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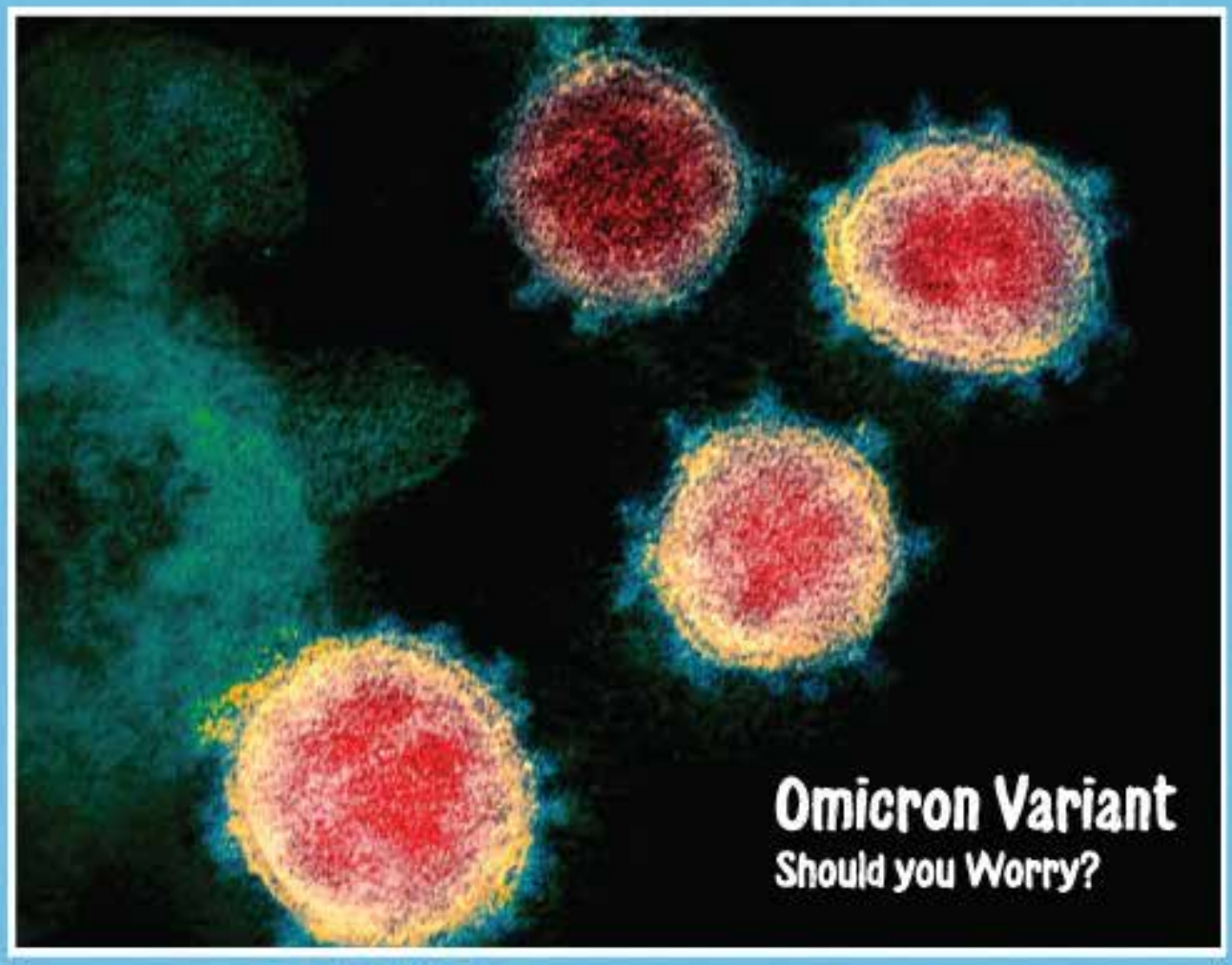

The Official Journal of the Gulf Federation For Cancer Control

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Original Article

Immunohistochemical Analysis Of Novel Biomarkers Cyclin D1, p53 And Ki67 In Endometrial Carcinoma: Clinicopathological Significance And Prognostic Value.

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Abstract:

Background: Endometrial carcinoma (EC) is the only gynecologic cancer with increasing incidence and mortality worldwide. This study aimed to determine association of cell proliferation marker CyclinD1, p53 and Ki67 with clinicopathological parameters and survival analysis in patients of EC.

Material and methods: One hundred twenty–four histological confirmed cases of EC treated at our institute were included in this study. The appropriate tissue blocks of cases which were retrieved from 2010 to 2015. The study period was from Jan 2018 to Jan 2020. Data pertaining to patient's clinical details, histopathological diagnosis, treatment and follow up was retrieved from Hospital information System. Immunohistochemical evaluation of Cyclin D1, p53 and Ki67 was done. Overall survival and Disease–free survival for each category were analyzed by the Kaplan–Meier method.

