ORIGINAL PAPER

Quantification of apoptotic phenomenon on endometrial biopsies in postmenopausal patients under hormonal replacement therapy (HRT)

DANA TERZEA, CRISTINA IOSIF, FLORINA VASILESCU, F. ANDREI, CAMELIA DOBREA, ALINA NICOLAE, ALINA GEORGESCU, F. MIRCEA, M. CEAUŞU, ARIANA VIŞAN, MIHAELA MIHAI, CARMEN ARDELEANU

Department of Histopathology and Immunohistochemistry, "Victor Babeş" National Institute for Research and Development in Pathology and Biomedical Sciences, Bucharest

Abstract

Aim. To quantify the apoptotic phenomenon on endometrial biopsies in postmenopausal patients under hormonal replacement therapy (HRT). *Material and methods*. The study lot consisted of 30 endometrial biopsies on which we studied the apoptotic phenomenon through morphological and molecular biology techniques (TUNEL reaction). Examination of endometrial biopsies before and post-therapeutically has been made. *Results and discussions*. From morphological point of view, pre-therapeutically, endometrial biopsies presented apoptotic changes in about 1–3% of cells and under TSH there have been observed apoptotic changes in about 1–2% of cells. In female reproductive system, we found out a raised rate of cellular proliferation and concurrently a raised rate of apoptosis. Apoptotic phenomenon can be observed in endometrium at every menstrual cycle. In proliferative endometrium apoptosis rate is low, but in endometrial carcinoma apoptosis rate grow up. Bcl2 and Bax are expressing in normal and hyperplastic endometrium, but in endometrial carcinoma Bcl2/Bax ratio decline. *Conclusions*. Quantification of apoptosis, using morphological and TUNEL reaction methods, on endometrial biopsies in postmenopausal patients before and after therapy indicate a low rate of apoptotic phenomenon.

***Keywords*:** apoptosis, endometrium, postmenopausal, TSH, TUNEL.

Introduction

From the clinical point of view, *menopause* is the last stage of a biological gradual process, which is initiated by the apparition of hormonal changes and represents permanent stop of menstruations. This process usually appears between 45–55 years and it is considered complete after six month of amenorrhea [1]. Because of increasing hope life to 80 years, a woman lives one third of her life after menopause.

Ovarian decline due to decrease of number of receptors for gonadotrophines and reduced number of ovarian follicles reduces production of steroid hormones. Steroid hormones insufficiency causes apparition of secondary changes: vasomotor, osteoporosis, genitor-urinary changes with reduced elasticity and thickness of vaginal mucosae, changes of urinary tract tissues (losing of elasticity and irretention), increase of cardiovasculary affections and cerebral strokes incidence, neuroendocrine changes, skin and pillar changes [2].

Due to this secondary change's major social and medical impact, hormonal replacement therapy (HRT) is recommended for raising life quality in postmenopausal patients [3, 4].

Hormonal replacement therapy (HRT) has short and long term, curative and prophylactic effects [1]. Recommendation of therapy may be widen on 15–20 years period. The therapy can be made with unique or combined scheme, sequential or continuous. This therapy influences endometrial and vaginal mucosae, having effects on epithelial cells, stromal cells and vascularisation.

Hormonal replacement therapy (HRT) with unique scheme and without preparats conteining progestatives has been associated with raising risk for endometrial carcinoma apparition. Combined therapy with administration has been introduced for removing this risk. This therapy includes progestatives, which induce secretory endometrial transformation, followed be apparition of deprivation hemorrhage.

Material and methods

The study of apoptotic phenomenon has been effectuated from morphological point of view and with molecular biology methods (TUNEL). On selected cases from our working lot (30 endometrial biopsy), we examined from morphological point of view the presence of apoptotic bodies.

The following criteria have been used for identification of apoptotic phenomenon in cases from our working lot: condensed chromatin and cytoplasm; presence of cytoplasmic fragments, which contain condensed chromatin.

