

Utility of PTEN, MLH1, ARID1A And B-Catenin, Biomarkers In Endometrial Hyperplasia and Endometrioid Endometrial Carcinoma

Endometriyal Hiperplazi ve Endometrioid Endometrium Karsinomunun Tanısında
PTEN, MLH1, ARID1A ve B-Katenin, Biyolojik Belirteçlerin Yararları

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ABSTRACT

Objective: Endometrial carcinomas are divided into two groups roughly: Endometrioid (Type I) and Non-endometrioid (Type II). The majority of the endometrial carcinomas are of endometrioid type and, this type of carcinoma is associated with hyperplasia which is formed due to unopposed estrogen. In this study, our purpose is to reveal the expression status of the PTEN, MLH1, ARID1A and β -catenin, biomarkers in all types of endometrial hyperplasia (EH) and endometrioid endometrial carcinoma (EC).

Material and Methods: We conducted a retrospective study with 202 cases including 82 EC cases, 71 simple EH cases without atypia, 16 complex EH cases without atypia, 9 simple atypical EH cases, 24 complex atypical EH cases which were diagnosed at Istanbul Civilization University Göztepe Training and Research Hospital. 10 normal endometrial tissues were used for the purpose of control.

Results: In our study, loss of PTEN ratio was 61%, loss of ARID1A ratio was 52,4%, loss of MLH1 ratio was 40,2% in patients with EC. These ratios were 87,5%, 41,7%, 20,8%, respectively in patients with atypical complex EH and 12,7%; 4,2%; 1,4% in patients with simple EH without atypia.

Conclusion: In the light of these findings it has been demonstrated that PTEN, ARID1A and MLH1 have a role in the transition from simple EH to atypical complex EH, and also MLH1 has a role in the transition from atypical complex EH to EC.

Keywords: endometrial hyperplasia, endometrioid endometrial carcinoma, PTEN, MLH1, ARID1A, β -catenin

ÖZET

Amaç: Endometriyal karsinomlar kabaca iki gruba ayrılır: Endometrioid (Tip I) ve Endometrioid olmayan (Tip II). Endometriyum karsinomlarının büyük çoğunluğu endometrioid tiptedir ve bu tip karsinoma, hiperplazi ile ilişkili olup, bu hiperplazi, karşılanmamış östrojen nedeniyle oluşur. Bu çalışmada amacımız, PTEN, MLH1, ARID1A ve β - kateninin; endometrial hiperplazi (EH) ve endometrioid endometrium karsinom (EC) tanısındaki yararını saptamaya amaçladık.

Materyal ve Metod: İstanbul Medeniyet Üniversitesi Göztepe Eğitim ve Araştırma Hastanesi' n de tespit edilen 82 EC olgu, atipik olmayan 71 basit EH olgusu, atipik olmayan 16 kompleks

EH olgusu, 9 basit atipik EH olgusu, 24 kompleks atipik EH olgusu içeren 202 olgu ile retrospektif bir çalışma yaptık. Kontrol amacıyla 10 normal endometriyal doku kullandık.

Bulgular: Çalışmamızda, EC'li hastalarda PTEN oranı kaybı% 61, ARID1A kaybı% 52,4, MLH1 kaybı% 40,2 idi. Atipik kompleks EH'li hastalarda bu oranlar sırasıyla% 87,5,% 41,7 ve% 20,8; Atipik olmayan basit EH'li hastalarda bu oran sırasıyla % 12,7; % 4,2; % 1,4 olarak saptandı.

Sonuç: Bu bulgular ışığında PTEN, ARID1A ve MLH1 'in basit EH' den atipik kompleks EH' ye geçişte rolü olduğu ve MLH1 'in atipik kompleks EH' den EC' ye geçişte rolü olduğu gösterilmiştir.

