PCNA and Ki-67 in endometrial hyperplasias and evaluation of the potential of malignancy

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Summary

Objective: The aim of this study was to investigate malignancy potential in endometrial hyperplasias and association with PCNA and Ki-67. *Methods*: Hysterectomy or probe curettage materials of 62 patients (20 simple hyperplasias (SH), six SH with atypical changes, five complex hyperplasias (CH), 11 CH with atypical changes, ten proliferative endometrium (PE) and ten secretory endometrium) were included in our study. Immunohistochemical staining for PCNA and Ki-67 protein was performed on formalin-fixed and paraffin-embedded tissue samples. *Results*: Immunoreactivity of PCNA was found to be significantly higher in atypical CH as compared to all other groups ($p < 0.05$). Also immunoreactivity of PCNA was significantly lower in SH as compared to atypical CH, and PE ($p < 0.05$). Average values showed that Ki-67 immunoreactivity is highest for atypical CH, and PE. Immunoreactivity of Ki-67 was found to be significantly higher in atypical CH as compared to other groups except PE ($p < 0.05$). *Conclusion*: PCNA immunoreactivity can be useful in patients with endometrial CH showing mild or moderate atypical changes in terms of prefering more conservative treatment modalities in those with low PCNA index. Also we suggest that Ki-67 could be insufficient to determine the potential of malignancy

Key words: Endometrial hyperplasia; Endometrial carcinoma; PCNA; Ki-67.

Introduction

Endometrial hyperplasia is the precursor lesion of endometrial carcinoma, which is the most frequent cancer of the female genital system in many countries [1]. It is generally believed that endometrial hyperplasias have the potential of premalignancy. Prospective studies to definitely determine the rate of progression to cancer of endometrial hyperplasias can only be performed in limited number of patients [2]. Simple hyperplasia has been shown to progress into cancer with a rate of 1%, complex hyperplasia with 3%, simple atypical hyperplasia with 8%, and complex atypical hyperplasia with 29%. The period of progression to cancer for hyperplasias without atypical changes is reported to be ten years on average, and four years for hyperplasias with atypical changes [2, 3].

Chronic anovulation (adolescence, polycystic ovarian syndrome, perimenaposal period), hyper production of endogenous eostrogen (tumors with granulosa cells, techoma, adrenocortical hyperplasia, obesity), and exogenous estrogen intake are meant to be the risk factors in endometrial hyperplasia etiology [4].

Ki-67 is a non histone protein and it is located on the 10th chromosome; 90% of Ki-67 is in the nucleolus and 10% is in the nucleoplasma. Ki-67 antigen is very quickly catabolized because it contains large amounts of serine, thyronine, proline and glutamic acid. Ki-67 antigen's half life is very short, approximately one hour. Ki-67 antigen exists in the proliferative phase cells. The tumors with high Ki-67 antigen expression are more agressive, have poor prognosis, more vascular invasion, and metastasize more than cells without Ki-67 antigen expression [5, 6].

Picartz *et al*. showed the growth fraction determined by Ki-67 in normal and neoplastic endometrial tissues in 1999 [7]. Thus, the cells that are immunoreactive to Ki-67 are thought to represent the growth fraction. In 1984 Gerdes *et al*. determined in detailed cell cyle anaylsis the presence of Ki-67 antigen in the cell nucleus in G1, S and G2 phases as well as mitosis through the cell cyle. On the other hand, they showed that Ki-67 antigen was not synthesized in the cells in the G0 phase [8]. Also, it was indicated that the Ki-67 index was an independent prognostic factor in breast cancer survival and recurrence [9]. Previously to those findings, in 1978, Miyachi *et al*. determined an antigen and its reactive antibody in the proliferating cell nucleus of the serum of patients with systemic lupus erythematosus. They named it "proliferating cell nuclear antigen" (PCNA) which was later shown to have a similar peptide structure with "cyclin" [10].

PCNA is an aspartic acid rich in glutamic acid which is a 36 kDa nuclear polypeptide located on the 20th chromosome and is 262 amino acids in length. It is the cofactor of DNA polymerase, responsible for DNA replication and it is located in the nucleus. PCNA is synthesized through cell cycling and regulates the cycle. The synthesis rate is correlated with cell proliferation and DNA synthesis. Although the proliferation potential of PCNA is directly correlated in normal cells, similar findings are not always observed in neoplastic cells [11, 12].