The examination has been made on selected cases before and after substitutive hormonal therapy.

On each histological sample have been studied 1000 nuclei of glandular cells on 40× magnification.

We also studied agglomerating chromatin, pinosis and the beginning of disappearance of small cytoplasm organites in cells, because these changes suggest beginning of apoptotic phenomenon.

In situ hybridization method

In situ hybridization is a technique that allows the visualization of specific nucleic acid sequences within a cellular preparation. Specifically, DNA fluorescence in situ hybridization (FISH) involves the precise annealing of a single stranded fluorescently labeled DNA probe to complementary target sequences. The hybridization of the probe with the cellular DNA site is visible by direct detection using fluorescence microscopy.

Formalin–fixed paraffin embedded tissue specimens are placed on slides. The DNA is denatured to single-stranded form, and subsequently allowed to hybridize with the Path Vysion probes. Following hybridization the unbound probe is removed by a series of washes and the nucleic are counterstained with DAPI (4,6 diamino-2-phenylinodole), a DNA-specific stain that fluoresces blue.

Results

In this study there have been followed from morphological point of view nuclear and cytoplasmatic changes suggestive for apoptosis in epithelial glandular cells, made on selected cases (30 endometrial biopsies) before and after therapy.

Before therapy

Biopsies with histological aspects of endometrium with low proliferation presented apoptotic changes in 2–3% of cells (Figure 1).

- biopsies with histological aspects of atrophic endometrium presented apoptotic changes in <1% of cells;
- biopsies with histological aspects of simple glandulary hyperplasia without atypia presented apoptotic changes in 3% of cells (Figure 2).

After therapy

Biopsies with histological aspects of mixed aspects of proliferative and low secretory presented apoptotic changes in 1–2% of cells (Figure 3).

- biopsies with histological aspects of atrophic endometrium presented apoptotic changes in <1% of cells;
- biopsies with histological aspects of mixed aspects of proliferative and hyperplastic presented apoptotic changes in 2% of cells (Figure 4).

Molecular biology tests (in situ hybridization through TUNEL reaction – terminal deoxynucleotidyl-transferase mediated dUTP nick end labeling- in situ enzymatic marking of DNA fragmentation induced by apoptosis) on selected cases (30 endometrial biopsies) revealed:

1. Nuclear staining in **1–3%** of endometrial biopsies taken **before therapy** (Figures 5 and 6).
2. Nuclear staining in **1–2%** of endometrial biopsies taken **after therapy** (Figures 7 and 8).

Discussions

In female reproductive system, there is a raised rate of cellular proliferation and at the same time a raised rate of apoptosis. At endometrial level, apoptotic phenomenon can be observed in every menstrual cycle. Apoptotic phenomenon is the one, which takes part in endometrial cells homeostasis regulation. In endometrium, apoptosis have a role to in endometrial mucosae regeneration and its preparation for a new menstrual cycle. Bcl2/Bax decrease before the apoptosis increasing [5, 6].

Errors appeared in this mechanism suggest the presence of endometrial affections like hyperplasia and endometrial carcinomas. During menstrual cycle, the highest rate of apoptosis has been observed during menstruation. Studies on endometrial level suggest that apoptosis rate in normal proliferative endometrium is similar with that of endometrium with hyperplasia (low rate), a significantly increase being observed in endometrial carcinomas grade II [7].

Granular mediated apoptosis in endometrial level involve cytotoxic granules action: perforine, granzime B, caspase3. These granules are released from natural-killer cells (NK) during late secretory phase of menstrual cycle. Apoptosis is a genetically planned mechanism through which unnecessary cells of organism are eliminated [8].

In last years, modern molecular biology techniques tried to identify molecular anomalies at any level, from DNA lesions to posttranslational changes, from punctiform mutations of some genes to lesions not caused by DNA lesions (not genotoxic).