Anahtar Kelimeler: endometriyal hiperplazi, endometrioid endometrial karsinom, PTEN, MLH1, ARID1A, β -katenin

INTRODUCTION

Endometrial carcinoma is the female genital malign tumor which is most often observed in the developed countries (1, 2). The majority of endometrium carcinomas are Type I endometrioid carcinomas and it is associated with endometrial hyperplasia (EH) resulted in unfulfilled estrogen excess (3). In last 30 years various genes have been examined. In recent years in the studies it has clearly been understood that p53, mismatch restore system genes (MSH2, MLH1, MSH6, PMS2), KRAS proto-oncogen mutation and β -catenin genes (CTNBN1) have taken a role in tumorigenesis and progression as well, along with changes that are most often observed in the endometrioid carcinoma improvement have been demonstrated in PTEN (phosphatase and tensin homolog) dominant tumor gen (4). ECs are formed of premalign cells having monoclonal changes by means of monoclonal biogenesis and frequently indicate microsatellite instability (MSI) with PTEN, β -katenin and K-ras mutation (5). In EC the gen which has most often undergone a change is the PTEN tumor suppressor gen and mutation rate changes between 30% and 83% approximately (6, 7). In the position of 10q23 it is localised and codes dual specific phosphatase. It is frequently observed in endometrioid endometrial carcinoma and associated with good prognosis. (6, 8). At the same time it has been reported in endometrial hyperplasia (9). In atypical and non-atypical hyperplasia mutation has been determined in proportion of 20-48 % (10). Although the specific results of PTEN has not fully been reported yet the frequency of PTEN mutation

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Makale Geliş Tarihi: 16.12.2017

Makale Kabul Tarihi: 13.02.2018

DOI: <http://dx.doi.org/10.16948/zktpb.367326>

has been found similar in each three grade group oftumor. Some of studies support that PTEN has played a role in the early phase of endometrial tumorigenesis (11, 12). However, epidemiological studies have shown that PTEN mutation in complex atypical EH has not provided for carcinoma progression (13). In one of the studies the results have also shown that there has been a significant conformity between microsatellite instability of the tumor (MSI) and PTEN mutations. PTEN mutations have been determined in 60-86% of the positive MSI cases, in 24-35% of negative MSI cases. This case makes think that PTEN would be one of genes which is taken aim at repair indeficiency of DNA (7, 8, 14). Microsatellite DNA sequences are short DNA runs presenting random dissociation along genom. During replication, damage possibility increases because of frequent recurrence in these areas. Mismatch repair genes are known as MLH1, MLH3, PMS1, PMS2, MSH2, MSH3, MSH4, MSH5, MSH6. When mutation takes place in these genes this damage can not be repaired. In endometrial carcinoma such as colon carcinoma high microsatellite instability level (MSI-high) leads to germline mutation or in DNA mismatch repair genes such as MLH1, MSH2 or MSH6 (HNPCC) or to methylation of MLH1 (15).

Microsatellite instability has been observed in proportion of 20-45% in ECs. (8). Abnormal methylation of MLH1 is the most common case generating a defect in the mechanism of DNA mismatch repair leading to microsatellite instability (16). In the same way, in sporadic endometrial carcinoma the most often reason of microsatellite instability is the hypermethylation of MLH1 promotor area, too (8).

CTNNB1 gen codes β -catenin which is an important member of Wnt signal pathway. β -catenin is a protein that takes charge in cell-cell adhesion, embryonic growth, cell differentiation and malign tumor transformation (11). It is known that β -catenin gen has an effect on adenomatous polyposis coli localised in 3p21 and on E-cadherin. β -catenin as an important component of E-cadherin-catenin unit plays a role in protection of cell differentiation and normal tissue. In the studies performed although β -catenin has been determined nuclear in the proliferative phase of menstrual cycle, has been observed basically as cytoplasmic and membranous in the secretory phase (17). This signifies that estrogen and progesterone provide stability of endometrial proliferation and differentiation by controlling activity of Wnt β -catenin (18).

