regime oferecido a um dos grupos de pesquisa (Item V.3.).

O CEP deve ser informado de todos os efeitos adversos ou fatos relevantes que alterem o curso normal do estudo (Res. CNS Item V.4.). É papel do pesquisador assegurar medidas imediatas adequadas frente a evento adverso grave ocorrido (mesmo que tenha sido em outro centro) e enviar notificação ao CEP e à Agência Nacional de Vigilância Sanitária – ANVISA – junto com seu posicionamento.

Eventuais modificações ou emendas ao protocolo devem ser apresentadas ao CEP de forma clara e sucinta, identificando a parte do protocolo a ser modificada e suas justificativas. Em caso de projeto do Grupo I ou II apresentados anteriormente à ANVISA, o pesquisador ou patrocinador deve enviá-las também à mesma junto com o parecer aprovatório do CEP, para serem juntadas ao protocolo inicial (Res. 251/97, Item III.2.e)

Relatórios parciais e final devem ser apresentados ao CEP, de acordo com os prazos estabelecidos na Resolução CNS-MS 196/96.

VII – DATA DA REUNIÃO

Homologado na IX Reunião Ordinária do CEP/FCM, em 22 de setembro de 2009.

Prof. Dr. Carlos Eduardo Steiner
PRESIDENTE do COMITÊ DE ÉTICA EM PESQUISA
FCM / UNICAMP

Comitê de Ética em Pesquisa - UNICAMP
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7.2. Anexo 2: Ficha de coleta de dados

INSTRUMENTO DE COLETA DE DADOS

HC: ___/___/___/___/___/___/___/___/___/___/ IDADE ___/___/ Nº Estudo: __/___/___/___/___

