ORIGINAL PAPER

RJME
Romanian Journal of Morphology & Embryology
http://www.rjme.ro/

Immunohistochemical reaction of the glandular epithelium in endometrial hyperplasia compared to endometrial carcinoma

VICTOR EMANUEL STOENESCU[1)], MIHAELA NICULESCU[2)], LILIANA NOVAC[3)], MARIA MAGDALENA MANOLEA[3)], PAUL IOAN TOMESCU[4)], ANDA LORENA DIJMĂRESCU[3)], MARIUS BOGDAN NOVAC[5)], ŞTEFANIA TUDORACHE[3)], DOMINIC GABRIEL ILIESCU[3)]

[1)]*PhD Student, University of Medicine and Pharmacy of Craiova, Romania*
[2)]*Department of Anatomy, University of Medicine and Pharmacy of Craiova, Romania*
[3)]*Department of Obstetrics and Gynecology, University of Medicine and Pharmacy of Craiova, Romania*
[4)]*Department of Urology, Faculty of Medicine, University of Medicine and Pharmacy of Craiova, Romania*
[5)]*Department of Anesthesiology and Intensive Care, University of Medicine and Pharmacy of Craiova, Romania*

Abstract

The histopathological and immunohistochemical diagnosis of endometrial biopsies is used for estimating the risk of progression in endometrial hyperplastic lesions in carcinoma and for guiding the clinical management. The objective of this study was to evaluate the immunohistochemical expression of the estrogen receptor (ER) and progesterone receptor (PR), p14, p53, phosphatase and tensin homolog (PTEN), Ki67, in patients with endometrial hyperplasia (EH) with/without atypia *versus* endometrioid endometrial carcinoma type 1. After the histopathological determining of the lesion type at endometrial level, the cases were studied using immunohistochemical methods, namely by the use of an antibody panel. The immunohistochemical staining of PR was nuclearly and cytoplasmatically positive in EH with/without atypia and cytoplasmatically negative in endometrioid carcinoma, and in ER, the immunohistochemical staining was cytoplasmatically negative in the forms of EH without atypia and positive in various stages of intensity in the rest of the cases. The immunohistochemical staining of p14 was moderately expressed in the endometrioid carcinoma and negative in EH without atypia at nuclear level, and at cytoplasm level, it generally had a positive expression. In our study, the nuclear and cytoplasmic study of immunoxpression p53, both in hyperplastic lesions and in the endometroid endometrial carcinoma, was negative, similar to the immunohistochemical expression of PTEN. At nuclear level, the immunohistochemical staining of Ki67 was positive in EH with atypia and in endometrioid endometrial carcinoma, while at cytoplasm level, it was positive only in endometrioid endometrial carcinoma. The nuclear and cytoplasmic study of this immunohistochemical marker panel shows a different reactivity in EH with/without atypia and endometrioid endometrial carcinoma.

Keywords: immunohistochemistry, biomarkers, endometrial hyperplasia, endometrial carcinoma.

Introduction

Endometrial pathology is most commonly manifested by abnormal uterine bleeding in the peri- and post-menopausal period. In about 15% of these cases, endometrial hyperplasia (EH) or carcinoma is diagnosed, especially in early stages, because bleeding is a symptom that manifests itself early [1].

EH, the early lesion of most endometrial carcinomas, is characterized by the proliferation of endometrial glands, leading to the change of their aspect. The risk for EH progression to endometrioid endometrial carcinoma varies from 1% in simple EH without atypia, up to 46.2% in EH with atypia [2]. The most frequently used classification system for EH is the one released by *World Health Organization* (WHO) in 1994, a classification system where the architectural disturbance and cytological atypia are used for identifying four types of EH: (*1*) simple hyperplasia, (*2*) complex hyperplasia, (*3*) simple hyperplasia with atypia, (*4*) complex hyperplasia with atypia [3].

Categories 1, 2 and 4 are accepted, but there are still debates regarding the group of type 3 hyperplasia [4]. Hyperplasias without atypia are considered benign pathologies, but approximately 60% of endometrial hyperplasias with atypia may coexist with an endometrial carcinoma or may develop an invasive endometrial carcinoma in only a few years [5].

There should be kept in mind that endometrioid endometrial carcinoma is the most frequent malignant pathology in women in Europe and North America. It is, also, on the fourth place in classifying neoplasms in women, after breast, lung and colon neoplasms [6].

The ever-increasing prevalence of obesity, high blood pressure, diabetes mellitus and life prolongation, led to an increase of frequency and mortality of endometrial cancer, with an onset tendency at a younger age [7].

The immunohistochemical methods tried to find a series of biomarkers that could play a prognosis part for investigating the evolution of endometrial hyperplasias to endometrial cancer and for establishing an optimal treatment.

Materials and Methods

The performed prospective study included a group of 106 patients, studied between October 2012–December

2016. The study was performed in the Clinic of Obstetrics and Gynecology, "Filantropia" Municipal Hospital of Craiova and in the Research Center for Microscopic Morphology and Immunology, University of Medicine and Pharmacy of Craiova, Romania.

There were selected patients in the pre-menopause and post-menopause period, who presented with bleeding and received a diagnosis of endometrial hyperplasia as a result of the histopathological diagnosis. We clinically defined menopause as a status after an amenorrhea lasting for at least 12 months in women over 40 years old, and pre-menopause as the life period of a woman shortly before the onset of menopause. The endometrial samples were obtained after dilatation and curettage performed in the 106 women that presented with abnormal uterine bleeding.

The study performance was done according to the ethical principles comprised by the "Human Rights Declaration" adopted in Helsinki, which are in accordance with the Rules for Good Practice in the Clinical Study, and with the approval of the Committee of Academic and Scientific Ethics and Deontology of the University of Medicine and Pharmacy of Craiova. The informed consent for using the data in this study was obtained from all patients.

The histopathological study was performed on endometrial fragments harvested by biopsy curettage, fixed in 10% neutral formalin and included in paraffin. For the classical histopathological study, there were used the Hematoxylin–Eosin (HE) and the Goldner–Szekely trichrome stainings.