In the first studies the mutation frequency of CTNNB1 (β -catenin) has been detected to 45% in all the endometrioid endometrial carcinomas. These are often together with nuclear accumulation of β -catenin in the tumors with gen mutation and these symptoms are frequently observed in ECs (19). Along with that dysregulation of CTNNB1/ β -catenin has been observed in early pathogenesis of EC it has also been determined in atypical hyperplasia, the squamous component of atypical complex endometrial hyperplasia and endometrial intraepithelial neoplasia (19). Immunohistochemical negativity of

β -catenin is associated with invasive growth pattern and bad prognosis (20). In a study it has been reported that inactivation of stromal β -catenin has resulted in degenerate progesterone signal transmission and complete stromal cell desidualisation loss. (21). ARID1A is localised in area of chromosomal 1p36 and this area is frequently deleted in various neoplastic diseases (22). ARID1A codes BAF250a interacting with a few proteins containing BRG, BRM and ATPase that take part in the formation of "switch/sucrose non fermentable (SWI/SNF) chromatin remodelling complex" and almost available in all eucaryotic cells (23). BAF250a is one of the alt units, which belongs to SWI/SNF complex and have ATPase activity, and coded by ARID1A and somatic mutation of this gen has been reported in numerous neoplasias (24). In the studies made in recent years almost in the half of ovarian clear cell carcinoma cases, inactivation has been identified as a result of ARID1A somatic mutation (25). In addition to that the inclination of ARID1A mutation has been found increased in EC. The changes in BAF250a (ARID1A) are often observed in high-grade endometrial carcinoma. In one of studies by Wiegand et al. in 39% of grade 3 endometrial carcinomas and 18% of serous carcinomas, 26% of carcinomas with clear cell, loss of expression have been observed (25).

The purpose of our study is to assess the expression patterns and diagnostic utility of MLH1, ARID1A, PTEN, β -catenin for transition from EH to EC. Furthermore, in our study we have studied whether these markers have interaction and consistency between each other, or not; we have also studied menopausal status and tumor FIGO grade, expression differences as well.

MATERIAL AND METHOD

CASE SELECTION: Between 2008-2013 in Göztepe Training and Research Hospital of Medeniyet University in İstanbul 82 cases with EC diagnosis, 71 cases with atypical simple EH diagnosis, 16 cases with non-atypical complex EH diagnosis, 9 cases with atypical simple EH diagnosis, 24 cases with atypical complex EH diagnosis in for total 202 cases were included in our study. 5 cases in the secretory phase and 5 cases in the proliferative phase endometrium cases were included for the purpose of control. Suitably designated for the operation abundant materials including blocks and glass allocated for representation of the diagnosis, blocks were prepared by the manual microdissection method, the cases divided into 5 groups of atypical simple EH, non-atypical simple EH, atypical complexed EH, non-atypical complexed EH and EC. Ages of patients, their menopausal situations, nuclear grades (according to grading 2009 system) were determined. In 2014 new WHO classification of endometrial hyperplasias is published but we have decided not to change the subgroups for enlightening the transition phase of hyperplasia to tumor. This study got approval by the clinical investigation ethical committee of Göztepe Training and Research Hospital of Medeniyet University in İstanbul with the decision numbered 2013/13 on 08.07.2013.

IMMUNOHISTOCHEMICAL STAINING TECHNIQUE:

After the suitable areas in H&E sections were examined and selected and marked in the blocks, the blocks consisting of 14 cases per block were formed by means of the microdissection method from these tissues and the sections at 3 micron thickness were prepared. As an immunohistochemical staining system, the kit (Bond™ polymer refine detection kit with catalogue number DS9800, Leica Biosystems) with HRP multimer basic and without biotin, which contains hydrogen peroxide substrate and 3,3'-diaminobenzidine tetrahydrochloride (DAB), and full automatic immunohistochemical dyeing device (Bond-Max, Leica) were used. The tissue sections were taken to electrostatic loaded glass slides (CITOGLAS, ca.75x25mm/3x1 inch, positive charged, LOT: 130984) and dried at 60°C two hours at least.

All the immunohistochemical staining process including deparafinisation and antigen release phase were realised by means of the full automatic staining device Bond-Max Leica. Primer antibodies were automatically dropped and incubated at 37°C for 25 minutes. On the device all the phases such as dehydration of the sections completed with opposite staining hematoxylin and blueing solution, making pellicle with xylene and covering lamella were automatically (Leica, CV 5030) were made and terminated. As primer antibody PTEN (Clon : 6H2.1, product no: PM278AA, Biocare, ready for use, lot number: 032013), ARID1A (product no: HPA005456, Atlas Antibodies, dilution 1:200, lot no: D81856), MLH1 (product no: G168-728, Cell Marque Antibody, dilution 1:200, lot no: 1313507D), β -catenin (product no: 14, Cell Marque Antibody, dilution 1:100, lot no:1325508A), swere used.

