UNIVERSITY OF MEDICINE AND PHARMACY CRAIOVA

DOCTORAL SCHOOL

PhD THESIS

ABSTRACT

THE MALIGN POTENTIAL OF ENDOMETRIAL PATHOLOGY IN PRE AND POST MENOPAUSE

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CONTENTS

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INTRODUCTION

The classification of endometrial hyperplasia according to glandular changes (simple hyperplasia versus complex hyperplasia) and nuclear atypical (HE with atypia versus complex atypical HE) should provide a perspective on the subsequent risk of endometrial carcinoma (EC). Even if we have an evolution of non-invasive techniques to detect the coexistence of endometrial cancer or the risk of progression of EC, studies conducted till now have failed to produce conclusive results. The risk of endometrial hyperplasia progressing to endometrial cancer is 5-10%, but these values vary according to the endometrial histology.

STATE OF KNOWLEDGE

CHAPTER I
EPIDEMIOLOGICAL DATA OF ENDOMETRIAL HYPERPLASIA

Endometrial hyperplasia (EH), which can be the precursor injury to endometrial cancers, is usually diagnosed when we evaluate women who have abnormal uterine bleeding through endometrial biopsy.

The most commonly used classification system for EH is WHO's since 1994, where glandular architectural modifications and cytology are used to identify four types of HE, namely simple or complex hyperplasia, with or without atypia (26). In particular, cytology is of great importance, not only for the evolution to endometrial cancer, but also for the risk of having an concurrent EC in women with EH (27). Most EH diagnoses are simple or complex EH without atypia and in this context there is no consensus on their management, but these lesions are considered to present a moderate risk of progression to endometrial carcinoma. For women with simple or complex EH with atypia, on the contrary, hysterectomy is generally recommended due to a high probability of associated carcinoma at the time of diagnosis of atypia (1). Women diagnosed with EH with atypia are 10 times more likely to develop EC compared to women diagnosed with EH without atypia (17). The overall incidence of endometrial hyperplasia in women aged 18 to 90 years was estimated to be 133/100,000 in general. Endometrial hyperplasia rates may be underestimated because most studies on the incidence of endometrial hyperplasia only referred to symptomatic women, with few studies on the incidence of endometrial

hyperplasia in asymptomatic women, because endometrial sampling is not performed on these categories of patients (20).

CHAPTER II

CONSEQUENCES OF ALTERATION OF STEROIDES ACTION AT ENDOMETRIAL LEVEL

Endometrial hyperplasia is the result of prolonged estrogen stimulation of the endometrium, the most common cause being the anovulatory cycles. Hyperplasia may also result from the increase in endogenous production or the exogenous administration of estrogens (23). Endometrial hyperplasia is thought to occur when estrogen, without the counteraction progesterone effects, stimulates the development of endometrial cells by binding estrogen receptors to endometrial cell nuclei. Exposure of these estrogen proliferative cells progesterone-dependent indicates the narrowing of the endometrial tissue both by reducing the number of estrogen receptors and by increasing estradiol conversion rate to estrone, a less effective estrogen, by increasing the dehydrogenase activity of estradiol (11).

Selective progesterone receptor antagonism in premenopausal women who actively produce estrogens is a concern because prolonged estrogen stimulation of the endometrium has been associated with an increased risk of endometrial cancer of 2 to 10 times greater (22).

CHAPTER III

ENDOMETRIAL DIAGNOSTIC EVALUATION FOR PREMALIGNANT AND MALIGNANT PATHOLOGY

Endometrial evaluation in women at risk for malignant lesions is done by endometrial biopsy, which is a minimally invasive maneuver. Hysteroscopic evaluation of abnormal uterine bleeding has the advantage of visualizing endometrial pathology and facilitates endometrial biopsy (9).

Transvaginal ultrasound is a non-invasive and easy to perform method of indirect endometrial visualization. Therefore, it is recommended to use it as a first method for assessing uterine pathology in premenopausal women with abnormal uterine bleeding.