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The immunohistochemical evaluation of the alteration of PCNA indicates both active DNA replication and DNA damage that might lead to carcinogenesis [13]. Immunohistochemical PCNA analysis has been done in carcinomas including the breast, and hepatocellular and gastric carcinomas previously [14].

Oncogenes have been studied to determine the malignancy potential of endometrial hyperplasias. Investigation of cell cycle-related antigens with immunohistochemical methods has become an important field in research for the development of malignancies. Proliferating cell nuclear antigen (PCNA) and Ki-67 are the two most emphasized cell cycle-related antigens [15].

Based on the above-mentioned data, we tried to investigate the malignancy potential in endometrial hyperplasias and an association with PCNA and Ki-67.

Materials and Method

Study Design

This study was performed on 62 patients who underwent hysterectomy for any reason or endometrial tissue sampling with probe curettage, and diagnosed with endometrial hyperplasia, proliferative or secretory endometrium with pathological examination in Dr. Zekai Tahir Burak Women's Health Education and Research Hospital, Ankara, Turkey. The subjects with polycystic ovarian syndrome, any malignant disease such as breast, colon, etc., diabetes mellitus, known cardiovasculer and metabolic diseases, body mass index > 28 kg/m^2, on medication such as thyroid hormone, oral contraceptives or HRT were excluded.

Hematoxylin-eosin sections were re-evaluated to confirm the histopathological diagnosis. Classification accepted by the World Health Organization (WHO) and International Society of Gyneocological Pathologists (ISGP) was used in the pathological definition and classification of the endometrial hyperplasias. The patients with endometrial hyperplasias were classified as having simple hyperplasia without atypia (n = 20), complex hyperplasia without atypia (n = 5), simple atypical hyperplasia (n = 6) and complex atypical hyperplasia (n = 11), as well as control groups with proliferative endometrium (n = 10) and secretory endometrium (n=10) after pathological evaluation.

The study protocol was approved by the ethical committee. All patients were clearly informed about the aim of the study, and written informed consent to the protocol was obtained from all patients.

Immunohistochemical staining

Immunohistochemical analyses for PCNA and Ki-67 were performed on formalin-fixed, paraffin-embedded archival tissue using the streptoavidin biotin-peroxidase technique. For all cases, a 4 μm histologic section was deparaffinized in xylene and rehydrated in descending dilutions of ethanol. For antigen retrieval, slides were treated by microwave heating in citrate buffer (pH 6.0) for 10 min. Endogenous peroxidase activity was blocked by 60 min of incubation with 0.3% hydrogen peroxidase. Slides were incubated with PCNA (1/100 dilution, Clone PC 10, DAKO) and Ki-67 (1/100 dilution, Clone MIB-1, DAKO). Sections were incubated with a streptavidin-biotin-peroxidase kit (Ultra Vision Large Volume Detection System Anti-Polyvalent, HRP, LabVision, USA), and after incubation the reaction product was detected using diaminobenzidine (DAB). Finally, the sections were counterstained with Mayer's hematoxylin, and mounted with mounting medium. Only nuclear PCNA and Ki-67 expression were accepted as specific. Appropriate positive and negative controls were stained for each antibody. For Ki-67, in microscopic analysis, the percentage of positive nuclei in 1000 consecutive cells of the most evenly stained areas of the tumor were counted. For PCNA, a total of 100 cells were counted at x10 magnification, and the percentage of the number of nuclei stained with PCNA was indicated as PCNA-LI.

Statistical analysis

For statistical analyses, SPSS for Windows Version 11.0 statistical software (SPSS Inc., Chicago, IL) was used. The groups were compared with Anova one-way variance analysis and the Tukey post-hoc test. A p value of 0.05 was taken as the threshold level for statistical significance.

Results

The mean age of the patients was 44.31 ± 7.54 years and the gravidity of the patients was 2.28 ± 1.43. According to pathological classification, 20 patients out of 62 included to our study were diagnosed with simple hyperplasia, six with simple hyperplasia with atypical changes, five with complex hyperplasia, 11 with complex hyperplasia with atypical changes, and ten with proliferative endometrium and ten with secretory endometrium.

Regarding immunohistochemical staining, PCNA immune reactivity was found to be the highest for complex atypical hyperplasia (9.91%) and the lowest for secretory endometrium (4.22%).