MICROSCOPIC EVALUATION OF PTEN, MLH1, ARID1A, β -CATENIN EXPRESSIONS:

PTEN were scored immunohistochemically as positive, negative and heterogenous (as both positive areas and negative areas) in tumoral, hyperplastic and normal endometrial glandular epithelium. It was accepted as stained when diffuse cytoplasmic and nuclear positivity were observed over 90% in proportion. Contrary situations are evaluated as loss of PTEN. The stromal cells indicating intensive positive expression and blood vessels were accepted as positive internal control (26). While MLH1 was being evaluated adjoin normal endometrium and lymphocytes were used as normal control. It was accepted as stained when nuclear expression was observed over 10% and as not stained 10% or less than 10% in proportion separately from intensity (27). ARID1A expression were evaluated as two different groups of dyeing intensity and the proportion of stained epithelial cells. During evaluation nuclear staining was accepted positively. The staining intensity were scored in four different groups as 0 for no staining, 1 for weakstaining, 2 for moderatestaining, 3 for strongstaining. As for staining rate, it was given points as scores 0 for 0.5% positive epithelial cell, 1 for 6-25% positive epithelial cells, 2 for 26-50% positive epithelial cells, 3 for 51-75% positive epithelial cells and 4 for more than 75% positive epithelial

cells. Thereafter, the result scores ranging from 0 to 12 were calculated by multiplying these scores. The data obtained from the calculation was evaluated as negative (no staining) for score 1 and less than 1 and positive for (staining) more than 1. Lymph node was used as positive control but endothelial used as positive internal control (25). β -catenin expression were evaluated separately as cytoplasmic and nuclear. Membranous and cytoplasmic staining were classified semiquantatively according to staining intensity. For cytoplasmic staining groups are classified as no staining, weak, moderate, strong and membranous staining groups classified in the same way as no staining, weak, moderate, strong. During statistical evaluation cytoplasmic the group indicating weak staining was included into the "no staining" group. When nuclear staining was observed and no any staining was observed it was accepted as no staining (28). As control tissue desmoid tumor obtained from the archive is evaluated.

Statistical Evaluation: During the evaluating of the results obtained from the study SPSS (Statistical Package for Social Sciences) for Windows 15.0 program was used for statistical analyses. While the study data were being evaluated Chi-square test, Continuity Correction (Yates) and Fisher's exact test were used for the comparison of qualitative data as well as definitive statistical methods (average, standard deviation). For the analysis of the relation between the markers Spearman's correlation index and Mc Nemar test were used. Significance was evaluated at the level of significance $p < 0.05$ (Mc Nemar significance level > 0.05).

RESULTS

Our study was conducted with 212 cases ages ranging from 25 to 62 years old. The rate of patients with menopause was found significantly higher than the other groups in EC cases ($p < 0.01$). In the groups of simple atypical EH and complex atypical EH the loss of PTEN expression was significantly higher in accordance with the other groups (Table 1) ($p < 0,01$). The loss of MLH1 expression was significantly higher than the other diagnosis groups in EC (%40,2). It is followed by complex atypical EH with the rate of %20,8 (Table 1) ($p < 0,01$). In EC and the group of complex atypical EH the loss of ARID1A expression is higher than the other hyperplasia and control groups and the expression is significantly high in the groups of complex non-atypical EH, simple non-atypical EH, simple atypical EH, and control group (Table 1) ($p < 0,01$).

β -catenin expression was examined in three different groups such as cytoplasmic, nuclear and membranous staining. In respect of cytoplasmic β -catenin expression in the groups of complex atypical EH and normal endometrium the rates of no staining and weakstaining cases are significantly higher than the other groups. In the groups of complex non-atypical EH (56,3%) and simple atypical EH weak staining rate was significantly found higher than the other ($p < 0,01$) (Table 2). As for the evaluation of membranous β -catenin expressions in

Table 1: Evaluation of staining characteristics of immunohistochemical markers according to diagnostic groups.