The immunohistochemical study was performed on the same biological material included in paraffin. The biological material sectioning was performed in the Microm HM350 rotary microtome, equipped with a section transfer system on water bath (STS, microM). The histological sections were collected on poly-L-lysine covered slides and dried in a thermostat at 37^0C for 24 hours. After that, the sections followed the classical protocol: deparaffinization, hydration, antigen demasking by boiling the slides in a sodium citrate solution, pH 6, for 21 minutes (seven cycles of 3 minutes) in a microwave oven. The endogenous peroxidase blocking was performed by incubating the histological sections in 3% oxygenated water for 30 minutes, at room temperature, followed by a wash in distilled water for 10 minutes and a wash in 1% phosphate-buffered saline (PBS) for 5 minutes. After this, there followed the blocking of non-specific sites by immersion of sections in 2% skimmed milk for 30 minutes. The sections were then incubated with primary antibodies, for 18 hours (over night), in a refrigerator, at 4^0C. The next day, there was applied the secondary biotinylated antibody for 30 minutes, at room temperature, followed by a wash in 1% PBS (three baths of 5 minutes), followed by application of Streptavidin–HRP (Horseradish peroxidase) for 30 minutes, at room temperature and slide wash in 1% PBS 3×5 minutes. The signal was detected by using 3.3'-Diaminobenzidine (DAB, Dako) and the reaction was stopped in 1% PBS. There followed the contrasting with Mayer's Hematoxylin, alcohol dehydration, xylene clarification and slide fixing using a DPX (Fluka) environment.

In our study, we used the following markers: anti-progesterone receptor (PR) (clone PgR 636, Dako, 1/50 dilution); anti-estrogen receptor (ER) (clone 1D5, Dako, 1/50 dilution); anti-p53 (clone DO-7, Dako, 1/100 dilution); anti-Ki67 (clone MIB-1, Dako, 1/50 dilution); anti-phosphatase and tensin homolog (PTEN, clone 6H2.1, Dako, 1/100 dilution); anti-p14 (clone SF3B14, Biocompare, 1/100 dilution).

The patients diagnosed with EH were classified with endometrial hyperplasia without atypia (simple and complex) and with atypia (simple and complex) or with endometrioid endometrial carcinoma.

Taking into consideration that we also found coexistent histological lesions, we classified the patients according to the highest stage histological lesion.

The tissue specimens included 54 cases of EH without atypia (39 cases of simple EH and 15 cases of complex EH), 42 cases of EH with atypia (24 cases of simple EH and 18 cases of complex EH), while 10 cases were endometroid endometrial carcinoma type 1.

The immunohistochemical study was classified at the nuclear and cytoplasm epithelium level on stages, as follows: stage 0 – negative reaction (-); stage 1 – poor reaction (+); stage 2 – moderate reaction (++); stage 3 – intense reaction (+++).

Results

The largest number of studied cases included women in the pre-menopause period (61.32%). This happened due to the fact that, during this period in a woman's life, there appear the most frequent situations of abnormal uterus bleeding, taking into consideration only the cases without an organic pathology. Menopause whose onset was less than 10 years before represented 18.87%, while the one >10 years was found in 19.81% of the cases. The average age of the studied groups was 52.29 years old ± 8.14 standard deviation.

Pathologically speaking, simple endometrial hyperplasia without atypia was characterized by a more glandular density than the normal proliferative endometrium, and by minimal changes of the glandular architecture, including the cytological ones. Numerous glands presented variable sizes and irregular shapes, some with a glandular dilation up to a cystic one, with a glandular-cystic aspect hyperplasia (Figure 1a). In the Gömöri staining, there is highlighted the gland/stroma ratio that maintains itself unitary or slightly sub unitary in favor of the glands (Figure 1b).

The immunohistochemical study for the progesterone and estrogen hormonal receptors was intensely positive (stage 3) at nuclear level (Figure 1, c and d). At cytoplasm level, it was moderately positive (stage 2) only to progesterone (Figure 1c) and negative to estrogen (Figure 1d). Immunostaining for p14 was poorly positive (stage 1) only at cytoplasm level (Figure 1e). The reaction to immunohistochemical biomarkers p53, PTEN and Ki67, both at nuclear and cytoplasmic level, was negative (Figure 1, f–h).

Simple hyperplasia with atypia was characterized by the presence of cytological and nuclear atypia, with a focal character, present in some cells of the glandular epithelium, who lost the nuclear polarity in relation to the basal membrane and who presented abnormal, round or irregular nuclei (Figure 2a). Most often, the glands of simple hyperplasia with atypia presented cellular stratifications. In the Gömöri staining, there is highlighted the gland/stroma ratio, which becomes slightly high in favor of the glands (Figure 2b). From the immunohistochemical point of view, the immunostaining of the progesterone and estrogen hormonal receptors was intensely positive (stage 3) at nuclear level (Figure 2, c and d). At cytoplasmic level, it was intensely positive (stage 3) only to progesterone (Figure 2c) and moderately positive (stage 2) to estrogen (Figure 2d). Immunostaining of p14 was stage 1 at nuclear level and stage 2 at cytoplasmic level (Figure 2e). The immunoexpressions of p53 and PTEN, both at nuclear and cytoplasmic level were negative (Figure 2, f and g). For Ki67, the immunoexpression was intense (stage 3) at nuclear level and negative (stage 0) at cytoplasmic level (Figure 2h).

Complex hyperplasia without atypia was identified in 15 cases. From the architectural point of view, this lesion presented a high glandular density in comparison to a simple form of EH, with important variations of the shape and size of the glands, branched glands with intraluminal papillae (Figure 3a). The cells presented uniform nuclei as shape and sizes with polarity preserved in relation to the basal membrane. The stroma between the proliferated glands was denser and presented frequent typical mitoses (Figure 3b). The progesterone immunoexpression at nuclear level was intense (stage 3) compared to the cytoplasmic one that was negative (Figure 3c).

At nuclear level, the ER immunoexpression was moderate (stage 2), in relation to the cytoplasmic one that was negative (Figure 3d). The immunostaining of p14 was negative (stage 0) at nuclear level and poor (stage 1) at cytoplasmic level (Figure 3e). For p53 and PTEN, both at nuclear and cytoplasmic level, the immunoexpressions were negative (Figure 3, f and g). The analysis of the activity of cellular proliferation Ki67 showed a moderately positive reaction (stage 2) at nuclear level and negative (stage 0) at cytoplasmic level (Figure 3h).