Cellular proliferation depends on a series of regulatory genes. They have a positive/stimulatory role on (oncogenic) proliferation or negative/inhibitory (tumoral suppressing genes). The following are part of first category: growth factors, growth factors receptors, transduction mithogen signal proteins and regulatory RNA transcription proteins with role in synthesis process in S phase of cellular cycle. Progression control of cellular cycle is made through other genes: tumoral suppressor genes. These genes determine cessation in control points G1/S, G2/M, check DNA replication fidelity, assure DNA repair and start apoptosis in irreparable lesions.

P53 oncoprotein is considerate the main gene of apoptosis, "the genome guardian" which prevents oncogenic mutations. P53 oncoprotein acts through some genes: Gadd45 (growth arrest/ DNA damage), P21WAF1/CIP1 (cyclin dependent protein kinases inhibitory), Bcl2/Bax (survival gene/ apoptosis gene), mdm2 (over expressed under p53 action, but which inactivate p53 through autoreglatory loop) and so on. In lack of apoptosis, DNA residual lesions may cause DNA synthesis inhibition, loss/altered expression of genetic information and malignisation [9].

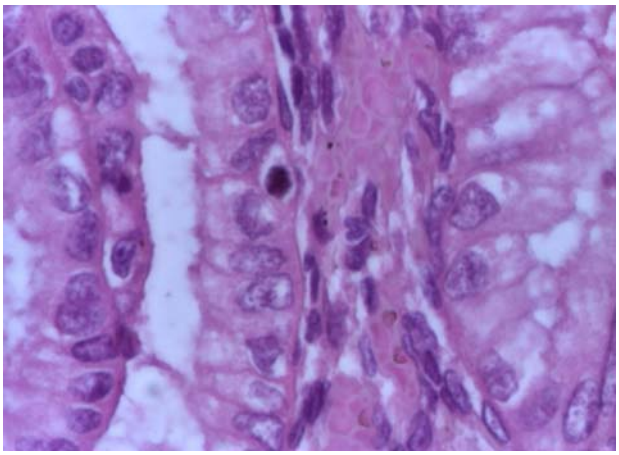

Figure 1 – ***Apoptotic changes (HE staining, 60×)***

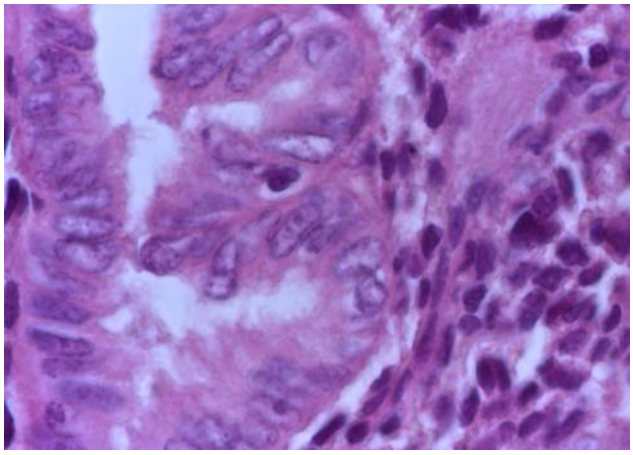

Figure 2 – ***Apoptotic changes (HE staining, 60×)***

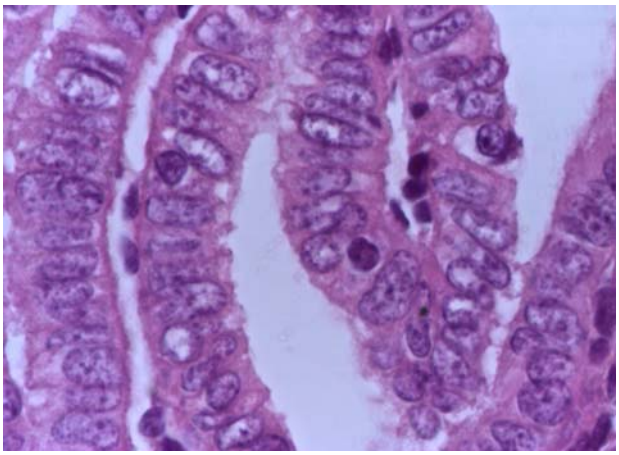

Figure 3 – ***Apoptotic changes (HE staining, 60×)***

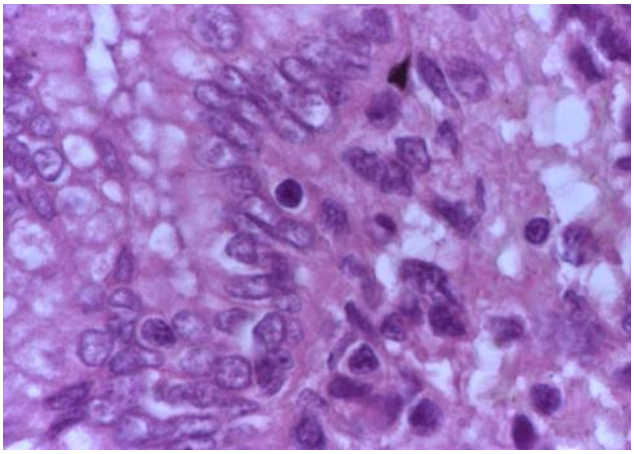

Figure 4 – ***Apoptotic changes (HE staining, 60×)***

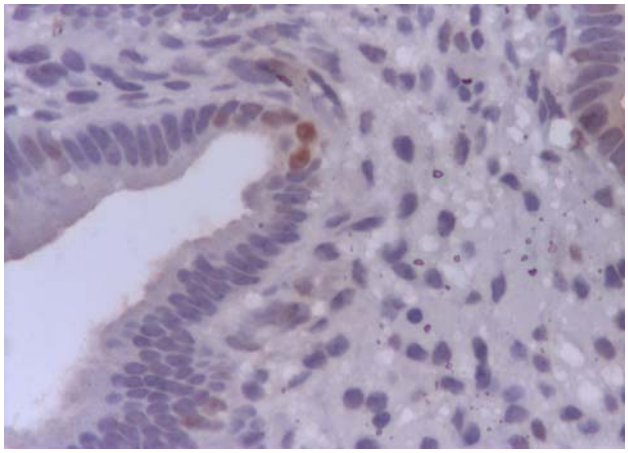

Figure 5 – ***Endometrium – nuclear staining, positive aprox. 1–3%, in situ hybridization (TUNEL, 40×)***

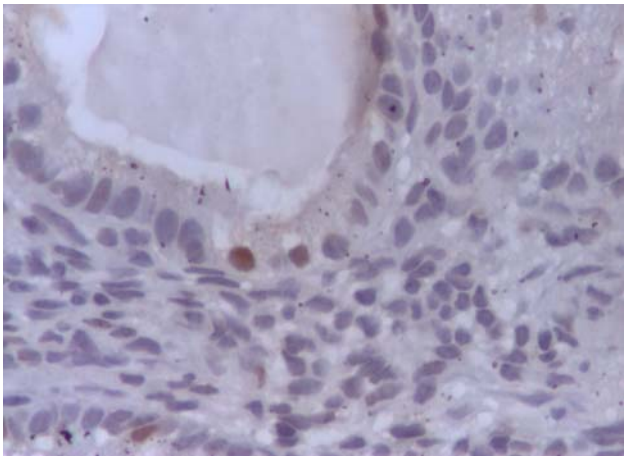

Figure 6 – ***Endometrium – nuclear staining, positive aprox. 1–3%, in situ hybridization (TUNEL, 40×)***