Marker		Endometrioid Carcinoma	Complex Atypical EH	Complex Non-Atypical EH	Simple Atypical EH	Simple Non-Atypical EH	Normal	p
		n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	
PTEN	No	50 (%61)	21 (%87,5)	3 (%18,8)	5 (%55,6)	9 (%12,7)	0 (%)	0,001**
	Yes	32 (%39)	3 (%12,5)	13 (%81,3)	4 (%44,4)	62 (%87,3)	10 (%100)	
MLH1	No	33 (%40,2)	5 (%20,8)	1 (%6,3)	0 (%)	1 (%1,4)	0 (%)	0,001**
	Yes	49 (%59,8)	19 (%79,2)	15 (%93,8)	9 (%100)	70 (%98,6)	10 (%100)	
ARID 1A	No	43 (%52,4)	10 (%41,7)	2 (%12,5)	0 (%)	3 (%4,2)	0 (%)	0,001**
	Yes	39 (%47,6)	14 (%53,8)	14 (%87,5)	9 (%100)	68 (%95,8)	10 (%100)	
Bcl-2 ratio	No	38 (%46,3)	11 (%45,8)	0 (%)	1 (%11,1)	5 (%7)	10 (%100)	0,001**
	Yes	44 (%53,7)	13 (%54,2)	16 (%100)	8 (%88,9)	66 (%93)	0 (%)	
Bcl-2 density	No	32 (%39)	6 (%25)	0 (%)	4 (%44,4)	2 (%2,8)	10 (%100)	0,001**
	Light	28 (%34,1)	14 (%58,3)	3 (%18,8)	1 (%11,1)	12 (%16,9)	0 (%)	
	Middle	17 (%20,7)	4 (%16,7)	9 (%56,3)	3 (%33,3)	45 (%63,4)	0 (%)	
	Severe	5 (%6,1)	0 (%)	4 (%25)	1 (%11,1)	12 (%16,9)	0 (%)	
Bax ratio	No	10 (%12,2)	2 (%8,3)	3 (%18,8)	0 (%)	2 (%2,8)	0 (%)	0,122
	Yes	72 (%87,8)	22 (%91,7)	13 (%81,3)	9 (%100)	69 (%97,2)	10 (%100)	
Bax density	No	8 (%9,8)	0 (%)	3 (%18,8)	0 (%)	2 (%2,8)	0 (%)	0,001**
	Light	21 (%25,6)	13 (%54,2)	4 (%25)	1 (%11,1)	14 (%19,7)	9 (%90)	
	Middle	21 (%25,6)	10 (%41,7)	5 (%31,3)	3 (%33,3)	26 (%36,6)	1 (%10)	
	Severe	32 (%39)	1 (%4,2)	4 (%25)	5 (%55,6)	29 (%40,8)	0 (%)	

Chi-square test was used. **p<0.01

Table 2: Evaluation of cytoplasmic, membranous and nuclear β-catenin expressions by diagnostic groups.

β-Katenin n (%)		Endometrioid Carcinoma	Complex Atypical EH	Complex Non-Atypical EH	Simple Atypical EH	Simple Non-Atypical EH	Normal endometrium	p
		n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	
cytoplasmic	No/ Light	8 (%9,7)	12 (%50)	1 (%6,3)	15 (%21,1)	3 (%33,3)	6 (%60)	0,001**
	Middle	37 (%45,1)	10 (%41,7)	9 (%56,3)	33 (%46,5)	1(%11,1)	2 (%20)	
	Severe	37 (%45,1)	2 (%8,3)	6 (%37,5)	23 (%32,4)	5 (%55,6)	2 (%20)	
nuclear	no	54 (%65,9)	20 (%83,3)	11 (%68,8)	60 (%84,5)	8 (%88,9)	8 (%80)	0,091
	yes	28 (%34,1)	4 (%16,7)	5 (%31,3)	11 (%15,5)	1 (%11,1)	2 (%20)	
membranous	No/ Light	2 (%2,4)	1 (%4,2)	0 (%)	6 (%8,5)	0 (%)	0 (%)	0,001**
	Middle	20 (%24,4)	7 (%29,2)	4 (%25)	41 (%57,7)	8 (%88,9)	2 (%20)	
	Severe	60 (%73,2)	16 (%66,7)	12 (%75)	24 (%33,8)	1 (%11,1)	8 (%80)	

Chi-square test was used. **p<0.01

Table 3: Examination of the correspondence between ARID1A and PTEN and MLH1 expressions.