Complex hyperplasia with atypia was characterized by numerous glands with irregular contour, papillary intraglandular proliferations, stratified epithelium (2–4 lines), with the loss of polarity and marked nuclear atypia, also presenting atypical mitoses (Figure 4a). The glands were extremely irregular in respect of shape and size, arranged "back-to-back" in most cases (Figure 4b). The progesterone immunoexpression was moderate at nuclear level (stage 2), in comparison to the cytoplasmic one that was poorly expressed (Figure 4c). The ER immunoexpression was similar to that of PR, stage 2 nuclear and stage 1 cytoplasmatic (Figure 4d). Immunostaining of p14 was negative (stage 0) at nuclear level and moderate (stage 2) at cytoplasmic level (Figure 4e). For p53 and PTEN, both at nuclear and cytoplasmic level, the immunoexpression was negative (Figure 4, f and g). The analysis of Ki67 immunoexpression showed an intense reaction (stage 3) at nuclear level and negative (stage 0) at cytoplasmic level (Figure 4h).

The 10 cases of endometrioid endometrial carcinoma type 1 were characterized by a glandular proliferation marked with intra-luminal papillary projections, with the presence of outgrowths and branches that realize a confluent pattern (Figure 5a). These are lined by stratified neoplastic epithelia with a thin fibrovascular axis (Figure 5b). Immunohistochemically speaking, the immunostaining of ER and PR was moderately positive (stage 2) at nuclear level (Figure 5, c and d). At cytoplasmic level, the immunostaining of PR was negative (stage 0) (Figure 5c) and poorly positive (stage 1) of ER (Figure 5d). Immunostaining of p14 was moderate (stage 2), both at nuclear and cytoplasmic level (Figure 5e). Similar to endometrial hyperplasias, independent of the atypia stage or complexity, the immunoexpression of p53 and PTEN, both at nuclear and cytoplasmic level, was negative, stage 0 (Figure 5, f and g). The Ki67 immunostaining was intense at nuclear level (stage 3) and poorly positive (stage 1) at cytoplasmic level (Figure 5h).

The results of the immunoexpression of the biomarkers used in our study are presented in the table below (Table 1).

Table 1 – ***Immunohistochemical study of the glandular epithelium from endometrial hyperplasias in comparison to endometrioid endometrial carcinoma***

Endometrial hyperplasia	PR		ER		p14		p53		PTEN		Ki67	
	nc	*cyto*	*nc*	*cyto*	*nc*	*cyto*	*nc*	*cyto*	*nc*	*cyto*	*nc*	*cito*
Simple hyperplasia	+++	++	+++	–	–	+	–	–	–	–	–	–
Simple hyperplasia with atypia	+++	+++	+++	++	+	++	–	–	–	–	+++	–
Complex hyperplasia	+++	–	++	–	–	+ focal	–	–	–	–	++	–
Complex hyperplasia with atypia	++	+	++	+	-	++	–	–	–	–	+++	–
Endometrioid endometrial carcinoma type 1	++	–	++	+	++	++	–	–	–	–	+++	+

PR: Progesterone receptor; ER: Estrogen receptor; PTEN: Phosphatase and tensin homolog; nc: Nucleus of glandular epithelial cell; cyto: Cytoplasm of glandular epithelial cell.