Mathew et al., found that the sensitivity of transvaginal ultrasound in detecting these abnormalities was 54%, and the specificity was 100%. Positive predictive values were 100% and negative predictive values were 81.1% (18). However, transvaginal ultrasound examination can not explain the whole endometrial pathology, so ultrasound findings do not always guide the

decision on invasive procedures. The histological classification of the samples sent for examination is very important because, in these situations, only the histological examination can establish the diagnosis. Kendall et al. found histological changes such as “glandular agglomeration” associated with the diagnosis of complex hyperplasia and the nucleoli were associated with pathologies called atypical cases (16). Bergeron and colleagues found the presence of “glandular agglomeration” significantly associated with the diagnosis of hyperplasia, while “nuclear pleomorphism” was more associated with the atipical classification (5).

The distinction between complex atypia and endometrial adenocarcinoma is sometimes problematic. Groups of back-to-back glands or cribriform arrays less than 2.1 mm in diameter are considered insufficient for the diagnosis of endometrial adenocarcinoma by some researchers and sufficient to be diagnosed as endometrial adenocarcinoma by other researchers (17).

CHAPTER IV

BIOMARKERS IN ENDOMETRIAL CANCER - POSSIBLE CLINICAL APPLICATIONS

Endometrioid endometrial carcinoma accounts for three-quarters of the total endometrial cancers,that may occur following the progression of premalignant lesions, but the lesion may range from endometrial hyperplasia without atypia to atypical hyperplasia and next to well-differentiated carcinoma (2). Studies have shown that atypical cytology is the most important criterion in the diagnosis of premalignant lesions (endometrial hyperplasia with atypia) and has a low reproducibility (22).

Endometrial cancer biomarkers, some of which have been identified yet: K-ras, HER2 / neu, epithelial growth factor receptor, catalytic 3-kinase phosphatidylinositol subunit (PI3KCA), fibroblast growth factor receptor 2 oncogenes (FGFR2), phosphatase and tensine homologue on chromosome 10 (PTEN), p53, p21, Ki-67, a cell proliferation index, BCL2-associated protein X (Bax), a promoter of the apoptosis gene , Bcl-2 an apoptosis suppressor, estrogen and progesterone receptor expression, and vascular endothelial growth factor A, known as VEGF-A (4). The interaction between genetic and hormonal events during the progression of premalignant lesions is linked to a number of hypotheses, but this interaction has never been fully understood until now. (18).

PERSONAL CONTRIBUTIONS

CHAPTER V
RESULTS OF THE CLINICAL STUDY AND STATISTICAL ANALYSIS

We conducted a prospective study involving a group of 106 patients, with premenopausal and postmenopausal patients who had at least one transvaginal ultrasound (TVU) for endometrial evaluation, which allowed us to classify the cases.

During this period, the patients were entered into the study by completing the initial assessment record and signing an informed consent, following an investigation protocol setting the set of quantifiable, specific parameters for case tracking. Clinical data has collected routine data on essential clinical information and the presence of risk factors for endometrial cancer.

Our study confirmed that women with a menopause installed for less than 10 years were predominantly HE lesions with / without atypia. In menopausal women for more than 10 years, histological lesions were relatively well distributed, but with the presence of 2 cases (9.52%) of EC. In the premenopausal women, EH was predominantly non-atypical but with 7 cases (10.77%) of EC.

The statistical correlation between age and endometrial carcinoma showed that there is no statistical significance, $p > 0.005$, so EC can occur both in premenopausal and menopausal women.

Recurrent bleeding episodes may be significant for malignant and premalignant endometrial lesions.

Statistical correlation between BMI and type of EH with / without atypia showed statistical significance, $p < 0.005$. Interpretation of results shows that with the increase in BMI, the percentage of atypical HE increases.

The statistical correlation between parity and EC showed statistical significance, $p < 0.005$. So we can say that in our study, parity and especially nulliparity can be a risk factor for the EC.

The DEFAB score is a parameter with a relatively high accuracy in the case of EH simple with atypia, EH complex with / without atypia for EC risk. The RHEA score is a high-precision parameter in the case of complex atypical EH with risk for EC.

CHAPTER VI

ULTRASONOGRAPHIC EVALUATION OF THE ENDOMETER

The endometrial thickness measurement was performed prospectively, through this imaging investigation, the transvaginal ultrasound, selecting the cases that were studied. Some of these cases were selected following the endometrial evaluation incidentally performed on the transvaginal ultrasound performed for another indication.

In the presence of postmenopausal bleeding, endometrial thickness greater than 5 mm was considered abnormal and further investigated. In cases where we did not have any risk at the start of the study, in asymptomatic women with an endometrial thickness greater than 10 mm, it was agreed to recommend further investigations. An 8 mm cut-off was used for premenopausal women, except women with uterine bleeding for other causes.

