Selected Immunohistochemical Prognostic Factors In Endometrial Hyperplasia versus Carcinoma

Amina A. Zidan, Amal A. Hassan, Shaimaa Sh. Abu Seadah, Eman H. Ibrahim and Samah M. Attiah

Department of pathology, Faculty of Medicine, Al-Azhar University, Cairo, Egypt
sh_sh_1977@hotmail.com

Abstract: Background and Objective: Endometrial hyperplasia develops in a setting of estrogen excess. Detection of high levels of steroid receptors denotes a good response to hormonal therapy namely progesterone in simple and complex hyperplasia. Decrease in receptor activity which is found in atypical hyperplasia results in low sensitivity to progesterone therapy. If a higher level is found in some rare incidence of atypical hyperplasia, they have a good chance of response to hormonal therapy and a radical surgery can be avoided. Higher positivity in malignant lesion usually correlates with better differentiation and better survival rates.ER and PR may be useful markers predicting therapy response in endometrial hyperplasia and endometrial carcinoma. **Aim of Work*:*** In this study, we aimed to assess the relationships between Ki-67, P53 expression and estrogen (ER), progesterone receptors (PR) in endometrial hyperplasia versus endometrial carcinoma. We also evaluated the relationship between Ki-67, P53, ER, PR expression and tumor grade in endometrial carcinoma. **Material and Methods:** Specimens included16 cases endometrial hyperplasia without atypia, 6 cases atypical endometrial hyperplasia and 18 endometrial carcinoma specimens. Immunohistochemical staining for Ki-67, P53, ER and PR was performed using Ultraview DAB detection kit on Ventana Bench Mark staining systems (Ventana Medical Systems, Tucson, Arizona, USA) on formalin-fixed and paraffin embedded tissue samples. Ki-67, P53, ER and PR expression was represented as the staining score. **Results:** mmunohistochemistry showed that Ki-67, P53, ER and PR were positive for nuclei of cells. The percentage of ER, PR were decreased significantly in atypical hyperplasia or endometrial carcinoma as compared to simple or complex hyperplasia (p= 0.050 and p=0.041 respectively). The P53score in atypical hyperplasia and endometrial carcinoma was significantly higher than those for hyperplasia without atypia (p= 0.020). The Ki-67 score in endometrial carcinoma was insignificantly higher than those for hyperplasia (p= 0.508). In endometrial carcinoma, Ki-67, P53overexpression was found to be related to poor differentiation (high-grade tumors). **Conclusions:** The study showed that estrogen and progesteronepositive receptors correlate significantly with hyperplasia without atypia and well differentiated tumors. The overexpression of p53 and Ki-67 seems to indicate a more malignant phenotype. The results suggest that decreased levels of ER and PR with an increasing risk of invasive cancer plays an important role in the occurrence and development of endometrial carcinoma.
[Amina A. Zidan, Amal A. Hassan, Shaimaa Sh. Abu Seadah, Eman H. Ibrahim and Samah M. Attiah. **Selected Immunohistochemical Prognostic Factors In Endometrial Hyperplasia versus Carcinoma.** *J Am Sci* 2015;11(4):14-22]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 3

Keywords: Endometrial hyperplasia, Endometrial carcinoma, Immunohistochemistry, Prognostic factors.

1.Introduction

Endometrial hyperplasia is a non-physiological and non-invasive proliferation at the endometrium level whose results consist in the growth of various forms and shapes of glands. The term of endometrial hyperplasia refers to an abnormality characterized by the increase of the endometrium quantity (volume), alteration of glandular architecture and change of glands/stroma ratio ***(Ismail, 2006).*** There are two forms of hyperplasia: the atypical form, representing a precursor lesion with certain characteristics found in relation to endometrial adenocarcinoma, and the non-atypical form, which is a self-limiting increase which do not seem to lead to cancer ***(Ilie et al., 2011).***

Endometrial carcinoma is the most common invasive carcinoma of the female genital tract and the fourth most frequently diagnosed cancer in North American women ***(Alkushi et al., 2007).***

In endometrial carcinoma, the prognostic impact of traditional clinicopathologic variables, such as International Federation of Gynecology and Obstetrics (FIGO) stage, histologic type and histologic grade, is well established. This information has been used to determine whether hysterectomy alone is likely to be curative. Nevertheless, there is a definite need for more specific prognostic markers to avoid over-treatment of low-risk groups and to ensure that patients with highly aggressive tumors receive adequate postoperative treatment ***(Salvesen et al., 1999).***

Immunohistochemical methods have been useful for detecting several biomarkers of possible prognostic importance for a number of cancer types. Regarding tumor cell proliferation, it is widely accepted that proliferative capacity may influence the clinical course, and hence patient prognosis. The nuclear Ki-67 antigen, which is expressed in all stages of the cell cycle except G0, may be detected by

immunohistochemistry to estimate proliferative activity in tumors. Over the past few years, it has been shown that p53 has a central role in the regulation of cell-cycle progression by the transcriptional activation of genes such as p21, followed by inhibition of cyclin-dependent kinases ***(Salvesen et al., 1999).***

Endometrial hyperplasia develops in a setting of estrogen excess. Detection of high levels of steroid receptors denotes a good response to hormonal therapy namely progesterone in simple and complex hyperplasia. Decrease in receptor activity which is found in atypical hyperplasia results in low sensitivity to progesterone therapy. If a higher level is found in some rare incidence of atypical hyperplasia, they have a good chance of response to hormonal therapy and a radical surgery can be avoided. Higher positivity in malignant lesion usually correlates with better differentiation and better survival rates***(Aparna, 2011).***