Results: Of the 124 cases of EC, 108(87.09%) cases were of type I and 16 (12.89%) cases of type II. Overall positive staining of cyclinD1, p53 and Ki67 were noted in 53.22%, 42.22% and 32.3% cases respectively. The clinicopathological parameters affecting disease–free survival were age (p=0.039) histological types (p=0.007), and FIGO stage (p<0.001). Elevated Ki67 index and p53 overexpression was associated with type II morphology (p= 0.001). Whereas Cyclin D1 expression was associated with type I morphology and poorly differentiated tumor.

Conclusion: Cyclin D1 positive staining, p53overexpression and an elevated Ki–67index all had an independent prognostic significance in endometrial cancer. This panel of biomarkers may help to differentiate tumor behavior, and necessity for more radical surgery and post– operative chemotherapy.

Key words: Endometrial carcinoma, cyclin D1, p53, Ki67, Survival analysis

Introduction:

Endometrial cancer (EC) is the most common gynecologic malignancy and fourth most common malignancy in women worldwide[1] .According to GLOBOCAN 2019, the incidence of endometrial carcinoma is 10–15 cases per 100,000 women, and most of these cases are sporadic[1,2]. Also, the incidence of EC is increasing in developing countries in the past few years, which has been attributed to an increase in life expectancy, obesity, diabetes mellitus, hormone replacement therapy etc.[3,4].

Although conventional FIGO (International Federation of Gynecology and Obstetrics) staging and hormone receptor status are used to guide surgical management and to predict outcome in patients with EC, same–stage patients often experience substantially different clinical courses[5]. Advanced disease also shows high failure rates in response to adjuvant therapies[1]. To resolve these discrepancies many investigators study pathogenesis of EC which involves stepwise acquisition of several genetic alterations in tumor suppressor genes and oncogene, such as p53, PTEN, cyclinD1etc.[6]. These novel biomarkers have been increasingly targeted to predict not only course of disease but also to plan treatment[5, 6].

In the present study we determined prognostic significance of Cyclin D1, tumor suppressor gene p–53, and proliferative index of Ki67 in endometrial carcinoma and their correlation with survival and relapse of tumor.

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Materials and Methods:

This was laboratory based cross–sectional study conducted in the Department of Pathology of tertiary care rural hospital of central India. The study was approved by institutional ethical committee prior to commencement. The appropriate blocks of 124 EC cases were retrieved from 2010 to 2015 in retrospective manner. The study period was from Jan 2018 to Jan 2020.These 124 tissue blocks were of cases diagnosed as EC on histopathology. They were further evaluated for immunohistochemical expression of cyclinD1, p53 and Ki67 without any extra cost to the patients.

Clinical details regarding patient's age, menopausal status, histological diagnosis, FIGO stage, treatment and follow–up were obtained from patient's case paper kept in Medical Record Department (MRD and also from Hospital Information system (HIS). Hematoxylin and eosin stained slides were reviewed for histological subtype, tumor grade, depth of myometrial invasion, cervical and adenexal involvement, lymphovascular and stromal invasion (LVSI) and lymph node metastasis (LN). The follow up period was for minimum 60 months. The patients who were lost to follow up were contacted directly from the patient's records in HIS.

The endometrial carcinoma had been classified into two main histological categories—endometrioid (Type I) and uterine papillary–serous (Type II) —and graded and staged asper current recommendations of the FIGO. Here we studied correlation of cyclin D1, p53 and Ki67positivity with histological type of EC, tumor grade, myometrial invasion, lymphovascular involvement, lymph node status, adnexal involvement, FIGO stage and clinical outcome in the form of survival analysis and tumor relapse.

Immunohistochemistry;

Immunohistochemical staining was performed on 4 µm sections of the 124 cases of EC by avidin biotin peroxidase complex method with proper controls. Antigen retrieval done with microwave oven in 0.01 M citrate buffer (PH 6.0) for 15–20 minutes.

Primary Antibody used for staining: Clone: EP1045Y, Species: Rabbit, Ig class: IgG, Protein Conc.: 50mg/ml, Catalog no.: AN471–5ME, Novacastra, USA for p53 and Antigen clone MIB–1 Dako for Ki67. Rabbit anti–human Cyclin D1 monoclonal antibody, Clone EP12, Master Diagnostica, Granada forcyclinD1.