Asymptomatic postmenopausal women have a low risk of premalignant or malignant histological lesions. Endometrial thickness varied between 8.2 and 13.5 mm. Of these 13 cases, only one case showed endometrial carcinoma at an early stage. In postmenopausal women with uterine bleeding, the mean endometrial thickness was 8.5 ± 3.64 mm, thus higher than the established cut-off. The mean endometrial thickness in premenopausal women was 12.3 ± 3.84 mm, well above the established cut-off, correlated with a histopathological diagnosis of atypical hyperplasia, and endometrial carcinoma was present in 2 cases.

We noticed an increase in endometrial thickness in cases that occurred with an increased BMI (overweight, obese) but not with those with diabetes.

CHAPTER VII

HISTOLOGICAL AND IMMUNOHISTOCHEMICAL ANALYSIS. IMMUNOHISTOCHEMICAL MARKERS

We used a panel of IHC biomarkers, namely:

1. Progesterone receptors
2. Estrogen receptors
3. P14 ARF
4. p53
5. PTEN
6. Ki 67

The immunohistochemical expression of the progesterone receptor at the nuclear level was detected in all the analyzed cases. In the cases studied, we have experienced a high immunohistochemical expression, which has allowed us some initial progesterone treatment in some cases.

The immunohistochemical expression of estrogen receptors was increased at the nuclear level in cases with simple EH and decreased in cases with complex EH and endometrioid endometrial carcinoma. Our results indicate that the loss of ER and PR in endometrial cells may be an important marker of evolution from hyperplasia without atypia to atipical hyperplasia and from hyperplasia with atypia to EC.

The immunohistochemical expression of the p14 ARF antibody, although rather poor, indicates that in the presence of atypia, this marker is expressed in most cases.

The immunohistochemical expression for the p53 nuclear protein was negative in EH without atypia and positive in 27.77% of cases with EH with atypia.

Immunohistochemical expression for PTEN was a negative grade, grade 0 (-) in all cases of EH with / without atypia. This observation may be due to the difference in the sensitivity of the immunohistochemical methods for assessing the staining results, as well as the antibodies used.

Immunohistochemical expression for the Ki 67 proliferation index was positive in most cases, increasing successively in endometrial tissue with EH without atypia, in endometrial tissue with atypical hyperplasia and in endometrial carcinoma tissue, this phenomenon being related to increased proliferation of cells.

General conclusions

Among the risk factors, the time elapsed since the menopause was relevant, with several EC cases in the menopause installed over> 10 years.

Episodes of recurrent bleeding are associated with severe endometrial lesions, EH with atypia and CE.

The presence of an elevated BMI statistically correlated, $p < 0.005$, with the type of EH and especially with EH with atypia and CE.

In our study, parity and especially nuliparity may be a risk factor for the EC, the statistical correlation between parity and EC, showing statistical significance, $p < 0.005$.

The DEFAB score is a parameter with relatively high accuracy in the case of simple EH with atypia, complex EH with / without atypia for EC risk. RHEA score is a high-precision parameter in complex EH with atypia for EC risk.

In postmenopausal asymptomatic women with a large endometrial thickness, between 8.2 and 13.5 mm, the risk of having a significant endometrial pathology was low.

In postmenopausal women with uterine bleeding, with an average endometrial thickness of 8.5 ± 3.64 mm, thus higher than the cut-off and in premenopausal women with a mean endometrial thickness of 12.3 ± 3.84 mm, these values correlated with a histopathological diagnosis of EH with atypia, and endometrial carcinoma was present in 2 cases.

Our results indicate that ER and PR loss in endometrial cells may be an important marker of evolution from hyperplasia without atypia to atypical hyperplasia and then to EC.

The immunohistochemical expression of the p14 ARF antibody was inversely proportional to the degree of endometrial histological lesion.

The immunohistochemical expression for the p53 nuclear protein was negative in EH without atypia and low positive grade 1 (+), in over 25% of cases with EH with atypia.

Immunohistochemical expression for PTEN was a negative grade, grade 0 (-) in all cases of EH with / without atypia.

We consider that the Ki 67 proliferation index may be an important index for assessing the proliferation and differentiation of endometrial carcinoma having a critical biological and clinical significance for its onset, progression and diagnosis.

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