The endometrial carcinoma is formed and develops in close relation to the plasma and tissue levels of sex steroidal hormones and their receptors. Also, the connection with the atypical endometrial hyperplasia is recognized, associated with a prolonged estrogen stimulation, of endogenous or exogenous origin, notbeing counterweight by progesterone ***(Stoian et al., 2011).***

Historically, estrogen has been seen as a direct promoter of endometrial carcinogenesis via the stimulation of rapid proliferation of epithelial cells,which is confirmed by the results of several studies ***(Cai et al., 2008).***

The estrogen can bind to nuclear estrogen receptors (ER), thus initiating the gene expression, and can increase the mutational rate by stimulating cell proliferation. Also, the expression of (PR) completes the picture for the hormonal levels in endometrial carcinoma, representing independent prognostic factors in several studies, alongside with the Ki67 proliferation index ***(Stoian et al., 2011).***

The Ki-67 antigen, a non-histone protein useful for the identification of proliferating cells that has no specific phase, is expressed in all active phases of cell cycle (Ki-67 is not expressed in G0 phase). An increase of Ki-67 expression shows an increased mitotic activity and cell proliferation ***(Taylor et al., 2003).*** Ki-67 expression is normally increased at endometrium level during the proliferative phase of the menstrual cycle ***(Ilie et al., 2011).***

Oncoprotein p53 is a phosphoprotein, encoded by p53 gene located on the short arm of chromosome 17 ***(Ilie et al., 2011).***

2.Material and Methods

Tissue Specimens:

Formalin-fixed and paraffin-embedded 40 specimens were collected and prepared for this study from AL-Zahraa University Hospital and from the archives of some private laboratories during the period 2010-2013.

Specimens included 16 cases endometrial hyperplasia without atypia (10 cases were simple and 6 cases were complex), 6 cases were atypical endometrial hyperplasia and 18 were endometrial carcinoma (EC) specimens.

All EC patients had undergone surgical intervention (Total abdominal hysterectomy and bilateral salpingio-ophrectomy). Endometrial hyperplasia samples were obtained either by curettage or biopsy specimens. Hyperplasia specimens were evaluated according to WHO classification **(Silverberg et al., 2003).** Regarding EC cases, grading was assessed according to the International Federation of Gynecology and Obstetrics criteria **(Mikuta, 1993), (Zaino et al., 1995);** Grade I (7 cases), Grade II (5 cases), Grade III (6 cases).

Multiple five micron sections were cut; one was stained by Hematoxylin and Eosin for histopathological examination, while the other sections were mounted on positive charged slide and immunostained by mouse monoclonal antibodies against ER, PR, Ki-67 and P53.

Immunohistochemistry:

Immunohistochemical stains were performed using Ultraview DAB detection kit on Ventana BenchMark staining system (Ventana Medical Systems, Tucson, Arizona, USA).

Immunohistochemistry for ER clone SP1, PgR clone 1E2 (Ventana Medical Systems), P53 clone Do-7 and Ki-67 clone MIB-1, ready to use (Dako autostainer).

Positive and Negative Control:

As a negative control for all markers, a tissue was processed through the above sequences but the primary antibody was omitted, instead phosphate buffer solution was added. Positive external control, represented by sections of breast carcinoma.

Evaluation of Immunostaining:

Positive staining was indicated as brown color in the nucleus of the cells. Quantification of positivity was expressed in percentages. Samples with nuclear staining of at least 20% of tumor cells were considered p53 positive and samples with 40% or higher were considered Ki-67 positive. The positivity limit for ER and PR was set to 5% based on numerous previous studies **(Markova et al., 2010).**

Statistical Analysis:

Data were analyzed using Statistical Program for Social Science (SPSS) version 18.0. Quantitative data were expressed as mean± standard deviation (SD). Qualitative data were expressed as frequency and percentage. The following tests were done: Chi-square (X^2) test of significance was used in order to compare proportions between two qualitative parameters.

Probability (P-value)

– *P*-value ≤0.05 was considered significant.

– *P*-value <0.001 was considered as highly significant.

– *P*-value >0.05 was considered insignificant.

3. Results

A total of 40 cases [22 cases (55%) of endometrial hyperplasia and 18 cases (45%) of endometrial carcinoma] were enrolled into this study. The cases were distributed as follow: simple hyperplasia (25%), complex hyperplasia (15%), atypical hyperplasia (15%), endometrial carcinoma (45%) according to the International Federation of Gynecology and Obstetrics criteria it was divided into grads: grade I (38.9%), grade II (27.8%) and grade III (33.3%) of lesions ***(Table 1).***

Table (1): Type of lesions distribution of the patients groups

Lesions	No.	%
Simple Hyperplasia	10	25.0
Complex Hyperplasia	6	15.0
Atypical Hyperplasia	6	15.0
Endometrial Carcinoma	18	45.0
Grade I	*7*	*38.9*
Grade II	*5*	*27.8*
Grade III	*6*	*33.3*

Immunohistochemical expression of ER:

ER staining was nuclear. 16 out of 40 (40%) of cases were positive for ER. Positive rate increased significantly in endometrial hyperplasia (54.5%) than endometrial carcinoma (22.2%), using Chi-square test with *(*P*-value 0.038)* ***(Table2).***

Table (2): ER expression in endometrial hyperplasia versus carcinoma

Lesions	ER			
	Positive		Negative	
	No.	%	No.	%
Hyperplasia	12	54.5	10	45.5
Endometrial carcinoma	4	22.2	14	77.8
Total	16	40.0	24	60.0
x2	4.310			
p-value	**0.038**			