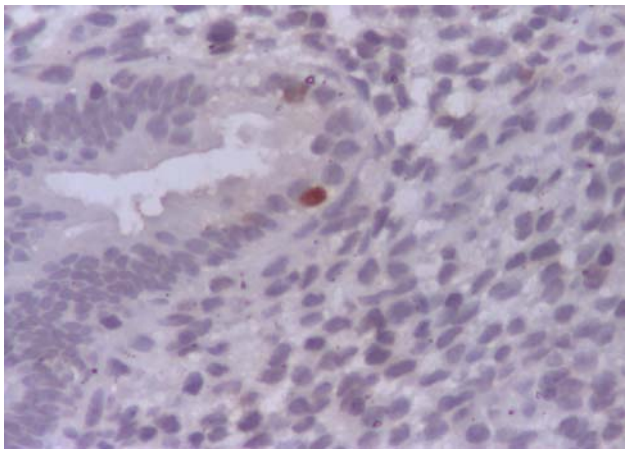

Figure 7 – ***Endometrium – nuclear staining, positive aprox. 1–2%, in situ hybridization (TUNEL, 40×)***

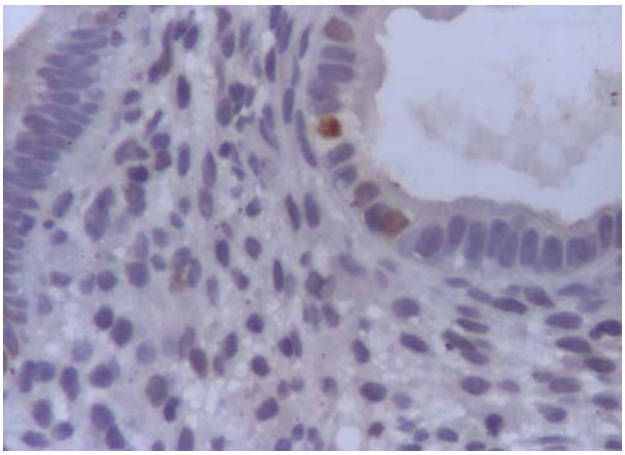

Figure 8 – ***Endometrium – nuclear staining, positive aprox. 1–2%, in situ hybridization (TUNEL, 40×)***

In endometrial glandular cells caspase-3 and TUNEL-positive cells significant raise from late secretory phase; cytotoxic granules (perforin and granzyme B) liberated from natural killer (NK) are initiating agents of apoptotic ways which induce menstruation.

In proliferative endometrium and in endometrial hyperplasia apoptosis rate is low, but in endometrial carcinoma apoptosis rate increase; Bcl2 and Bax are expressed in normal and hyperplastic endometrium; in endometrial carcinoma Bcl2/Bax ratio decreases.

These phenomenons are caused by progesteron decreasing and having a long lasting vasoconstriction as effect. After hemorrhage stopping, endometrial surface is recovering through epithelial proliferation from glandular sack bottom in basal layer. Epithelial glandular cells will overtake this level, covering stromal spaces between glands and merging each glandular epithelium [10].

Thereby will be covered too spiralate arterioles producing hemostasis. Endometrial structure is recovering by this proliferative phenomenon and from histophysiological point of view menstrual cycle is beginning again.

Conclusions

Morphological quantification of apoptosis on endometrial biopsies before and after therapy marking out a low rate of apoptotic phenomenon.

No important differences of apoptotic rate on endometrial biopsies before and after therapy through morphological examination have been observed.

No important differences of apoptotic rate on examined endometrial biopsies through in situ hybridization before and after therapy have been observed.

Low apoptotic rate observed with morphological methods has been confirmed by *in situ* hybridization technique.

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Corresponding author

Dana Terzea, MD, Department of Histopathology and Immunohistochemistry, "Victor Babeş" National Institute for Research and Development in Pathology and Biomedical Sciences, 99–101 Independenței Avenue, 050 096 Bucharest, Romania; Phone +4021–319 27 34, E-mail: danaterzea@gmail.com

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