Evaluation of immunohistochemical staining:

Cyclin D1 staining was evaluated in the epithelial component for intensity of nuclear staining and the extent (percentage of positive cells). The intensity of nuclear staining was graded as: No staining: (0) Weak: (1+) Moderate: (2+) Strong: (3+). When less than 10% of cells were positive for Cyclin D1 staining, a score of 0 was used, 10% to 30% cell positivity was scored as 1, 31% to 60% positivity scored as 2, and more than 60% positive cells was labeled as 3[7].

Alkushi et al method was used for evaluation of p–53[8]. A p53 score of 0, 1+, 2+, 3+ was given when <10% of tumor cells, 10–30%, 31–50% and>50% tumor cells were p–53 positive. A cut off level of > 50% positive nuclear staining was considered as overexpression of p53.

The hot spot method (the area with the most intense staining) was used for Ki–67 scoring, the percentage of Ki67 positively stained nuclei within three high–powered fields (× 40 magnifications) randomly selected across the tumor, ensuring at least 1000 nuclei were counted.

A cutoff value of ≥ 30% nuclear staining was considered to separate the negative (low proliferating index) from the positive (high proliferating index for ki67) [9] .

Statistical analysis:

SPSS (Statistical Package for Social Sciences) version 20.0 software was used for the statistical analysis. For the comparison of quantitative data (clinicopathological parameters), Chi–square test and cross tabulation tests were used. Univariate Cox proportional hazard analysis was used the prognostic significance of different parameters. Overall survival (OS) and Disease–free survival (DFS) of each group were analyzed by the Kaplan–Meier method and were compared between categories with log–rank test. P value< 0.05 was considered statistically significant.

Results

During the period from 2010 – 2015, 141cases were diagnosed on histopathology as endometrial carcinoma. Seventeen cases were either inoperable at the time of presentation or not willing for further treatment hence excluded from the study. Thus, present study included 124 patients of EC. Of the 124 cases, 108 cases were of type I and 16 cases of type II EC. The mean age at the time of presentation was 58.6 years with age ranged from 38 to 84years. Most of the (90, 72.6%) patients in study group were postmenopausal. The commonest risk factor associated with EC was obesity (34.67%) followed by diabetes mellitus (17.74%) and hormone replacement therapy (15.32%).

Fifty–eight cases (48.18%) were of grade I, 38 (16.66%) grade II, and 18 (35.18%) cases were of grade III EC. Of the 16 cases of Type II EC, eight cases of carcinosarcoma and four each case of papillary serous carcinoma and clear cell

carcinoma were noted. Tumor cell infiltration in outer half of myometrium was noted in 52 cases. Adnexal involvement was seen in 26(20.96%) cases, 87 cases were free from involvement and in 5 cases adnexal involvement was not assessed due to suboptimal surgery. Seventy–three (58.87%) cases were of FIGO stage I, and only 10 (8.06%) cases had stage IV disease at the time of presentation. The distribution of clinicopathological variables, such as age, risk factors, histological type, tumor grade and FIGO stage shown in Table 1.

The positive staining of cyclin D1 (3+) was observed in 66/124(53.22%) cases of EC of which 64 (59.25%) cases were of type I and 2(12.5%) cases of type II EC .Cyclin D1 positivity (3+) was significantly correlated with type I EC (p = 0.011), poorly differentiated tumor (p = 0.0012), LVI (p = 0.004), LN metastasis (p = 0.001) and advanced stage (0.0015) but not correlated with patient age (p = 0.148). Type I EC showing cyclin D1 positivity shown in Figure 1a and b.

Fifty–six (45.2%) cases showed over expression of p53 (>50% nuclear staining). p53 overexpression was significantly higher in type II EC 93.8% (15/16; p=0.0024), poorly differentiated tumor 77.1% (37/48; p=0.0002) and FIGO stage III, IV (p=0.002). Over expression of p53 was associated with poor survival in univariate analysis (p= 0.003). Overexpression of p53 in poorly differentiated EC shown in Figure 2a and weak expression of p53 in type I EC shown in Figure 2b.

High proliferating index (> 30% nuclearstaining) of Ki67 was noted in 32.2 % (40/124) of EC cases. A positive correlation was noted between histological type and elevated Ki67 index. Higher Ki67 proliferating index was significantly associated with type II EC (p=0.0018), higher grade (p=0.001), lymph node metastasis (p=0.0008), adnexal involvement (p=0.0001) and advanced FIGO stage (p=0.0002). IHC showing high proliferative index of Ki67 in poorly differentiated EC and uterine serous carcinoma shown in Figure 3a and Figure 3b respectively.

The five–year disease–free survival (DFS) was significantly higher (85.73 %, p= 0.001) with low proliferating index of Ki67 (<15%). Expression of Cyclin D1, p53 and Ki67 according to clinico–histopathological variables is outlined in table 1.