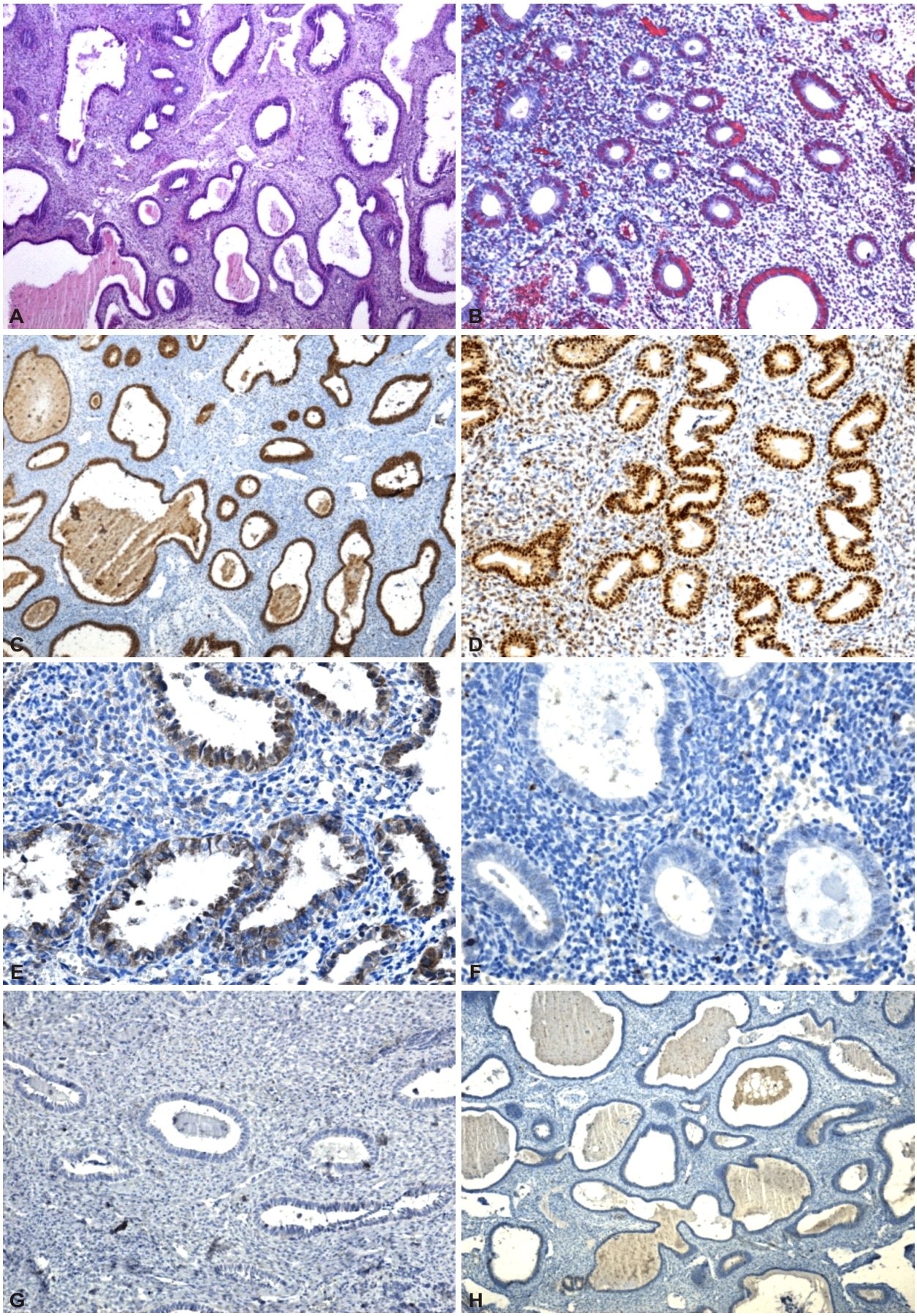

Figure 1 – ***Simple EH without atypia: (A) HE staining, ×100; (B) Gömöri staining, ×100; (C) Anti-PR antibody immunostaining, ×100; (D) Anti-ER antibody immunostaining, ×100; (E) Anti-p14 antibody immunostaining, ×200; (F) Anti-p53 antibody immunostaining, ×200; (G) Anti-PTEN antibody immunostaining, ×100; (H) Anti-Ki67 antibody immunostaining, ×100.***

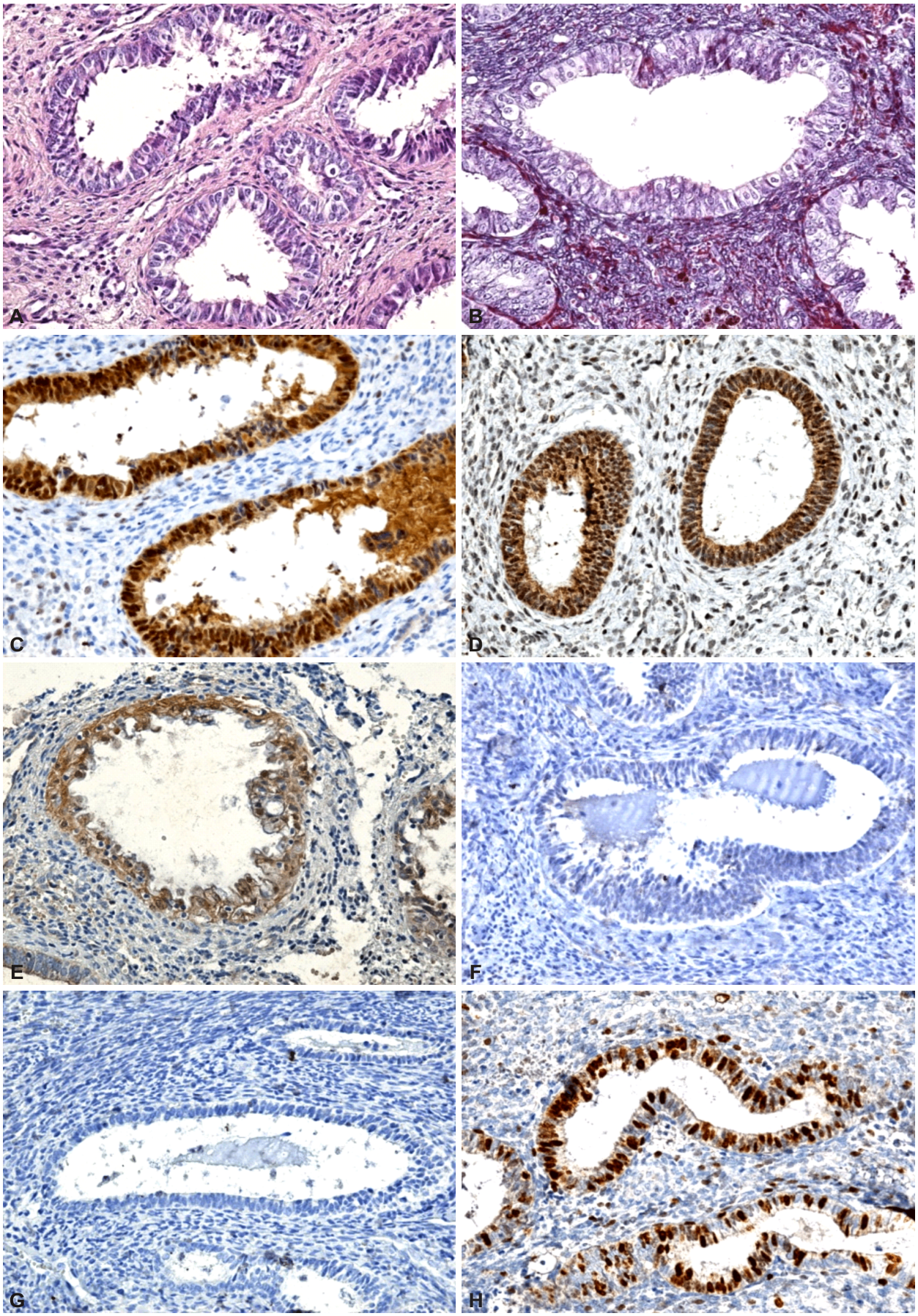

Figure 2 – ***Simple EH with atypia: (A) HE staining, ×200; (B) Gömöri staining, ×200; (C) Anti-PR antibody immunostaining, ×200; (D) Anti-ER antibody immunostaining, ×200; (E) Anti-p14 antibody immunostaining, ×200; (F) Anti-p53 antibody immunostaining, ×200; (G) Anti-PTEN antibody immunostaining, ×100; (H) Anti-Ki67 antibody immunostaining, ×200.***