There was also statistically significant difference between types of lesion and ER expression of the patients groups, using Chi-square test with (*P*-value 0.050); the expression decreased in atypical hyperplasia (33.3%) and endometrial carcinoma (22.2%) than simple hyperplasia (60%) and complex hyperplasia (66.7%) ***(Fig. 1A,B,C)***. As regarded the cases of endometrial carcinoma, the expression of ER decreased in high grade: Grade I (28.6%), Grade II (20%) and Grade III (16.7%) ***(Table 3).***

Table (3): Relation between ER expression and types of lesion

Lesions	ER			
	Positive		Negative	
	No.	%	No.	%
Simple Hyperplasia	6	60.0	4	40.0
Complex hyperplasia	4	66.7	2	33.3
Atypical hyperplasia	2	33.3	4	66.7
Endometrial carcinoma				
Grade I	2	28.6	5	71.4
Grade II	1	20.0	4	80.0
Grade III	1	16.7	5	83.3
x2	7.131			
P-value	**0.050**			

Immunohistochemical expression of PR:

PR staining was nuclear.20 out of 40 (50%) of cases were positive for PR. Positive rate increased significantly in endometrial hyperplasia (68.2%) than endometrial carcinoma (27.8%), using Chi-square test with (*P*-value 0.011) ***(Table 4).***

Table (4): PR expression in endometrial hyperplasia versus carcinoma

Lesions	PR			
	Positive		Negative	
	No.	%	No.	%
Hyperplasia	15	68.2	7	31.8
Endometrial carcinoma	5	27.8	13	72.2
Total	20	50.0	20	50.0
x2	6.465			
p-value	**0.011**			

Table (5): Relation between type PR expression and types of lesion

Lesions	PR			
	Positive		Negative	
	No.	%	No.	%
Simple Hyperplasia	7	70.0	3	30.0
Complex hyperplasia	5	83.3	1	16.7
Atypical hyperplasia	3	50.0	3	50.0
Endometrial carcinoma				
Grade I	3	42.9	4	57.1
Grade II	1	20.0	4	80.0
Grade III	1	16.7	5	83.3
x2	8.876			
p-value	**0.041**			

There was also statistically significant difference between types of lesion and PR expression of the patients groups (*P*-value 0.041); the expression decreased in atypical hyperplasia (50%) and endometrial carcinoma (27.8%) than simple hyperplasia (70%) and complex hyperplasia (83.3%) ***(Fig. 2 A,B,C).*** As regarded the cases of endometrial carcinoma; the expression of PR decreased in high grade: Grade I (42.9%), Grade II (20%) and Grade III (16.7%) ***(Table 5).***

Immunohistochemical expression of P53:

P53 staining was nuclear.21 out of 40 (52.5%) of cases were positive for P53. Positive rate increased significantly in endometrial carcinoma (77.8%) than endometrial hyperplasia (31.8%) (*P*-value 0.004) ***(Table 6).***

There was also statistically significant difference between types of lesion and P53 expression of the patients groups (*P*-value 0.020); the expression increased in atypical hyperplasia (66.7%) and endometrial carcinoma (77.8%) than simple hyperplasia (10%) and complex hyperplasia (33.3%) ***(Fig. 3 A,B,C).*** As regarded the cases of endometrial carcinoma; the expression of P53increased in high grade: Grade I (71.4%), Grade II (80%) and Grade III (83.3%) ***(Table 7).***

Table (6): P53 expression in endometrial hyperplasia versus carcinoma

Lesions	P53			
	Positive		Negative	
	No.	%	No.	%
Hyperplasia	7	31.8	15	68.2
Endometrial carcinoma	14	77.8	4	22.2
Total	21	52.5	19	47.5
x2	8.386			
p-value	**0.004**			

Table (7): Relation between type P53 expression and types of lesion

Lesions	P53			
	Positive		Negative	
	No.	%	No.	%
Simple Hyperplasia	1	10.0	9	90.0
Complex hyperplasia	2	33.3	4	66.7
Atypical hyperplasia	4	66.7	2	33.3
Endometrial carcinoma				
Grade I	5	71.4	2	28.6
Grade II	4	80.0	1	20.0
Grade III	5	83.3	1	16.7
x2	13.419			
p-value	**0.020**			

Immunohistochemical expression of Ki-67:

Ki-67 staining was nuclear.10 out of 40 (25%) of cases were positive for Ki-67. Positive rate increased insignificantly in endometrial carcinoma (38.9%) than endometrial hyperplasia (13.6%) (*P*-value 0.067) ***(Table 8).***

There was also statistically insignificant difference between types of lesion and Ki-67 expression of the patients groups, using Chi-square test with (P-value 0.508); the expression increased in atypical hyperplasia (16.7%) and endometrial carcinoma (38.9%) than simple hyperplasia (10%) ***(Fig. 4 A,B,C).*** As regarded the cases of endometrial carcinoma; the expression of Ki-67 increased in high grade: Grade I (28.6%), Grade II (40%) and Grade III (50%) ***(Table 9).***

Table (8): Ki-67 expression in endometrial hyperplasia versus carcinoma

Lesions	Ki-67			
	Positive		Negative	
	No.	%	No.	%
Hyperplasia	3	13.6	19	86.4
Endometrial carcinoma	7	38.9	11	61.1
Total	10	25.0	30	75.0
x2	3.367			
p-value	0.067			