At the time of this report, 23 patients were dead of which 15 were dead due to complication of EC within 60 months of diagnosis. Eighteen patients had recurrence and salvage treatment. The 5–year DFS and 5–year OS (Overall survival) were 81.56% (95% confidence interval (CI), 78.41%–84.56%) and 83.13% (95% CI, %79.74%–86.52%), respectively.

In addition, longer DFS and OS was noted for patients younger than 60 years (P=0.002), type I histology (P=0.001), low grade (p=0.001)), superficial myometrial infiltration(p=0.039), no lymph–vascular invasion (p= 0.015) and early stage(p=0.0001) and Low levels (<15% positive cells) of Ki–67 protein expression (p=0.004). Univariate analysis of survival according to clinicopathological variables have been shown in Table 2.

Clinicopathological parameters	Patients no.
Age : < 60 years	86 (70.16%)
>60 years	38 (29.83%)
Histological Type	
Endometriod (Type I)	108 (87.09%)
Non– Endometrioid (Type II)	16 (12.09%)
Serous Papillary	04 (3.22%)
Clear cell	04 (3.22%)
MMMT	08 (2.41%)
Tumor Grade	
FIGO Grade I	52 (48.18%)
FIGO Grade II	24 (16.66%)
FIGO Grade III	18 (35.18%)
MI in Type I tumors (108)	
< inner half	66 (53.22%)
> inner half	42 (33.87%)
MV in Type II tumors (16)	06 (4.83%)
< inner half	10 (8.06%)
> inner half	
LVI in Type I Tumors (108)	
Present	22 (17.74%)
Absent	86 (69.35%)
LVI in Type II Tumors (16)	
Present	12 (9.6%)
Absent	4 (3.22%)
LN Type I tumors (108)	
Present	20 (16.12%)
Absent	88 (70.96%)
LN in Type II tumors (16)	
Present	14 (11.29%)
Absent	2 (1.61%)
FIGO Stage	
Stage I	73 (58.87%)
Stage II	17 (13.70%)
Stage III	24 (19.35%)
Stage IV	10 (8.06%)
Clinical outcome	
Alive without disease	96(77.41)
Alive with disease (Recurrence)	08(4.83)
Died of disease	15 (7.25)
Died of other cause	08 (4.83)

Table 1: Clinicopathological features of 124 patients of endometrial carcinoma

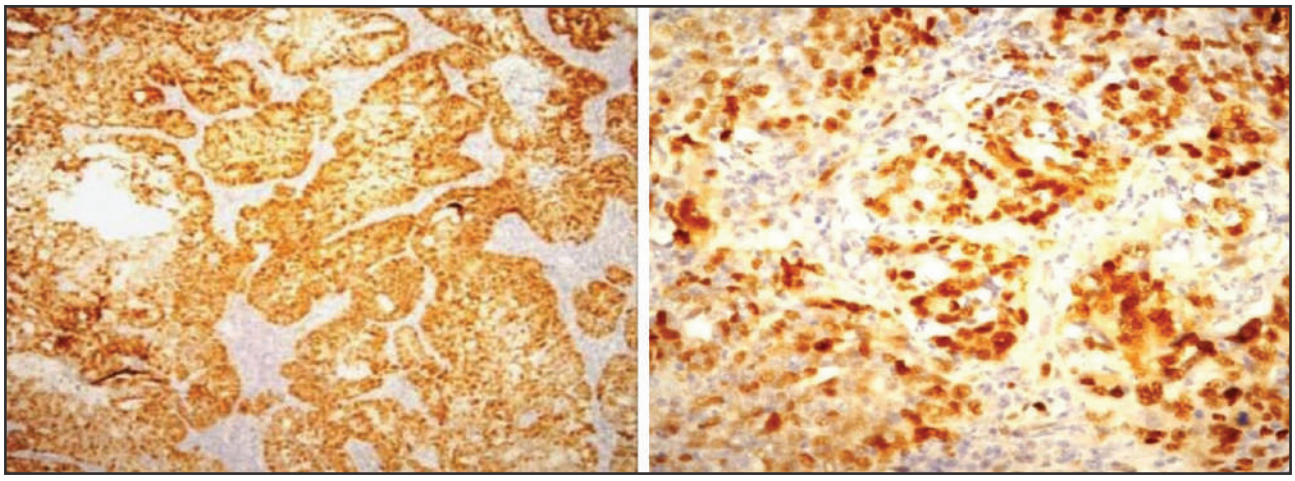

Figure 1: a) Photomicrograph Type I Endometrial carcinoma showing positive reaction with Cyclin D1. b) Photomicrograph of Type I Endometrial carcinoma showing positive reaction with Cyclin D1.