Nome: ___/

Menopausa ☐ Peri Menopausa ☐

Uso de TH ☐ SIM ☐ NÃO **Se SIM:** Há quanto tempo? ☐☐☐ Meses

Tipo de TH: ☐ E ☐ E + P ☐ Outros

Se NÃO: ☐ Nunca Usou ☐ Já Usou

Usou antes por ☐☐☐ Meses e parou há ☐☐☐ Meses

Tipo de TH: ☐ E ☐ E + P ☐ Outros

Sangramento Pós-Menopausa: ☐ SIM ☐ NÃO Medicações:_____

Patologias e Medicações: **HAS:** ☐ SIM ☐ NÃO ☐

DM: ☐ SIM ☐ NÃO ☐

CA Mama: ☐ SIM ☐ NÃO

Uso de TAMOXIFENO: ☐ SIM ☐ NÃO

PESO: ☐☐☐ Kg ALTURA ☐☐☐ cm

Ant. Obst: G ☐ P ☐ C ☐ A ☐

Última USG pélvica antes da histeroscopia diagnóstica: ☐☐ / ☐☐ / ☐☐

Linha endometrial: ☐☐

Pólipo endometrial: ☐ SIM ☐ NÃO Tamanho: ☐☐ mm

Outros achados: ☐ espessamento endometrial ☐ sangramento pós-menopausa

☐ Pólipo endometrial ☐ hipermenorragia/metrorragia

Conteúdo intraútero a esclarecer ☐ outros ☐

Endométrio: ☐ Proliferativo

☐ Secretor: Superfície ☐ Lisa ☐ Polipóide

☐ Atrófico ☐ Cística ☐ Irregular

☐ Hipotrófico: Vascularização ☐ Normal ☐ Ausente

☐ Hipertrófico: ☐ Atípica

Orifícios tubáreos: **D:** Normal ☐ Visível ☐ Vascularizado ☐

E: Normal ☐ Visível ☐ Vascularizado ☐

Pólipos: ☐ Endocervical ☐ Endometrial ☐ Ístimico ☐ Cornual ☐ OTD ☐ OTE

Sinéquias: TIPO: ☐ Mucosa ☐ Fibrosa ☐ Mista

LOCALIZAÇÃO: ☐ Corporal ☐ Marginal ☐ Central Múltipla ☐ Fúndica

☐ Cornual D ☐ Cornual E ☐ OTD ☐ OTE

☐ Cérvico-ístimica

DIU: ☐ In situ ☐ Deslocada ☐ Deformado

BE: ☐ SIM ☐ NÃO **Se Sim:** ☐ Abundante ☐ Escasso

AP: _____

Outros Achados: _____

Histeroscopia cirúrgica: ☐☐ / ☐☐ / ☐☐

Histerometria: ☐☐, ☐☐ cm

Tamanho do pólipo: ☐☐☐ mm Tipo: ☐ Pediculado ☐ Séssil

Localização: ☐ Fúndico ☐ Parede Lat. D. ☐ Parede Lat. E. ☐ Parede Ant. ☐ Parede Post.

☐ Ístimico ☐ Cornual D ☐ Cornual E

Superfície: ☐ Lisa ☐ Irregular ☐ Cística

Vascularização: Aumentada ☐ Típica ☐ Atípica ☐ Ausente

Conduta: POLIPECTOMIA ☐ SIM ☐ NÃO

BIÓPSIA DE ENDOMÉTRIO ☐ SIM ☐ NÃO

CURETAGEM UTERINA ☐ SIM ☐ NÃO

AP: ☐ Pólipo atrófico-cístico

☐ Pólipo da mucosa endometrial

☐ Pólipo atrófico da mucosa endometrial

☐ Pólipo com hiperplasia simples

☐ Pólipo com hiperplasia complexa sem atipia

☐ Pólipo com hiperplasia simples e focos de atipia

☐ Pólipo com hiperplasia complexa e focos de atipia

☐ Endométrio proliferativo

☐ Endométrio secretor

☐ Endométrio atrófico

☐ Carcinoma endometrial

☐ Outros achados: _____

GRAU HISTOLÓGICO: ☐ Bem diferenciado ☐ Mod. diferenciado ☐ Pouco diferenciado

Expressão dos receptores de estrógenos:

	Epitélio Glandular	Estroma
% de células coradas (score):		
Intensidade de coloração (score):		

Expressão dos receptores de progesterona:

	Epitélio Glandular	Estroma
% de células coradas (score):		
Intensidade de coloração (score):		

Expressão de Bcl-2:

	Epitélio Glandular	Estroma
% de células coradas (score):		
Intensidade de coloração (score):		

Expressão de COX-2:

	Epitélio Glandular	Estroma
% de células coradas (score):		
Intensidade de coloração (score):		

Expressão de Ki-67:

	Epitélio Glandular	Estroma
% de células coradas (score):		

7.3. Anexo 3: Artigo 1. submetido a revista Reproductive Sciences

Reproductive Sciences

Thank you for submitting your manuscript to *Reproductive Sciences*.

Manuscript ID:	RSCI-11-363
Title:	Immunohistochemical expression of estrogen and progesterone receptors in endometrial polyps: comparison between benign and malignant polyps in the postmenopause
Authors:	Antunes-Junior, Armando Vassallo, Jose De Brot, Louise Godoy, Carlos Pinto-Neto, Aarão Costa-Paiva, Lucia
Date Submitted:	24-Dec-2011

Print Return to Dashboard

7.4. Anexo 4: Artigo 2 submetido a revista Gynecologic Oncology

Processed for Author Lucia Helena Simões Costa-Paiva, PhD, MD

Page: 1 of 1 (1 total submissions) Display 10 results per page.

Action	Manuscript Number	Title	Initial Date Submitted	Status Date	Current Status
Action Links View Submission Send E-mail		Is the immunohistochemical expression of proliferation (Ki-67) and apoptosis (Bcl-2) markers, and cyclooxigenase-2 (COX-2) related to carcinogenesis in postmenopausal endometrial polyps?	Dec 27, 2011	Dec 27, 2011	Submitted to Journal