Table (9): Relation between type Ki-67 expression and types of lesion

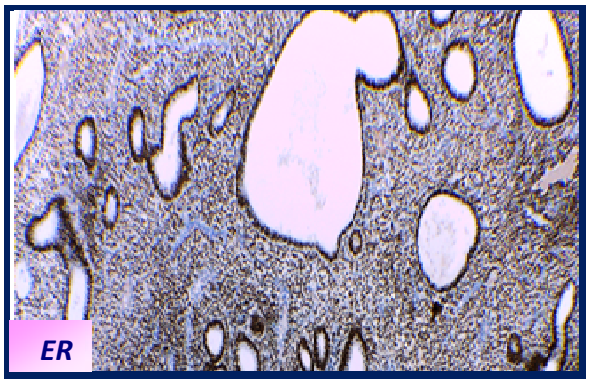
Lesions	Ki-67			
	Positive		Negative	
	No.	%	No.	%
Simple Hyperplasia	1	10.0	9	90.0
Complex hyperplasia	1	16.7	5	83.3
Atypical hyperplasia	1	16.7	5	83.3
Endometrial carcinoma				
Grade I	2	28.6	5	71.4
Grade II	2	40.0	3	60.0
Grade III	3	50.0	3	50.0
x2	4.292			
p-value	0.508			

4. Discussion

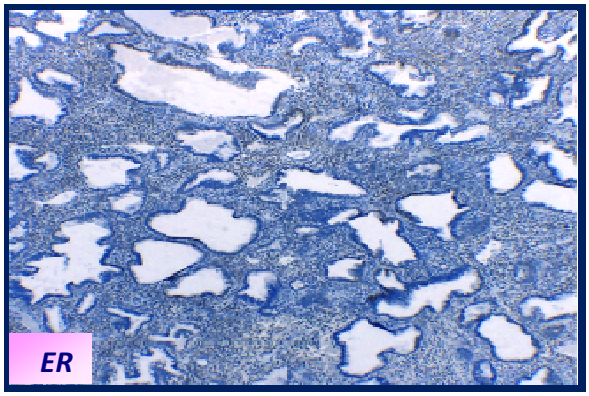
Endometrial carcinoma is one of the most common gynaecologic malignancies in industrialized and developing countries and is generally accepted to be an endocrine related neoplasm. In order to improve the treatment and follow up of these patients, various prognostic factors have been extensively studied ***(Aparna, 2011).***

Our immunohistochemical study over a period of three years included 40 cases with a histological diagnosis of 22 cases of endometrial hyperplasia (55%) and 18cases of endometrial carcinoma (45%). These findings are nearly in agreement with ***Aparna (2011),*** who reported that 64.3% % of his cases were endometrial hyperplasia and 35.7% were endometrial carcinomas.

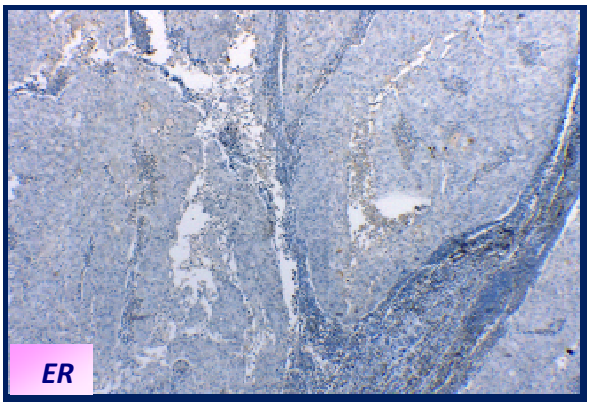
Our cases of endometrial hyperplasia were subdivided into endometrial hyperplasia without atypia (ten cases were simple and six cases were complex) (40%) and six cases of atypical endometrial hyperplasia (15%). These findings are in line with ***Aparna (2011)*** who reported that 57.1% % of his cases were endometrial hyperplasia without atypia and 7.2% were atypical endometrial hyperplasia.



1A

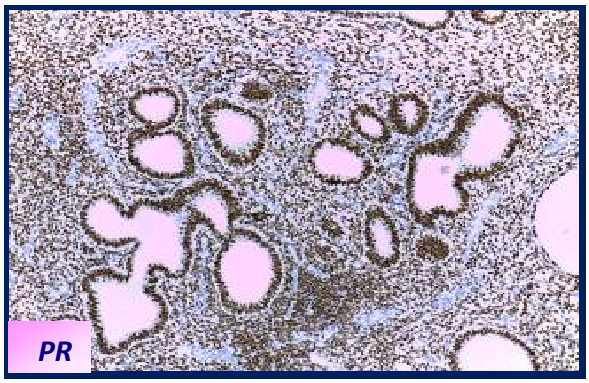


1B

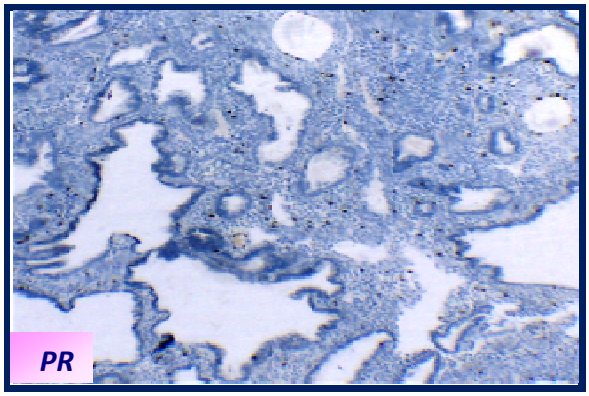


1C

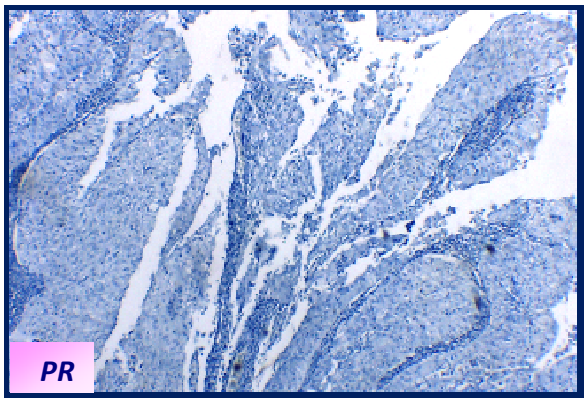
Figure 1: (A) Simple endometrial hyperplasia showing positive nuclear expression of ER. (B) Complex endometrial hyperplasia with atypia showing no expression of ER. (C) Endometrial carcinoma GII showing no expression of ER. (Original magnifications, 200)