ARID1A	PTEN		Total	MLH 1		Total
	No staining	Staining		No staining	Staining	
	n (%)	n (%)		n (%)	n (%)	
EC						
No staining	32 (%64)	11 (%34,4)	43 (%52,4)	19 (%57,6)	24(%49)	43 (%52,4)
Staining	18 (%36)	21 (%65,6)	39 (%47,6)	14 (%42,4)	25 (%51)	39 (%47,6)
Total	50 (%100)	32 (%100)	82 (%100)	33 (%100)	49 (%100)	82 (%100)
Complex Atypical EH						
No staining	9 (%42,9)	1 (%33,3)	10 (%41,7)	5 (%100)	5 (%26,3)	10 (%41,7)
Staining	12 (%57,1)	2 (%66,7)	14 (%58,3)	0 (%)	14 (%73,7)	14 (%58,3)
Total	21 (%100)	3 (%100)	24 (%100)	5 (%100)	19 (%100)	24 (%100)

Mc-Nemar was used.

Table 4: Examination of the correspondence between PTEN and MLH1 expressions.

	MLH1	PTEN		Total
		No staining	Staining	
		n (%)	n (%)	
EC	No staining	24 (%48)	9 (%28,1)	33 (%40,2)
	Staining	26 (%52)	23 (%71,9)	49 (%59,8)
	Total	50 (%100)	32 (%100)	82 (%100)
Complex Atypical EH	No staining	5 (%23,8)	0 (%)	5 (%20,8)
	Staining	16 (%76,2)	3 (%100)	19 (%79,2)
	Total	21 (%100)	3 (%100)	24 (%100)

the groups of EC, complex atypical EH and complex non-atypical EH the intense staining rates were significantly higher than the other groups ($p < 0,01$) (Table 2). Significant difference was not determined statistically among the diagnosis groups in respect of nuclear β -catenin expression ($p > 0,05$) (Table 2).

A consistency between PTEN and ARID1A expressions was determined in ECs (Mc Nemar: 0.265). 32 of 50 cases (64%) indicating PTEN expression loss were indicating ARID1A expression loss as well at the same time (Table 3). However, in the group of complex atypical EH an inconsistency between PTEN and ARID1A expression loss (Mc Nemar: 0.003). 9 (42.9%) of 21 cases indicating PTEN expression loss showed ARID1A expression (Table 3).

When consistency between MLH 1 and ARID1A expression loss was examined in EC consistency was not determined (Mc Nemar: 0.143). In (57.6%) 19 of 33 cases indicating MLH1 expression loss, ARD1A expression loss was determined (Table 3). However, in complex atypical EH a consistency was determined in respect of expression loss (Mc Nemar: 0.063). All non-staining 5 cases in MLH1 was not stained in ARID1A (ratio), either (Table 3).

There is an inconsistency between MLH1 and PTEN expression loss in EC group. (Mc Nemar: 0.006). 24 (48%) of 50 cases indicating PTEN expression loss, also reveals MLH1 expression loss (Table 4). Between PTEN expression loss and MLH1 expression loss in complex atypical EH an inconsistency was determined as well (Mc Nemar: 0.001). 5 (23.8%) of 21 cases which was not stained with PTEN, was not stained with MLH1, either (Table 4).

DISCUSSION

PTEN gen as a suppressor tumor gen which takes charge in cell growth and regulation of apoptosis, is one of the genes which is most often mutated in EC and their precursor lesion (Endometrial intraepithelial neoplasia, EH) (10). In the literature there have been studies reporting that evaluation of PTEN expression could be helpful in respect of distinction among normal, plastic and neoplastic endometrium (29-31).