Immunoreactivity of PCNA was significantly higher in complex atypical hyperplasia (9.91%) as compared to simple hyperplasia (9.02%), simple hyperplasia with atypical changes (6.88%), complex hyperplasia (7.72%), proliferative endometrium (8.66%) and secretory endometrium (4.22%) ($p < 0.05$). On the other hand, the PCNA-LI index was found to be significantly lower in secretory endometrium as compared to all other groups ($p < 0.05$). According to statistical analyses, PCNA immune reactivity was significantly lower in simple hyperplasia as compared to simple hyperplasia with no atypical changes, complex atypical hyperplasia, and proliferative endometrium ($p < 0.05$) (Figure 1).

The mean percentual grades of Ki-67 expression were 26.2% for simple hyperplasia without atypia, 15.75% for complex hyperplasia without atypia, 23.32% for simple atypical hyperplasia, 41.88% for complex atypical hyperplasia patients, as well as 42.05% for proliferative endometrium and 14.95% for secretory endometrium (control groups). Comparing the mean values of Ki-67 immune reactivity in the study groups, the highest immunoreactivity values were observed in complex atypical hyperplasia and proliferative endometrium. According to statistical analyses, immune reactivity of Ki-67 was found to be significantly higher in complex atypical hyperplasia compared to all other groups ($p < 0.05$). However, no statistically significant difference could be found in proliferative endometrium (Figure 2).

Fig. 1

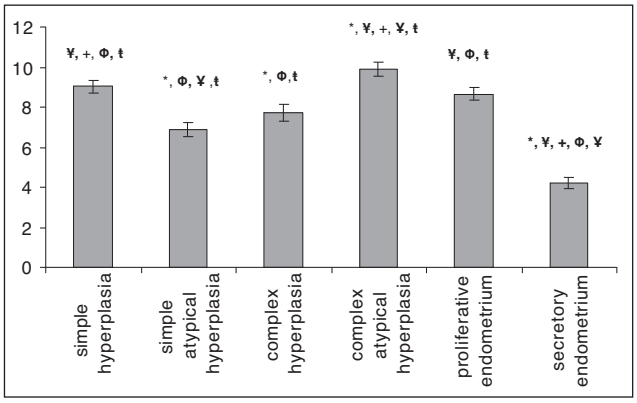
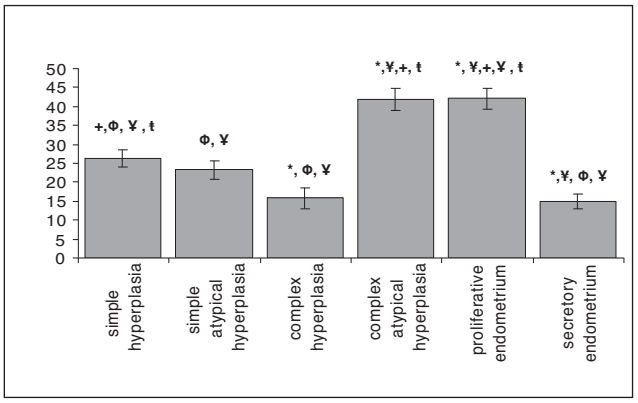


Fig. 2



* $p < 0.05$ simple hyperplasia versus other groups; **¥** $p < 0.05$ simple atypical hyperplasia versus other groups; **+** $p < 0.05$ complex hyperplasia versus other groups; **Φ** $p < 0.05$ complex atypical hyperplasia versus other groups; **¥** $p < 0.05$ proliferative endometrium versus other groups; **ŧ** $p < 0.05$ secretory endometrium versus other groups.

Figure 1. — PCNA-LI indices of the patient and control groups.

Figure 2. — Ki-67 indices of the patient and control groups.

Discussion

It was generally believed that the majority of hyperplasias had precancerous potential [2]. However, this concept of continuity changed in time, and more importance is now given to the existence of atypical cytological changes. Currently, it is believed that the most important factor regarding progression of endometrial hyperplasia to endometrial carcinoma is the existence of atypical cytological changes [3].