2A



2B

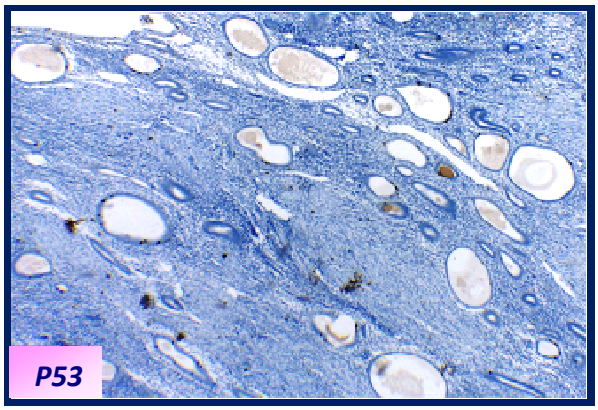


2C

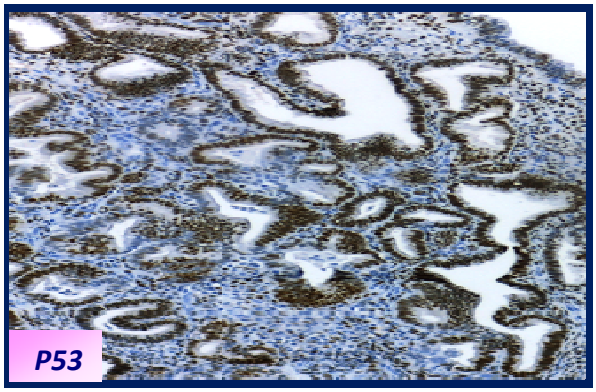
Figure 2: (A) Simple endometrial hyperplasia showing positive nuclear expression of PR. (B) Complex endometrial hyperplasia with atypia showing no expression of PR. (C) Endometrial carcinoma GII showing no expression of PR. (Original magnifications, 200)

The cases of endometrial carcinoma were subdivided into 7 cases (38.9%) well differentiated endometrial carcinoma (G1), five cases (27.8%) moderately differentiated (G2) and six cases (33.3%) poorly differentiated endometrial carcinoma (G3). These findings are nearly in agreement with ***Stoian et al. (2011)*** who reported that 50% of their endometrial carcinoma cases were G1, 31.8% were G2 and 18.2% were G3. Also these findings are in agreement with ***Aparna (2011)*** who reported that 26.7% of their endometrial carcinoma cases were G1, 26.7% were G2 and 46.6% were G3. Also these findings are in line with ***Markova et al. (2010)*** who reported that 78% of their endometrial carcinoma cases were G1 & G2 and 22% were G3.

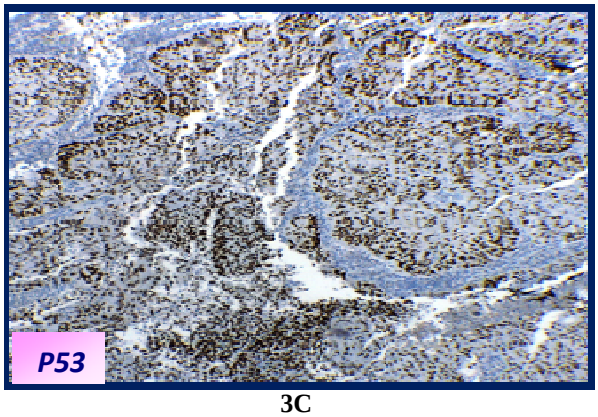
The current study has been undertaken to evaluate the relationships between Ki-67, P53 expression and estrogen (ER), progesterone receptors (PR) in endometrial hyperplasia versus endometrial carcinoma. We also evaluated the relationship between Ki-67, P53, ER, PR expression and tumor grade in endometrial carcinoma.