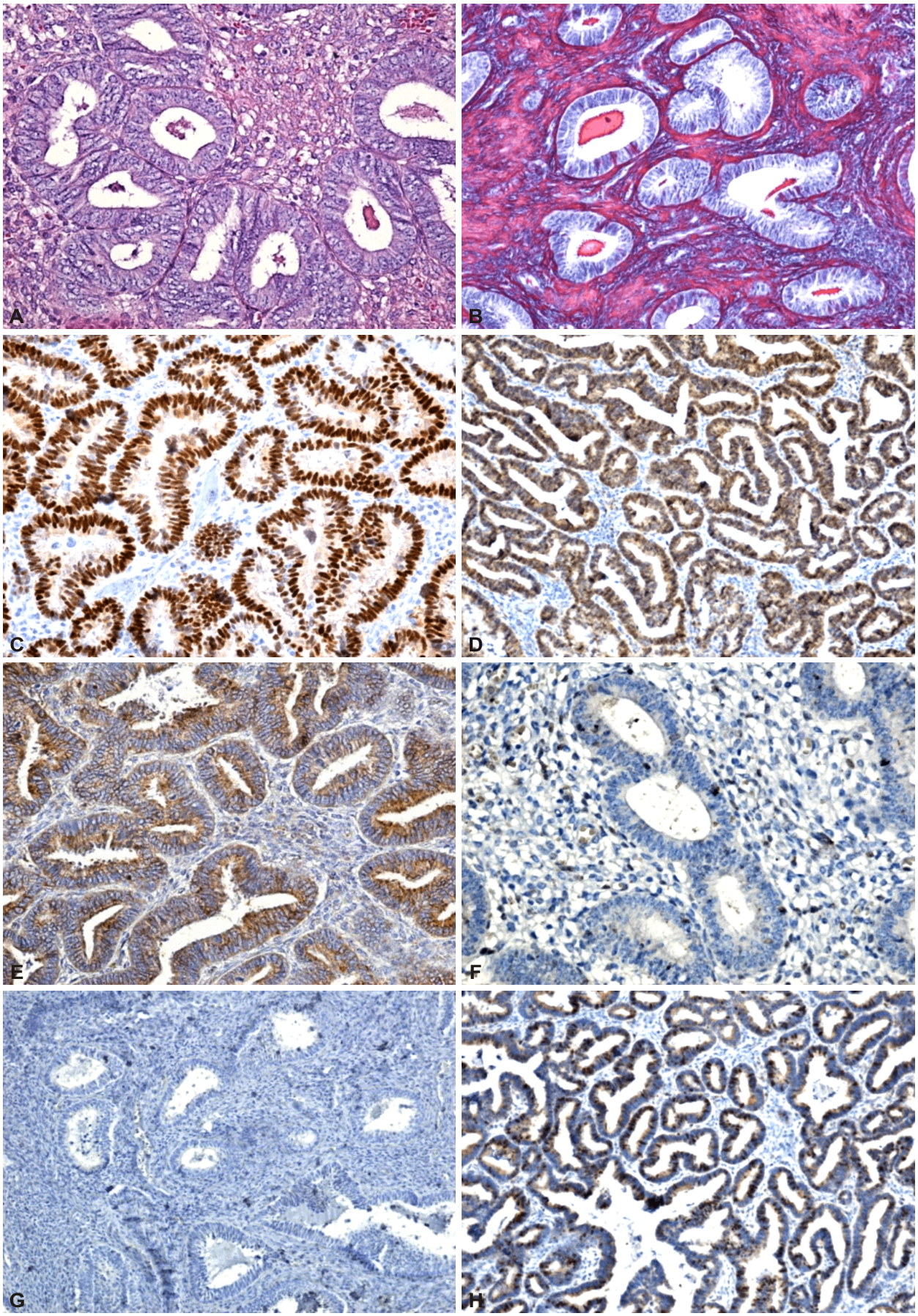

Figure 3 – ***Complex EH without atypia: (A) HE staining, ×200; (B) Gömöri staining, ×200; (C) Anti-PR antibody immunostaining, ×200; (D) Anti-ER antibody immunostaining, ×100; (E) Anti-p14 antibody immunostaining, ×200; (F) Anti-p53 antibody immunostaining, ×200; (G) Anti-PTEN antibody immunostaining, ×100; (H) Anti-Ki67 antibody immunostaining, ×100.***