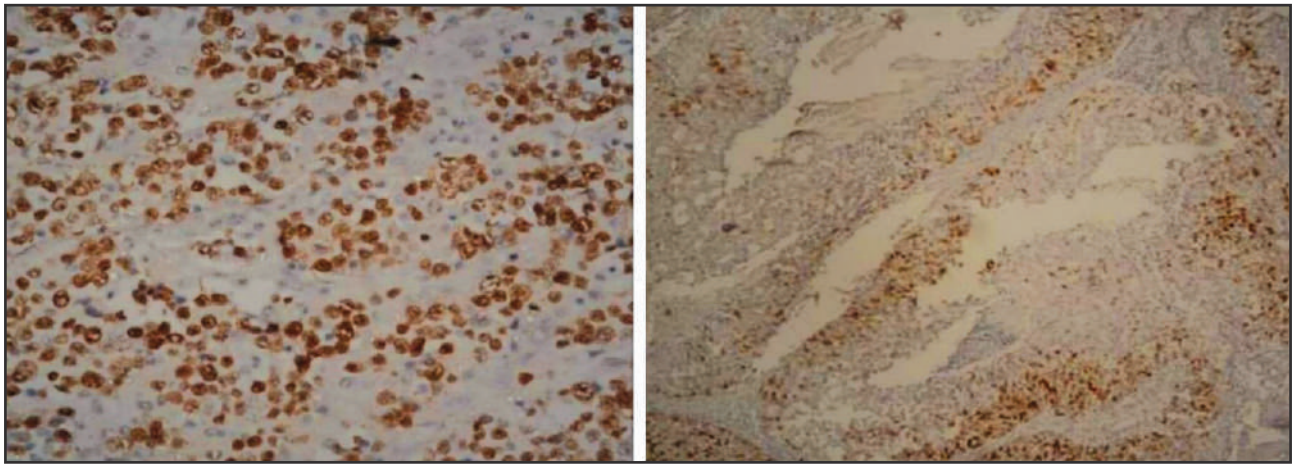

Figure 2: a) Photomicrograph of poorly differentiated endometrial carcinoma showing overexpression of p–53 (IHCX400). b) Photomicrograph of moderately differentiated endometrial carcinoma showing weak positivity of p– 53for p 53 (IHC x 200)

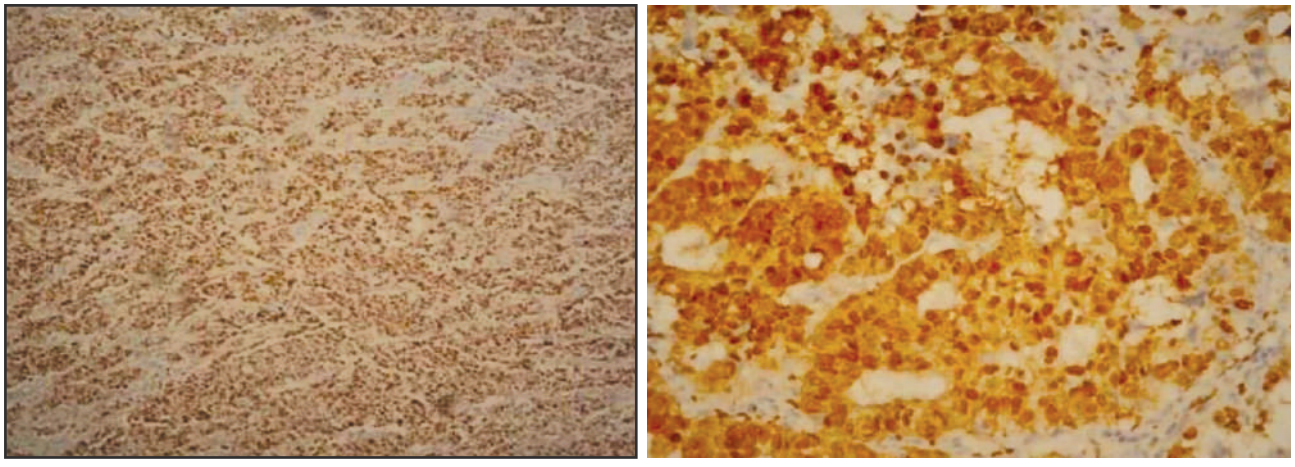

Figure 3: a) Photomicrograph of poorly differentiated Endometrial carcinoma showing high proliferative index of Ki67 (100X). b) Photomicrograph of uterine serous carcinoma showing high proliferative index of Ki67 (x 400)

Discussion

Endometrial carcinoma accounts for approximately 76, 000 deaths annually worldwide. Increasing incidence and disease mortality make EC an important health issue in women's, particularly in developed countries where the incidence is highest. It is also major health problem in developing countries like India as well where most of the women are from remote area and reported with advanced stage of disease[10].