Determining the malignancy potential of endometrial hyperplasias, it is important to avoid unnecessarily excessive treatment, particularly in young patients, and studies with that purpose are still kept up to date [2, 3]. Immunohistochemical measurement of the proliferative activity of cells has been widely used to assess the biological behavior of human tumors [15]. Ito and colleagues [16] investigated the immune reactivity of PCNA in various endometrial lesions and they found no significant difference between the PCNA index of the groups they classified as simple hyperplasia (n = 4), complex hyperplasia (n = 14), and atypical hyperplasia (n = 10). Furthermore, no significant difference was found between the PCNA index of endometrial hyperplasias [16]. Yu and colleagues [17] studied the PCNA index in cases with proliferative endometrium, secretory endometrium, and invasive adenocarcinoma and the highest immunoreactivity was found in proliferative endometrium. The PCNA index was higher in atypical hyperplasias as compared to simple hyperplasias. In addition, PCNA staining was observed in a patchy fashion in atypical hyperplasias, especially in regions where atypical cytological characteristics were present. They concluded that according to this finding atypical hyperplasia developed in a patchy fashion [17]. Different from Yu *et al's*. study, we classified atypical hyperplasias as complex and simple, and also found PCNA immunoreactivity significantly higher in complex atypical hyperplasia as compared to other groups. Cinel and colleagues [18] studied PCNA immunoreactivity in simple endometrial hyperplasias and complex endometrial hyperplasias without typical nuclear changes. They found a significantly higher PCNA index in complex hyperplasia as compared to simple hyperplasia. Terlikowski *et al*. [19] studied PCNA immune reactivity in simple hyperplasia, simple atypical hyperplasia, complex hyperplasia and complex atypical hyperplasia in a study they performed in 2001. They found the PCNA index in a average of 23% of simple hyperplasia, 28% of simple atypical hyperplasia, 35% of complex hyperplasia, and 39% of complex atypical hyperplasia. According to these findings, they suggested that the PCNA index was a reliable index for the determination of differentiation of premalign/malign endometrial changes. They suggested that performing PCNA analysis would be beneficial, especially in patients under 40 years of age, diagnosed with endometrial hyperplasia, while more conservative treatment methods could be selected for those with lower proliferation indices [19]. Therefore, our findings are similar to those in this study. However, in contrast to previous findings, we have found significantly lower PCNA immunoreactivity in simple atypical hyperplasia as compared to simple hyperplasia without atypical changes and complex atypical hyperplasia. Thus, we suggest that PCNA immunoreactivity can be useful in patients under 40 years of age with complex endometrial hyperplasia showing mild or moderate atypical changes. More conservative treatment modalities would be preferred in patients with a low PCNA index, however, we can evaluate this as a negative factor for those with high PCNA indices. Furthermore studies with greater numbers of patients should be performed, and certain cut-off values should be determined for the use of PCNA index in practice. Findings of lower PCNA immunoreactivity in simple atypical hyperplasia as compared to other hyperplasia groups indicate that the precancerous potential of this pathology should be reviewed.

Another cell cycle-related antigen, Ki-67, has been shown to be an independent prognostic factor for survival and recurrence in many recent studies [20-22]. Risberg and colleagues [23] analyzed Ki-67 expression in proliferative endometrium, secretory endometrium, simple hyperplasia, complex hyperplasia, and atypical hyperplasia and found the highest expression in proliferative endometrium. However, no statistically significant differences in the Ki-67 index of endometrial hyperplasias were found [23]. In the study of Ambros [24], the highest Ki-67 index was found in proliferative endometrium with an average of 23.2%. They obtained the mean values of 9.8% in simple hyperplasia, 12.7% in complex hyperplasia, and 10% in atypical hyperplasia [24]. Ioffe and colleagues [25] similarly found the highest Ki-67 index in proliferative endometrium (38.4%).

Our findings are concordant with these three studies. In our study, the significantly higher values of Ki-67 expression in complex atypical hyperplasia and absence of any significant difference between proliferative endometrium may indicate that Ki-67 could be insufficient to determine the potential of malignancy. Although Ki-67 has been shown to be an independent prognostic factor in terms of survival and recurrence in prostate carcinoma and breast carcinoma, any finding indicating that Ki-67 is capable of showing the relation of endometrial lesions with malignancy has not been found in the few performed studies. Our findings also support this view.

In conclusion, PCNA immunoreactivity can be useful in patients with complex endometrial hyperplasia showing mild or moderate atypical changes in terms of preferring more conservative treatment modalities in those with a low PCNA index. We also suggest that Ki-67 could be insufficient to determine the potential of malignancy. However, the main limitation of this study is its sample size. With our small sample size the data cannot exactly determine any specific importance of PCNA and Ki-67. Further larger series investigations are needed to clarify this subject.

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