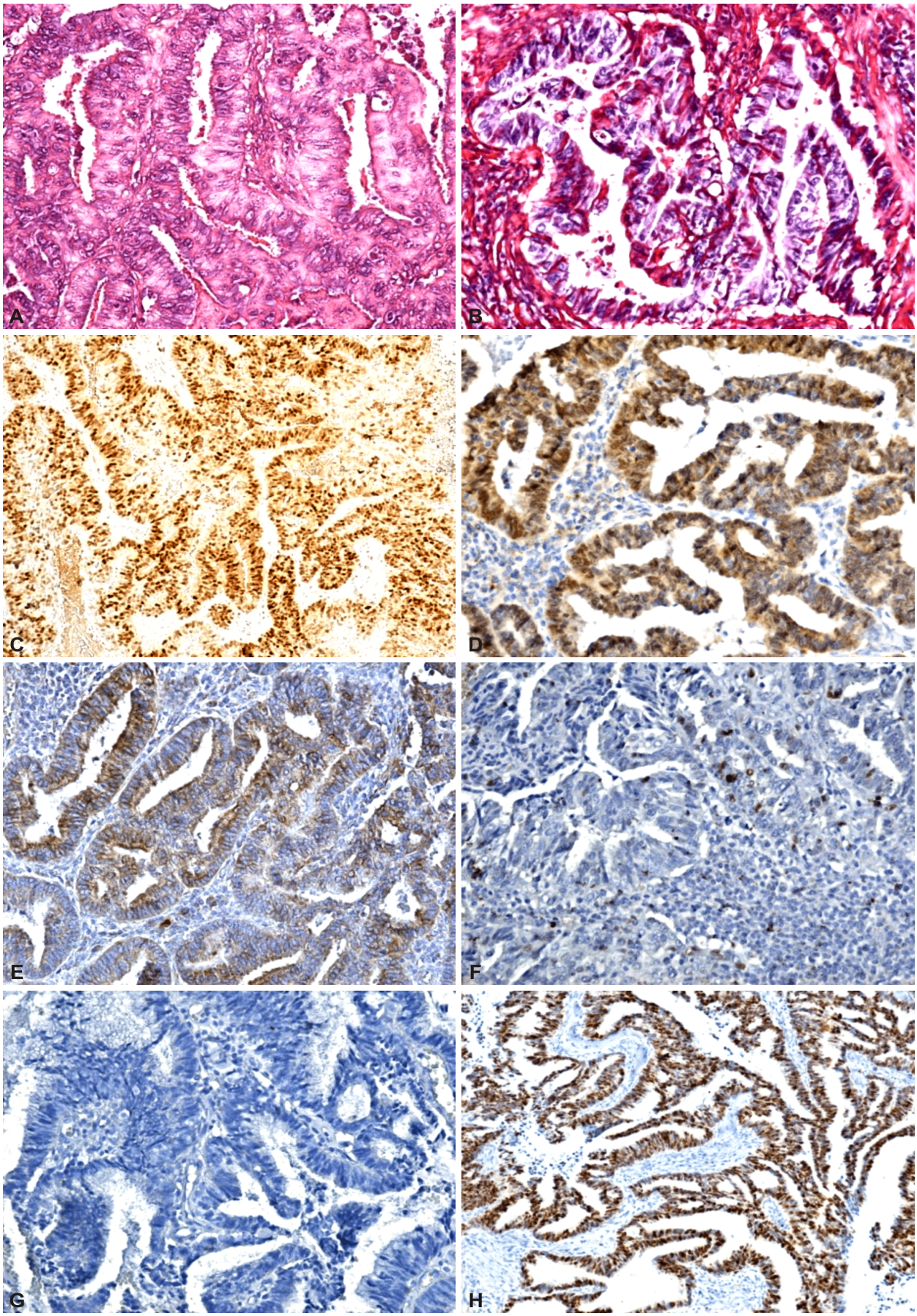

Figure 4 – ***Complex EH with atypia: (A) HE staining, ×200; (B) Gömöri staining, ×200; (C) Anti-PR antibody immunostaining, ×100; (D) Anti-ER antibody immunostaining, ×200; (E) Anti-p14 antibody immunostaining, ×200; (F) Anti-p53 antibody immunostaining, ×200; (G) Anti-PTEN antibody immunostaining, ×200; (H) Anti-Ki67 antibody immunostaining, ×100.***

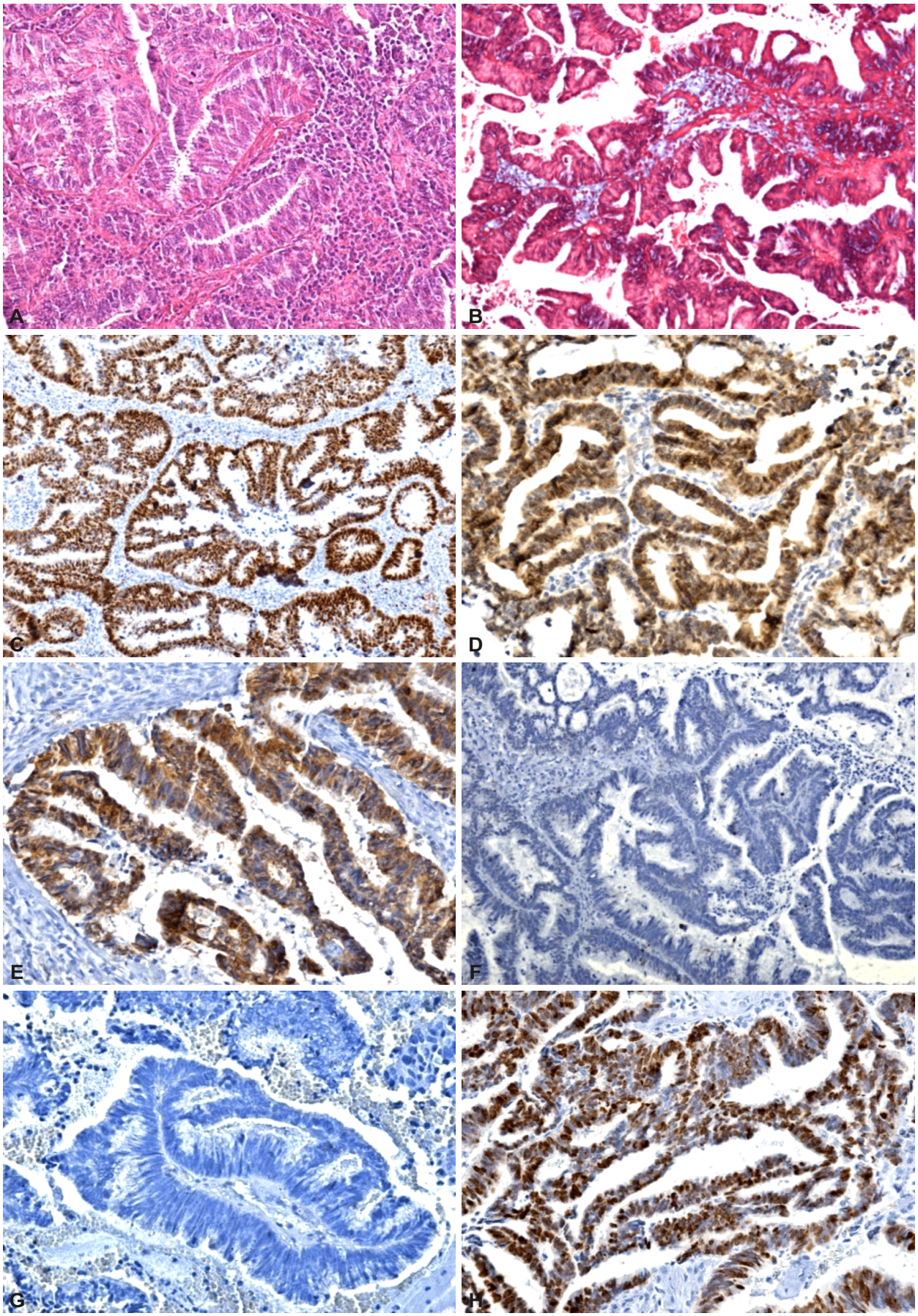

Figure 5 – ***Endometrioid endometrial carcinoma: (A) HE staining, ×200; (B) Gömöri staining, ×200; (C) Anti-PR antibody immunostaining, ×100; (D) Anti-ER antibody immunostaining, ×100; (E) Anti-p14 antibody immunostaining, ×200; (F) Anti-p53 antibody immunostaining, ×100; (G) Anti-PTEN antibody immunostaining, ×200; (H) Anti-Ki67 antibody immunostaining, ×100.***

Discussion

Endometrial hyperplasias are morphological notions indicating a proliferation stage of endometrial glands with or without cyto-nuclear atypia that most often progress to a malignant lesion. The cases with problems of differential diagnosis were represented by the ones with atypia, especially when presenting foci of endometrial endometrioid carcinoma. EH with atypia, as well as endometrial endometrioid cancer, are most often diagnosed on samples of endometrial biopsy. In clinical practice, there is sometimes a disparity between the histological diagnosis of EH with atypia on the endometrial biopsy tissue and the piece harvested after surgery, with a different expression of certain immunological markers [8].