In our study even though PTEN expression loss is not observed in the control group The expression loss was significantly high in the EC and atypical EH groups ($p < 0,01$). This verity makes think that PTEN mutation could specially play key role in transition from hyperplasia to carcinoma, contrary to this thesis it plays a role in early phase of EC improvement but not effective in transition to carcinoma. Lee et al. have reached similar conclusions (32). However, a significant relation between histological grade of EC and PTEN expression has not been determined ($p > 0,05$). Although it is emphasized that PTEN mutation has more often determined in low grade histological tumors backwards (33, 34) in company with similar results which have been obtained recently (35) data of our study has suppor-

ted the result suggesting that PTEN change have not a significant effect in phases after EC generation. It has recently been reported that there have been inactivating mutations in ARID1A and the gen as a tumor suppressor gen has functioned in 57% of clear cell ovary carcinomas (36). In addition, it has been found that inclination of being mutated has increased in ARID1A in endometrioid endometrial carcinoma. Changes in BAF250a (ARID1A) are frequently observed in high grade endometrial carcinoma (25). Fedare et al. has demonstrated that ARID1A expression associated with phase of endometrial clear cell carcinoma (37). Wiegand et al. have reported that expression loss has been observed in 39% of grade 3 endometrial carcinomas, 18% of serous carcinomas, 26% of clear cell carcinomas (25). Guan et al. have determined the expression loss in 26% of ECs (38) the while, Maeda et al. have observed intense positivity in normal endometrial tissue (39).

In our study the significant difference has statistically been observed in respect of ARID1A expression loss among the groups examined ($p < 0,01$). The expression loss in the groups of EC (52.4%) and complex atypical EH (41.7%) has been higher according to the other groups, but significant expression loss has not been observed in the groups of complex non-atypical EH (12.5%), simple non-typical EH (4.2%), simple atypical EH (0%) and control (0%) (Table 7). The significant increase of ARID1A loss in complex atypical EH and EC groups makes think that ARID1A could play a role in transition from hyperplasia to EC and early phases of EC tumorigenesis. The way same as that in our study Rahman et. al have not determined any relation between ARID1A expression and tumor grade (40).

β -catenin is the second gen area mutation of which is often observed in ECs. It is also possible to determine nuclear positiveness in type I carcinomas (31-47%) in comparison with Type II immunohistochemically (41). According to literature β -catenin has immunohistochemically indicated positive cytoplasmic and membranous staining in endometrium in proliferative period. Cytoplasmic and membranous positive staining has been observed in ECs subject to tumor differentiation. Positiveness decreases as long as tumor differentiation decreases (8, 42). In the studies by Moreno-Bueno et al. which consist of 21 atypical EH, 95 EA, 33 nonendometrioid endometrial carcinomas cases, β -catenin expression in atypical EHs has been observed in higher incidence and a significant relation between β -catenin and histological type of lesion has been statistically determined (43). The relation between β -catenin expression and phase of tumor has not been demonstrated but almost significantly close relation with tumor grade has been determined ($p: 0,058$). In a study performed by Saegusa et al. normal proliferative endometrium, atypical hiperplasia and endometrium carcinoma cases have been examined in respect of nuclear β -catenin expression. In this study any staining has not been determined in normal proliferative endometrium, however, in simple or complex non-atypical hyperplasia in proportion to 10.8%, in atypical hiperplasia in proportion to 31,3% and in adenocarcinoma in proportion to 27.6% determined

(19). In our study β -catenin expression have been examined in three different groups as cytoplasmic, nuclear and membraneous. The significant differences between cytoplasmic and membraneous β -catenin expressions and the diagnosis groups have been determined statistically ($p < 0,01$) the while, a significant difference between nuclear expressions and the diagnosis groups has not been determined ($p > 0,05$). When cytoplasmic β -catenin expression has been examined in the groups of the complex atypical EH (%50) and normal (proliferative and secretory) endometrium (%60) the rates of non-stained cases and weakstained cases are significantly higher than the other groups (Table 8, Figure 3). In our study data in comparison with the studies we have reported, intense cytoplasmic expression in high incidence has indicated a consistency, however, high incidence of weak staining propotion in the complexed atypical EH group has not indicated consistent with the other studies. In the examination of the membraneous β -catenin expression in EC (%73,2), complex non-typical EH (%75) and complex atypical EH (%66,7) in comparison with simple non-typical EH (%33,8) and simple atypical EH (%11,1) the intense expression incidence is significantly higher ($p < 0,001$) (Table 8, Figure 3). According to these results between EC and EH groups statistical β -catenin significance has not been observed.