3A

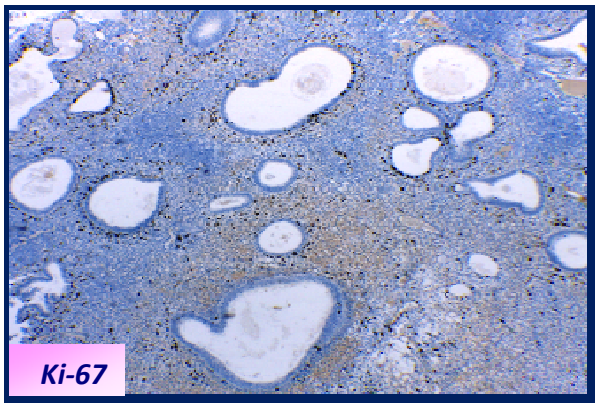


3B

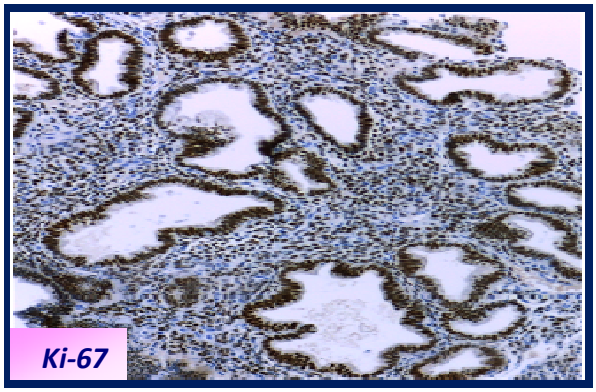


3C

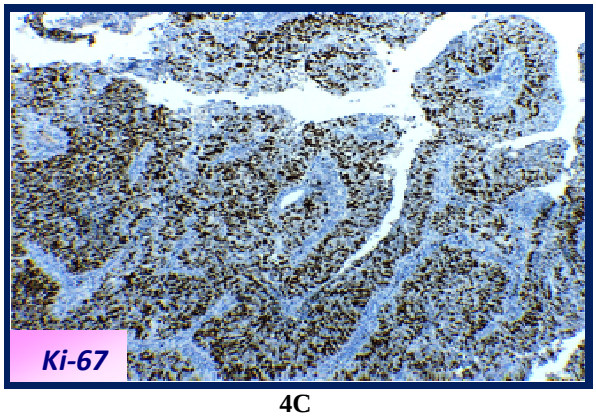
Figure 3: (A) Simple endometrial hyperplasia showing no expression of P53. (B) Complex endometrial hyperplasia with atypia showing positive nuclear expression of P53. (C) Endometrial carcinoma GII showing positive nuclear expression of P53. (Original magnifications, 200)



4A



4B



4C

Figure 4: (A) Simple endometrial hyperplasia showing no expression of Ki-67. (B) Complex endometrial hyperplasia with atypia showing positive nuclear expression of Ki-67. (C) Endometrial carcinoma GII showing positive nuclear expression of Ki-67. (Original magnifications, 200)

Estrogen and progesterone receptors are present in both normal endometrial tissue and endometrial cancer. Based on the results of various authors, the presence and the amount of steroid receptors correlate with the clinical stage of the disease, the histological grade, and survival. The absence of steroid receptors is considered

a negative prognostic factor for aggressive growth and poor prognosis ***(Ferrandina et al., 2005).***

In our study, ER were positive in 40% and negative in 60% of the analyzed cases, while PR were positive in 50% and negative in 50% of the analyzed cases. These findings are lower than the frequency found by ***Aparna (2011)*** who found that ER & PR were positive in 71% &74% respectively of studied cases. The higher percentage of positivity might be related to increased numbers of his studied cases.

In the current study, P53 was positive in 52.5% and negative in 47.5% of the analyzed cases, while Ki-67 was positive in 25% and negative in 75% of the analyzed cases. Our results differ from that reported by ***Markova et al. (2010)*** who found that Ki-67 expression (45.1%) was higher than P53 expression (24.3%) among the analyzed cases. The discrepancy of immunostaining might be due to all cases in ***Markova et al. (2010)*** study were endometrial carcinomas.

Nunobikio et al. (2003) said that estrogen and progesterone receptors were present in endometrial carcinomas and were reported to be at lower levels compared with endometrial hyperplasia. We also found significantly high levels of ER and PR in endometrial hyperplasia (54.5% & 68.2% respectively) compared with endometrial carcinomas (22.2% &27.8% respectively). Also these findings are in agreement with ***Pieczynska etal. (2011)*** who reported that significantly high levels of ER and PR in endometrial hyperplasia (97.7% & 99.2% respectively). Also these findings are in line with ***Aparna (2011),*** who reported that significantly high levels of ER and PR in endometrial hyperplasia (100% & 100% respectively) compared with endometrial carcinomas (20% & 26.7% respectively).

A number of works confirm an association between elevated P53 expression and unfavourable prognostic factors in women with primaryendometrial cancer. Elevated p53 expression significantly correlated only with poor differentiation of endometrial tumors ***(Erdem et al., 2003).***

Mariani *et al.* (2003) described P53 as the only molecular marker able to predict distant metastases independent of other histopathological, molecular and cytokinetic parameters.

The Ki-67 antigen, a non-histone protein useful for the identification of proliferating cells that has no specific phase, is expressed in all active phases of cell cycle (Ki-67 is not expressed in G0 phase). An increase of Ki-67 expression shows an increased mitotic activity and cell proliferation. A number of studies have shown that Ki-67 is an independent prognostic indicator of survival ***(Markova et al., 2010).***

In our study there was an increased expression of the p53 tumorsuppressor gene in endometrial carcinomas compared with endometrial hyperplasia (77.8% & 31.8% respectively).These findings are consistent with ***Ilie et al. (2011).***

***Uchikawa et al. (2003) and Ilie et al. (2011)* stated that** the immunoexpression of Ki-67 in endometrial hyperplasia was lower compared with the immunoexpression of Ki-67 in endometrial carcinomas. These findings are consistent with the current study which detected higher expression of Ki-67 in endometrial carcinomas compared with endometrial hyperplasia (38.9% & 13.6%respectively).