In our study, the analysis of the PR immunoexpression shows the highest stage (3) at nuclear level in the glandular epithelia from EH without atypia (simple and complex) and simple EH with atypia, decreasing in complex hyperplasia with atypia, namely in the endometrial endometrioid carcinoma. At cytoplasmic level, the immunoexpression was expressed differently, in variable stages, from simple with atypia (stage 3) to poor (stage 1) in complex hyperplasia with atypia and negative in endometrial endometrioid carcinoma. Some authors communicate an intense immunoexpression only in EH without atypia [9]. The values of our study are, though, in accordance with the values communicated by Teleman & Mihailovici, in 2003 [10].

The ER immunoexpression at nuclear level was intense (stage 3) in simple hyperplasias with and without atypia, presenting moderate reactions in complex hyperplasias and endometrial endometroid carcinoma. At cytoplasmic level, the immunoexpression was negative in EH without atypia, of moderate and poor intensity in those with atypia and in endometrial endometrioid carcinoma. Studies performed simultaneously on the analysis of hormonal receptors reported that the ER and PR immunoexpression was positive in simple and complex hyperplasia and low in EH with atypia [11]. Other studies mention a low immunoexpression of ER and PR in advanced tumors and less differentiated with a severe prognosis [12, 13]. Recent studies on the immunoexpression of estrogen/progesterone receptors in the endometrial polyp suggest that the low expression of these hormonal biomarkers may be a risk indicator for malignity [14]. A series of studies mention that the presence of ER in endometrial carcinomas is associated with a less aggressive phenotype, and the absence of the PR immunoreactivity represents an independent risk factor for survival [15]. In the same way, other authors showed that tumors with positive estrogen/progesterone expression have a significant association with the clinical and pathological parameters showing a better prognosis [16].

The study of the p14 immunoexpression at nuclear and cytoplasmic level shows different expressions in hyperplasias with/without atypia. This protein acts as a tumor suppressor by inhibiting the ribosome biogenesis or by stopping the cellular cycle dependant on p53, and cellular apoptosis, respectively. In our study, the immunoexpression is moderately expressed (stage 2) in the endometrioid endometrial carcinoma and negative in hyperplasias without atypia at nuclear level, and at cytoplasmic level it is generally expressed in variable stages (2 or 1). Other authors mentioned in their studies that this protein appears as a response to the inadequate hyperproliferative signals, such as cancer tissues [17, 18].

p53 is a suppressor gene that controls cellular proliferation and suffers mutations by losing its functions in human cancers. In our study, the study of p53 immunoexpression at nuclear and cytoplasmic level, both in hyperplastic lesions and in endometrial endometroid cancer, was negative. Other studies showed that the p53 expression was low in a reduced number of complex hyperplasia with atypia (30%) in comparison to carcinomas (65%) [19]. Other studies suggested that endometrial hyperplasia is not always accompanied by the p53 expression, that is why its immunoexpression does not represent a marker of progression to malignity [20]. Nevertheless, other studies confirm an association between a high expression of p53 and the unfavorable prognosis in women with primary endometrial cancer, thus correlating with an aggressive histological type, an advanced stage of the disease and a decrease of the survival rate [21].

PTEN is a tumor suppressor gene that is involved in the control of cellular proliferation and in cellular differentiation and apoptosis [22]. In our study, the expression was negative at nuclear and cytoplasmic level, both in endometrial hyperplastic lesions and in type I endometrial cancer, not correlating thus with the histological lesion. Some authors showed in their studies that the loss of PTEN expression may be used as an argument in the diagnosis of atypia hyerplasias, as the atypia glands are PTEN negative in comparison to the adjacent, normal ones that are PTEN positive. Thus, there is performed a heterogeneous PTEN expression, whose etiology still remains unknown [23]. Other authors showed that the PTEN loss is detectable both in the benign and in the malignant endometrium, but still not in the same intensity, uncorrelated with the clinical and histological risk factors [24]. This situation was also found in our study.

Ki67 refers to the non-histone proteins and nowadays it is considered a proliferation marker of the cellular activity. In our study, at nuclear level the proliferation index Ki67 is intensely positive (stage 3) in hyperplasias with atypia and in endometrioid endometrial carcinoma, moderate in complex hyperplasia without atypia. However, at cytoplasmic level, it is positive only in endometrioid endometrial carcinoma (stage 1). This study is in correlation with the observations of other authors that showed a higher positive expression of Ki67 in the endometrium with atypical hyperplasia and endometrial carcinoma, than in the normal endometrial tissue [12]. Thus, Ki67 may be used as a valuable marker in the differential diagnosis of hyperplasias without atypia in comparison to those with atypia and endometrial carcinoma, as well as in endometrial carcinogenesis [25].

Conclusions

The analysis at nuclear and cytoplasmic level of these immunohistochemical markers shows a different reactivity in hyperplasias without atypia, in comparison to those with atypia and endometrioid endometrial carcinoma. An additional evaluation of the factors associated with the

immunohistochemical expression, together with a long-term monitoring of women, may be useful in understanding the processes that take place in the progression of endometrial lesions to neoplastic lesions.

Conflict of interests

The authors declare that they have no conflict of interests.

References

[1] Gredmark T, Kvint S, Havel G, Mattsson LA. Histopathological findings in women with postmenopausal bleeding. Br J Obstet Gynaecol, 1995, 102(2):133–136.

[2] Laas E, Ballester M, Cortez A, Gonin J, Canlorbe G, Daraï E, Graesslin O. Supervised clustering of immunohistochemical markers to distinguish atypical and non-atypical endometrial hyperplasia. Gynecol Endocrinol, 2015, 31(4):282–285.

[3] Silverberg SG, Mutter GL, Kurman RJ, Kubik-Huch RA, Nogales F, Tavassoli FA. Tumors of the uterine corpus: epithelial tumors and related lesions. In: Tavassoli FA, Devilee P (eds). Pathology and genetics of tumours of the breast and female genital organs. World Health Organization (WHO) Classification of Tumours, International Agency for Research on Cancer (IARC) Press, Lyon, 2003, 221–232.

[4] Emons G, Beckmann MW, Schmidt D, Mallmann P; Uterus commission of the Gynecological Oncology Working Group (AGO). New WHO classification of endometrial hyperplasias. Geburtshilfe Frauenheilkd, 2015, 75(2):135–136.