Clinoco– pathological characteristics	Cylin D1			P53			Ki67		
	Posi% 66(53.22)	Neg% 58(46.77)	Pvalue	Posi% 56(45.2)	Neg% 68(54.8)	Pvalue	Pos% 40(32.3)	Neg% 84(67.7)	pvalue
Age (in years)									
<=60(n=86, 70.16%)	42(48.82)	44(51.17)	0.1428	27(31.4)	59(68.6)	0.0035	17(19.8)	69(80.2)	0.0001
>60(n=38, 29.83%)	24(63.15)	14(36.84)		29(76.3)	9(23.7)		23(60.5)	15(39.5)	
Histology									
Tpye I(n=108,87.1%)	64(59.2)	44(40.7)	0.001	41(38.0)	67(62.0)	0.0024	26(24.1)	82(75.9)	0.0018
Type II (n=16, 12.9%)	2(12.5)	14(87.5)		15(93.8)	1(6.2)		14(87.5)	2(12.5)	
Grade									
I–II(n=76,73.4%)	28(36.8)	48(63.1)	0.0012	19(25.0)	57(75.0)	0.0002	7(9.2)	69(90.8)	0.0001
III(n=48,26.8%)	37(77.1)	11(22.9)		37(77.1)	1(22.1)		33(93.8)	15(31.2)	
Myometrial invasion									
<1/2(n=72, 58.1%)	29(40.3)	43(59.7)	0.002	10(13.9)	62(86.1)	0.0001	8(11.1)	64(88.9)	0.005
>1/2(n=52, 41.9%)	37(71.2)	15(28.8)		46(88.5)	6(11.5)		32(61.5)	20(38.5)	
LVSI									
Yes (n=34, 27.4%)	26(76.5)	8(23.5)	0.004	30(88.2)	4(11.8)	0.0005	28(82.4)	6(17.6)	0.0005
No (n=90, 72.6%)	40(44.4)	50(55.6)		26(28.9)	64(71.1)		12(13.8)	78(86.7)	
Cervixinvolvement									
Yes (n=36, 29.03%)	27(75)	9(25)	0.005	25(27.8)	65(72.2)	<0.001	7(19.4)	29(80.6)	0.0012
No (n=88, 70.93%)	39(43.3.7)	49(57.7)		31(91.2)	3(8.8)		11(12.5)	77(87.5)	
LN metastasis									
Yes (n=32, 25.80%)	26(81.4)	6(18.6)	0.001	31(91.2)	3(8.8)	0.008	31(91.2)	3(8.8)	0.0008
No (n=92, 74.19%)	40 (43.5)	52(56.5)		25(27.8)	65(72.2)		9(10.0)	81(90.0)	
Adenexal involvement									
Yes–(n=26, 20.96%)	21(80.8)	5 (19.2)	0.005	22(82.6)	4(15.4)	<0.001	23(88.5)	3(11.5)	0.0001
No.(n= 98, 79.03%)	45(45.9)	53(54.1)		56(35.7)	68(65.3)		17(17.3)	81(82.7)	
FIGO Stage									
I–II(n=90,72.6%)	38(42.2)	52(57.8)	0.0015	25(27.8)	65(72.2)	0.002	9(10.0)	81(90.0)	0.0002
III–IV (n=34, 27.4%)	28(82.2)	6(17.6)		31(91.2)	3(8.8)		31(91.2)	3(8.8)	

Table 2: Expression of Cyclin D1, p53, and Ki67 in 124 patients of endometrial cancer according to the clinico– pathological characteristics (N=124)

Endometrial carcinoma is a disease of older, postmenopausal women and more than 90% EC are sporadic[2]. Women who were diagnosed at an early stage with type I histology have long survival time, than those with type II histology or at an advanced FIGO stage[11]. Although FIGO staging system and histopathology helps us to treat the EC patients, it remains insufficient to predict clinical outcome and to advise neoadjuvant therapy due to molecular heterogeneity of EC[12]. Therefore, there is utmost need to identify specific biomarkers to achieve personalized treatment and to improve clinical outcome[11]. Here we studied tumor biomarkers related to cell cycle progression (Cyclin D1), cell proliferation (Ki67) and cell–cycle regulation (p53).

The mean age of the patients at the time of presentation was 58.6 for type I carcinoma whereas it was 66.4 years for type II which was similar to study by Stavropaulos A et al[13] and Shevra C R et al.[14]. In our study, common risk factors associated with EC were obesity (34.67%), diabetes mellitus (17.74%) and hormone replacement therapy (15.32%).This was in line with study of Schmandt RE et al[15], who concluded obesity as commonest risk factor associated with EC.