The present study reported that ER and PR levels were high in simple and complex hyperplasia (60%, 70% & 66.7%, 83.3% respectively) and low in atypical hyperplasia (33.3% &50% respectively). These findings are in accordance with ***Nyholm et al. (1995)*** who reported that ER and PR are frequently more often positive in non-atypical endometrial hyperplasia compared with atypical hyperplasia. Our results are in agreement with ***Cai et al. (2008)*** who found that the expression of ER was markedly decreased in atypical hyperplasia and endometrial carcinoma as compared to simple hyperplasiaor complex hyperplasia.These findings are lower than the frequency found by ***Bozdogan et al. (2002), Aparna (2011) and Ilieet al. (2011)*** who found that all cases of endometrial hyperplasia were ER and PR positive.

Number of studies published since 1980 have shown an inverse correlation between steroid hormone receptors presence and grade of the tumor ***(Nyholm et al., 1992) & (Aparna, 2011). Gul et al. (2010)*** also analyzed steroid hormone receptor content by immunohistochemical methods in endometrial carcinomas and have demonstrated an inverse correlation between ER/PR status and tumor grade. These findings are in agreement with our study which showed ER expression in 28.6% of G1, 20% of G2 and 16.7% of G3. Our findings also revealed PR expression 42.9% of G1, 20% of G2 and 16.7% of G3. Our results are in agreement with ***Aparna (2011),*** who reported that decreased expression of ER and PR with increasing grade of endometrial carcinomas. Our findings are also consistent with ***Stoian et al. (2011)*** who reported that well-differentiated tumors had a higher number of receptors for estrogen and progesterone (39% & 49%,respectively) which was not the case in poorly differentiated tumors. ***Stoian et al. (2011)*** revealed ER expression in 39% of G1, 22% of G2 and 9% of G3 and PR expression in 49% of G1, 28% of G2 and 12% of G3.

Uchikawa et al. (2003)found the correlation of the hormone receptors content (estrogen and progesterone) withseveral histopathological features, and especially the tumor differentiation. The well-differentiated tumors aremore frequently positive for the estrogen and theprogesterone receptors than the poorly differentiated lesions.

Many studiesby ***Ferrandina et al. (2001), Sivridis et al. (2001) and Stoian et al. (2011)*** have found that the hormone dependence, and thus the response to the hormonaltherapy or the chemotherapy for the endometrialcarcinoma decreases in aggressive tumors, the survivalrate improves at every stage in the case of the patients with receptor-positive tumors compared with tumors that are receptor-negative.

Our study showed higher expression ofP53 in the endometrial with complex (33.3%) and atypical hyperplasia (66.7%) than that found in simple hyperplasia (10%). This findings are in agreement with ***Ilie et al. (2011)*** who found that positive immunoreactivity to p53 belonged to complex hyperplasia endometria (30%) and atypical hyperplasia endometria (60%) and was absent in simple hyperplasia.

The current study showed higher expression of P53 in grade 3 tumors (83.3%) compared withthe groups with G 1 and G2 (71.4% & 80%). These findings are in agreement with ***Markova et al. (2010)*** who found a significantly greater p53 positivity in grade 3 tumors compared with grades 1 and 2 (42.3% vs 20.3%,). These findings arealso in agreement with ***Alkushi et al. (2007)***who found that higher expression of P53 in Grade 3 (64%) compared with grades 1 and 2 (2.8% &38%).

In the present study, we have shown that theexpression of Ki-67 in the lesions of atypical hyperplasia (16.7%) was dramatically increased as compared to thelesions of simple hyperplasia (10%). These findings are in agreement with ***Roger et al. (2001), Pathirage et al. (2006)and Cai et al. (2008)*** who demonstrated the same results. On the contrary, ***Ilie et al. (2011)***analysed the activity of cell proliferation for various types of endometrial hyperplasia, they found that Ki-67 expression decreased with the hyperplasia advancement. They obtained the highest expression in simple hyperplasia (8%), followed by complex hyperplasia (5%) and atypical hyperplasia (3%).

In our study, Ki67 expression was correlated with the tumor grade, there was higher Ki-67 positivity in G3(50%) compared with the groups with grade 1 (28.6%) and G2 tumors (40%). These findings are in line with ***Markova et al. (2010)*** who found a correlation between Ki-67 positive tumors and grading, in the group with grade 3 tumors, a significantly higher Ki-67 positivity was seen compared with the groups with grade 1 and 2 tumors (65.3% vs 40.6%). Our results arepartly in accordance with works by ***Salvesen et al. (1998),*** who demonstrated a correlation among elevated expression of Ki-67 with grading of endometrial carcinoma. On the contrary, ***Pansare et al. (2007)*** did not show any correlation among Ki-67 expression and grade of tumor.