In our study a significant difference in all the marker expressions in respect of EC FIGO grades has not been determined statistically ($p > 0,05$). The molecular basic studies have revealed that endometrial carcinomas have ingenerated as a result of tumor supresor inactivation process and oncogenic activation which consists of many phases (44). In the literature it has been determined a significant consistency between microsatellite instability of the tumor (MSI) and PTEN mutations in the studies in which these pathways have examined as well (7, 8, 45). In the study of Nelson et. alit has been found that mismatch repair defect is related to ARID1A and PTEN expression loss (46). In the same way in the study of Xiao et al. PI3K/AKT (PTEN loss or PIK3CA activating mutation) pathway defects in tumors having ARID1sA expression loss in EC have been determined in high incidence (47). In Mouse models in a study in which molecular inter-course of ARID1A and PTEN pathways it has been reported that only the defect in ARID1A pathway has not generated histological changes , but poor differentiated ovarian tumor along with the defect in ARID1A and PTEN pathways has generated (48). Rahman et al. have examined p53, Her/neu, PTEN, MLH1, and ARID1A expressions and evaluated relation among these markers in their article they have not demonstrated any significant consistency among ARID1A, MLH1 and PTEN expressions statistically (40). However, in our study when EC and complex atypical EH groups have been examined in respect of MLH1 and PTEN expression loss any significant consistency among these markers has not determined statistically. (Mc Nemar $p: 0,006$ /Mc Nemar: 0,001 ; $p < 0,05$). When the consistency between PTEN and ARID1A expression loss has been examined the consistency in EC group has been determined, the while, this consistency in

the complexatypical EH has not been observed (Mc Nemar: 0.003). 32(64%) of 50 cases have not been stained with PTEN, not with ARID1A either. In the complex atypical EH group 9 (42.9%) of 21 cases which have not been stained with PTEN, have not been stained with ARID1A, either. In a study operated by Jones et al. on the gastrointestinal tumors in 2012, it has been reported that ARID1A mutation has frequently been observed in tumors that indicate microsatellite instability at high level (24). In addition it has been reported that ARID1A mutation in various cancer types including indel and frameshift mutation gynecological malignities often observed in mismatch repair defect, isthe most common type of mutation (49).

In our study a significant consistency between MLH1 and ARID1A expression loss has been determined statistically (Mc Nemar $p: 0,143$; $p > 0,05$). 19 (57.6%) of 33 cases indicating MLH1 expression loss have also been observed in ARID1A. Different from the others in the complex atypical EH group only between MLH1 and ARID1A expression loss a consistency has been determined (Mc Nemar: 0,063). All 5 cases that have not revealed MLH1 expression have not revealed ARID1A expression, either. Wang et al. have reported that significantly high level of ARID1A mutation has been observed in gastric cancer related to MSI as a result of clonal selection of manager gen because of degenerated DNA mismatch repair mechanism (50). The results of our study have made think that the same mechanism could also be valid for EC as well. In our study when the consistency among ARID1A, PTEN and MLH1 expressions have been examined a significant consistency between both ARID1A and PTEN, and ARID1A and MLH expression losses in EC group has been determined (respectively Mc Nemar $p: 0,265$; $p > 0,05$ and $p: 0,143$; $p > 0,05$). However, in examination in terms of MLH1 and PTEN expression losses any significant consistency has not been determined among these markers statistically (Mc Nemar $p: 0,006$; $p < 0,05$).

ARID1A expression loss in accordance with PTEN and MLH1 expression losses in EC has preoccupied that ARID1A gen could interrelate with PI3K/AKT/PTEN pathways and DNA mismatch repair genes (MLH1 etc) or tumor could make multifunctional progress including the pathways of these three tumorigenesis in EC. We suggest that larger laid studies in which molecular genetic method participates should be performed in order to reveal relation of these pathways more clearly.

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