Type I, mostly endometrioid carcinomas (108 /124; 87.09%) are hormone dependent cancers, more common and got good prognosis than type II (16 /124; 12.90%) which affect older age group. Our findings are in agreement with Stavropaulos A et al[13], Liange S et al.[16]and Yu CG et al [17].

Clinicopathological Characteristics	5yrs DFS%	95%CI	P–value	5yrs OS%	95%CI	p–value
Age(in yrs) <=60(n=86, 69.4%)	87.20	84.6–89.8	<0.001	96.15	93.22–99.07	0.002
>60(n=38, 30.6%)	67.09	60.56–73.6		82.70	73.69–91.70	
Histological type Type I (n=108, 87.1%)	83.47	80.62–86.31	0.001	96.55	94.04–99.06	<0.001
Type II (n=16, 12.9%)	63.68	50.29–77.06		60.66	47.25–74.07	
Histological Grade I II(n=76,61.3%)	86.71	83.77–89.65	<0.001	97.30	94.63–99.98	0.001
III (n=48, 38.7%)	72.03	66.09–77.70		83.70	76.30–91.11	
Myometrial Invasion < ½ (n=72, 58.1%)	87.44	84.52–90.36	<0.001	95.81	92.26–99.36	0.039
> ½ (n=52, 41.9%)	72.72	67.17–78.82		85.78	79.72–91.82	
LVI Present (n=34, 27.4%)	67.01	60.44–73.58	<0.001	82.07	73.14–91.00	0.015
Absent (n=90, 72.6%)	86.48	83.60–89.37		95.25	91.94–78.56	
LN Metastasis Present (n=32, 25.8%)	69.40	62.11–76.69	<0.001	79.94	70.15–89.74	0.002
Absent (n=92, 74.2%)	85.15	82.11–88.18		96.25	93.49–99.02	
Cervical invasion Present (n=36, 29.0%)	66.71	59.81–73.60	<0.001	83.30	74.84–91.79	0.026
Absent (n=88, 71.0%)	87.13	84.58–89.78		95.16	91.80–98.52	
Adenexal involvement Present (n=26,21%)	68.82	61.75–75.89	<0.001	78.77	68.99–85.77	0.009
Absent (n=98, 79%)	84.37	81.16–87.59		95.48	92.36–88.45	
FIGO Stage I, II (n=90, 72.4%)	86.23	83.53–88.93	<0.001	96.67	93.99–99.35	<0.001
III, IV (n=34, 27.6%)	66.88	59.60–74.16		78.79	68.62–87.95	
Cyclin D1 Positive (n=66, 53.2%)	76.32	71.54–81.11	0.001	89.15	83.68–94.62	0.305
Negative (n=58.46.8%)	87.04	83.56–90.53		96.12	92.79–99.45	
P53 Positive (n=56, 45.2%)	71.85	66.52–77.18	<0.001	83.86	77.50–90.21	0.003
Negative (n=68, 54.8%)	88.57	85.89–91.24		97.55	94.82–100.28	
Ki67 High index (n=40, 32.3%)	71.82	64.87–78.62	0.001	82.47	74.72–90.22	0.004
Low index (n=84, 67.7%)	85.73	82.82–78.63		96.31	93.28–99.35	

Table 3: Univariable Analysis of Survival according to Clinicopathological Characteristics in 124 patients of endometrial carcinoma.
LVI – lymphovascular invasion
LN – lymph node

Grade I, II E correspond to early stage and had good survival rate, compared with poorly differentiated and type II carcinomas in advanced stage with poor survival outcome[18]. In this study Type I EC cases had a DFS rate of 83.47% which was significantly higher than 63.68% (p<0.001) for Type II clear cell carcinoma, carcinosarcoma etc. Five–year DFS for grade I was 86.71% which was decreased to 72.03% for grade III (p< 0.001). Our findings of adverse outcome of type II and poorly differentiated carcinoma were similar to

Suthipintawong C[18] TejarizoGarcia et al[19]. Also, more than half of myometrial invasion, lymphovascularinvasion, and cervix involvement were found in advanced tumor stage, suggesting important prognostic parameters in term of survival and recurrence of EC. These observations were similar to Wang et al[20] who concluded that poorly differentiated tumor, MI> 50% and adnexal involvement are significant risk factors affecting DFS and OS in their study on 117 patients of grade III EC.

Cyclin D1, encoded by the proto– oncogene CCND1 located on 11q13 and plays an important role during the rate limiting point G1S phase transition in cell cycle[16]. It acts along with Cyclin–dependent kinases (CDKs) CDK4 and CDK6 to phosphorylate the retinoblastoma protein (Rb) during the G1 phase to promote DNA synthesis and cell cycle proliferation. Cyclin D1 amplification and overexpression have been frequently described in solid tumors including endometrial cancers and others such as carcinoma breast, colon, urinary bladder, lung, prostate etc.[16, 21].