[5] Antonsen SL, Ulrich L, Høgdall C. Patients with atypical hyperplasia of the endometrium should be treated in oncological centers. Gynecol Oncol, 2012, 125(1):124–128.

[6] Jemal A, Murray T, Ward E, Samuels A, Tiwari RC, Ghafoor A, Feuer EJ, Thun MJ. Cancer statistics, 2005. CA Cancer J Clin, 2005, 55(1):10–30.

[7] Bray F, Loos AH, Oostindier M, Weiderpass E. Geographic and temporal variations in cancer of the corpus uteri: incidence and mortality in pre- and postmenopausal women in Europe. Int J Cancer, 2005, 117(1):123–131.

[8] Kisielewski F, Gajewska ME, Marczewska MJ, Panek G, Wielgoś M, Kamiński P. Comparison of endometrial biopsy and postoperative hysterectomy specimen findings in patients with atypical endometrial hyperplasia and endometrial cancer. Ginekol Pol, 2016, 87(7):488–492.

[9] Zidan AA, Hassan AA, Abu Seadah SS, Ibrahim EH, Attiah SM. Selected immunohistochemical prognostic factors in endometrial hyperplasia *versus* carcinoma. J Am Sci, 2015, 11(4): 14–22.

[10] Teleman S, Mihailovici MS. [Value of steroid receptors assessment in atypical endometrial hyperplasia]. Rev Med Chir Soc Med Nat Iaşi, 2003, 107(4):813–816.

[11] Nunobiki O, Taniguchi E, Ishii A, Tang W, Utsunomiya H, Nakamura Y, Mori I, Kakudo K. Significance of hormone receptor status and tumor vessels in normal, hyperplastic and neoplastic endometrium. Pathol Int, 2003, 53(12):846–852.

[12] Yu CG, Jiang XY, Li B, Gan L, Huang JF. Expression of ER, PR, C-erbB-2 and Ki-67 in endometrial carcinoma and their relationships with the clinicopathological features. Asian Pac J Cancer Prev, 2015, 16(15):6789–6794.

[13] Mohsin SK, Weiss H, Havighurst T, Clark GM, Berardo M, Roanh le D, To TV, Qian Z, Love RR, Allred DC. Progesterone receptor by immunohistochemistry and clinical outcome in breast cancer: a validation study. Mod Pathol, 2004, 17(12): 1545–1554.

[14] Antunes A Jr, Vassallo J, Pinheiro A, Leão R, Neto AM, Costa-Paiva L. Immunohistochemical expression of estrogen and progesterone receptors in endometrial polyps: a comparison between benign and malignant polyps in postmenopausal patients. Oncol Lett, 2014, 7(6):1944–1950.

[15] Ito K, Utsunomiya H, Yaegashi N, Sasano H. Biological roles of estrogen and progesterone in human endometrial carcinoma – new developments in potential endocrine therapy for endometrial cancer. Endocr J, 2007, 54(5):667–679.

[16] Kumari PR, Renuka IV, Apuroopa M, Chaganti PD. A study of expression of estrogen and progesterone receptor, in atrophic, hyperplastic and malignant endometrial lesions, with emphasis on relationship with prognostic parameters. Int J Res Med Sci, 2015, 3(11):3318–3325.

[17] Olcha P, Cybulski M, Skomra D, Obrzut B, Ignatov A, Jóźwik M, Schneider-Stock R, Semczuk A. The pattern of p14ARF expression in primary and metastatic human endometrial carcinomas: correlation with clinicopathological features and TP53 pathway alterations. Int J Gynecol Cancer, 2010, 20(6):993–999.

[18] Sano T, Masuda N, Oyama T, Nakajima T. Overexpression of p16 and p14 ARF is associated with human papillomavirus infection in cervical squamous cell carcinoma and dysplasia. Pathol Int, 2002, 52(5–6):375–383.

[19] Elhafey AS, Papadimitriou JC, El-Hakim MS, El-Said Ai, Ghannam BB, Silverberg SG. Computerized image analysis of p53 and proliferating cell nuclear antigen expression in benign, hyperplastic, and malignant endometrium. Arch Pathol Lab Med, 2001, 125(7):872–879.

[20] Terlikowski S, Lenczewski A, Famulski W, Sulkowska M, Dobrzycka B, Stasiuk-Barmuta A, Kulikowski M. Patterns of immunohistochemical staining for p53 expression in hyperplastic endometrium and adenocarcinoma. Folia Histochem Cytobiol, 2002, 39(3):195–196.

[21] Li M, Zhao L, Shen D, Li X, Wang J, Wei L. Clinical implications and prognostic value of single and combined biomarkers in endometrial carcinoma. Chin Med J (Engl), 2014, 127(8):1459–1463.

[22] Kapucuoglu N, Aktepe F, Kaya H, Bircan S, Karahan N, Ciriş M. Immunohistochemical expression of PTEN in normal, hyperplastic and malignant endometrium and its correlation with hormone receptors, bcl-2, bax, and apoptotic index. Pathol Res Pract, 2007, 203(3):153–162.

[23] Djordjevic B, Hennessy BT, Li J, Barkoh BA, Luthra R, Mills GB, Broaddus RR. Clinical assessment of PTEN loss in endometrial carcinoma: immunohistochemistry outperforms gene sequencing. Mod Pathol, 2012, 25(5):699–708.

[24] Yang HP, Meeker A, Guido R, Gunter MJ, Huang GS, Luhn P, d'Ambrosio L, Wentzensen N, Sherman ME. PTEN expression in benign human endometrial tissue and cancer in relation to endometrial cancer risk factors. Cancer Causes Control, 2015, 26(12):1729–1736.

[25] Shevra CR, Ghosh A, Kumar M. Cyclin D1 and Ki-67 expression in normal, hyperplastic and neoplastic endometrium. J Postgrad Med, 2015, 61(1):15–20.

Corresponding author

Marius Bogdan Novac, Assistant Professor, MD, PhD, Department of Anesthesiology and Intensive Care, University of Medicine and Pharmacy of Craiova, 2 Petru Rareş Street, 200349 Craiova, Dolj County, Romania; Phone +40744–278 405, e-mail: mariusnovac2005@yahoo.com

Received: February 1, 2017 *Accepted: September 25, 2017*