References

1. Alkushi A, Clarke BA, Akbari M, Makretsov N and Miller D (2007): ‘’Identification of prognostically relevant and reproducible subsets of endometrial adenocarcinoma based on clustering analysis of immunostaining data" Modern Pathology., 20: 1156–1165.
2. Aparna S (2011): ‘’An immunohistochemical study of steroid hormone receptors (estrogen and progesterone) in endometrial hyperplasia and neoplasia (malignancy)’’ rguhs.
3. Bozdogan O, Atasoy Pand Braila M (2002): ‘’Apoptosis-related proteins and steroid hormone receptors in normal, hyperplastic and neoplastic endometrium” Int J Gynecol Pathol., 21: 375-382.
4. Cai H, Zhou Y, Wang B and Gong L (2008): "Expression of estrogen receptors alpha and beta protein inendometrial carcinoma" Cancer Therapy., 6: 907-912.
5. Erdem O, Erdem M andDursum A (2003): ”Angiogenesis, p53 and bcl-2 expression as prognostic indicators in endometrial cancer: comparison with traditional clinicopathological variables” Int J Gynecol Pathol., 22:254Y260.
6. Ferrandina G, Ranelletti FO, Larocca LM, Maggiano N, Fruscella E, Legge F, Santeusanio G, Bombonati A and Mancuso S (2001): “ Tamoxifen modulates theexpression of Ki67, apoptosis, and microvessel density incervical cancer” Clin Cancer Res., 7(9):2656–2661.
7. Ferrandina G, Ranelletti FO andGallotta V (2005): ”Expression of cyclooxygenase-2 (COX-2), receptors for estrogen (ER) and progesterone (PR), p53, Ki-67, and neu protein in endometrial cancer” Gynecol Oncol., 98:383-389.
8. Gul AE, Keser SH, Barisik NO, Cakir C and Sensu S (2010): “The relationship of cerb B2 expression with estrogen receptors and progesterone receptors and prognostic parameters in endometrial carcinoma” Diagnostic pathology, Feb 5-13.
9. Ilie D, Georgescu CV, Simionescu C, Braila AD and Braila M (2011): "Immunohistochemical Aspects of Endometrium Hyperplasias in Perimenopause" Current Health Sciences Journal., 37(2).
10. Ismail SM (2006): "Histopathological challenges in the diagnosis of endometrial hyperplasia and carcinoma" Current Diagnostic Pathology., 12:312–324.
11. Mariani A, Sebo TJ and Webb MJ (2003):”Molecular and histopathologic predictors of distant failure in endometrial cancer” Cancer Detect Prev., 27:434Y441.

12. Markova I, Duskova M, Lubusky M, Kudela M, Zapletalova J and Prochazka M(2010):“Selected Immunohistochemical Prognostic Factors in Endometrial Cancer” Int J Gynecol Cancer., 20: 576-582.
13. Mikuta JJ (1993): ‘’International Federation of Gynecologyand Obstetrics staging of endometrial cancer 1988’’ Cancer, 71: 1460-63.
14. Nunobiki O, Taniguchi E, Ishii A, Tang W, Mori I and Kakudo K (2003): ‘’Significance of hormone receptors status and tumor vessels in normal, hyperplastic and neoplastic endometrium” pathology Int. 53(12): 846-52.
15. Nyholm HC, Christensen IJ and Nielsen AL (1995): “Progesterone receptorlevels independently predict survival in endometrialadeno carcinoma” GynecolOncol., 59:347-351.
16. Nyholm HC, Nielsen AL and Lyndrup J(1992): “Biochemical and immunohistochemical estrogen and progesterone receptors in adenomatous hyperplasia and endometrial carcinoma: correlation with stage and other clinicopathologic features” Am J Obstet Gynecol., 167: 1334-1342.
17. Pansare V, Munkarah AR andSchimp V(2007): ‘’Increased expression of hypoxia-inducible factor 1alpha in type I and type II endometrial carcinomas” Mod Pathol., 20:35Y43.
18. Pathirage N, Di Nezza LA, Salmonsen LA, Simpson ER and Clyne CD (2006): “Expression of aromatase, estrogenreceptors, and their coactivators in patients with endometrial cancer” Fertility Sterility,86:469-472.
19. Pieczynska B, Wojtylak S, ŻawrocKIki A and Biernat W (2011): “Analysis of PTEN, esrtogen Receptor α and progesterone receptor in endometrial hyperplasia using tissue microarray” Pol J Pathol., 3: 133-138.
20. Roger P, Sahla ME, Mäkelä S, Gustafsson JA, Baldet P and Rochefort H (2001): “Decreased expression of estrogen receptor! protein in proliferative preinvasive mammary tumors” Cancer Res.
21. Salvesen HB, Iversen OE and Akslen LA (1998): “Identification ofhigh-risk patients by assessment of nuclear Ki-67 expressionin a prospective study of endometrial carcinoma” Clin Cancer Res., 4:2779Y2785.
22. Salvesen HB, Iversen OE and Akslen LA (1999): "Prognostic Significance of Angiogenesis and Ki-67, p53, and p21 Expression: A Population-Based Endometrial Carcinoma Study "JCO.,17 (5)1382.
23. Silverberg SG, Kurman RJ, Nogales F, Mutter GL, Kubik-Huch RA and Tavassoli FA (2003): ‘’ World Health Organization Classification of Tumors. Pathology and genetics of tumors of the breast and female genital organs: Epithelial tumours and related lesions’’ Lyon: IARC Press.
24. Sivridis E, Giatromanolaki A, Koukourakis M and AnastasiadisP(2001):‘’Endometrial carcinoma: association of steroid hormonereceptor expression with low angiogenesis and bcl-2expression” Virchows Arch., 438(5):470–477.
25. Stoian SC, Simionescu C, Stepan A and Nurciu M (2011): ‘’Endometrial carcinomas: correlation between ER, PR, Ki67 status and histopathological prognostic parameters’’ Rom J Morphol Embryol., 52(2):631–636.
26. Taylor RJ, Jackson TC, Reid JG and Duffz SRG (2003): ‘’The differential expression of oestrogen receptors, progesterone receptors, Bcl2 and Ki-67 in endometrial polips’’ BJOG., vol.110:794-798.
27. Uchikawa J, Shiozawa T and Fruscella E(2003): “Expression of steroid receptor coactivators and corepressors in human endometrial hyperplasia and carcinoma with relevance to steroid receptors and ki-67 expression” Cancer, 98 (10): 2207-2213.
28. Zaino RJ, Kurman RJ, Diana KL and Morrow CP (1995): ‘’The utility of the revised International Federation of Gynecology and Obstetrics histologic grading of endometrial adenocarcinoma using a defined nuclear grading system’’ Cancer., 75: 81-86.

3/8/2015