Sixty six (53.22%) cases of EC showed nuclear staining of cyclin D1, including 52(48.1%) cases of type I and2(12.5%) cases of type II EC .Our findings are consistent with Shevra CR et al[14], Khabaz MN[21]and Liang S et al[16], who found overall cyclin D1 expression in 57%, 56.3% and 52% respectively in type I EC. Khabaz MN et al[21] also noticed high score of cyclin D1 has been significantly linked with patient age (p=0.0001). We concluded high score of Cyclin D1 staining was significantly correlated with histological type I EC (p = 0.011), high grade tumors (p = 0.0012), lymph node metastasis (p = 0.001), adenexal involvement (p = 0.005) and advanced tumor stage (p =0.0015), but not with patient age (p = 0.142). Similar findings were noticed by Fangfang Nan et al[22] in study of 126 patients of EC. Our findings of negative staining for cyclin D1 was correlated with longer DFS and OS was similar to study done by Liang S et al[16]. However, Khabaz MN et al[21] observed no significant association between higher cyclin D1 staining and grade, and survival outcome and recurrence.

The p53 protein is a transcription factor and initiates apoptosis in case of failed DNA repair. Inactivation of p53 gene provides the tumor cell with higher capacity for cell division and proliferation, thus contributing to malignant change and tumor formation. Mutant p53proteins are non– degradable and accumulates in the nucleus thereby detectable by IHC [22] .

In present study IHC scores of p53 over expression were significantly associated with increasing age of patients (p = 0.0035), type II EC (p = 0.0024), poorly differentiated tumor (p = 0.0002), outer half of myometrial invasion (p = 0.0001), adenexal involvement (p = 0.0001) and advanced FIGO stage (p = 0.002). Our findings are consistent with previous studies done by Suthipintawong C et al[18] Akiyama et al[24] and Jastania R and Nageeti.T[25]. In contrast Stavropaulos A et al[13] did not find significant association of p53 over expression with the mean age of the patients (P=0.131), histological types (P=0.349), histological grades (P=0.165) and clinical stages (P=0.100).

Furthermore, in agreement with Akiyama et al[24], univariate survival analyses revealed that p53 overexpression was a significant and independent prognostic factor for poor DFS (p=0.009). Talhouk A et al[26] also concluded, p53 protein mutation is one of the most important molecular factors, which determined prognosis in EC, as presence of p53 mutation being associated with unfavorable outcome.

The Ki67 is a proliferation associated nuclear antigen, which is detected only in dividing cells and not in quiescent cells (G0 phase). The levels of Ki67protein are low in the G1 and S phases and peak early in mitosis [27]. Thus, expression of the Ki67 protein is correlated with the proliferative activity of intrinsic cells in malignant tumors, allowing it to be used as a marker of tumor aggressiveness [27].

In this study, Ki–67 high score (>30%) was strongly associated with well–known pathological prognostic parameters including high tumor grade (p= 0.0001), more than half of myometrial invasion (p= 0.005), lymph node involvement (0.0008). Also high proliferating index of Ki67 was strongly associated with type II histology (p= 0.0018),advanced FIGO stage(p= 0.0002) and poor DFS (p= 0.001) and OS (p= 0.004) .These observations were similar to various studies in the literature by Yu CGet al(17), Suthipintawong C et al (18) and Kitson SJ (28)who found Ki–67 as a prognostic biomarker in EC. Fanning and colleagues (29) had not found any correlation between Ki67 over expression and tumor recurrence in high risk EC this may be due to small sample size and exclusion of low risk tumors.

Apart from routine hormonal receptors, these biomarkers (cyclin D1, p53 and Ki67) may indicate tumor behavior, presence of adverse risk factors and the necessity for more radical surgery and post–operative radiotherapy and or chemotherapy. However, the present study has specific limitations. This study was conducted in a single institution and the sample size was relatively small. Therefore, further studies are required to strengthen the current findings.

Conclusion:

Due to molecular heterogeneity of EC further development of molecular biomarkers may be the next

step in refining our classification of EC in general and on a case–by–case basis. Our finding of Cyclin D1 staining, p53overexpression and an elevated Ki–67staining all had an independent prognostic impact, in addition to age and conventional FIGO stage, in patients of endometrial cancer. This implies that these biomarkers might be helpful in identifying high–risk patients who could be benefited by postoperative neoadjuvant chemotherapy and radiotherapy.

Funding and Conflict of Interest:

No

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