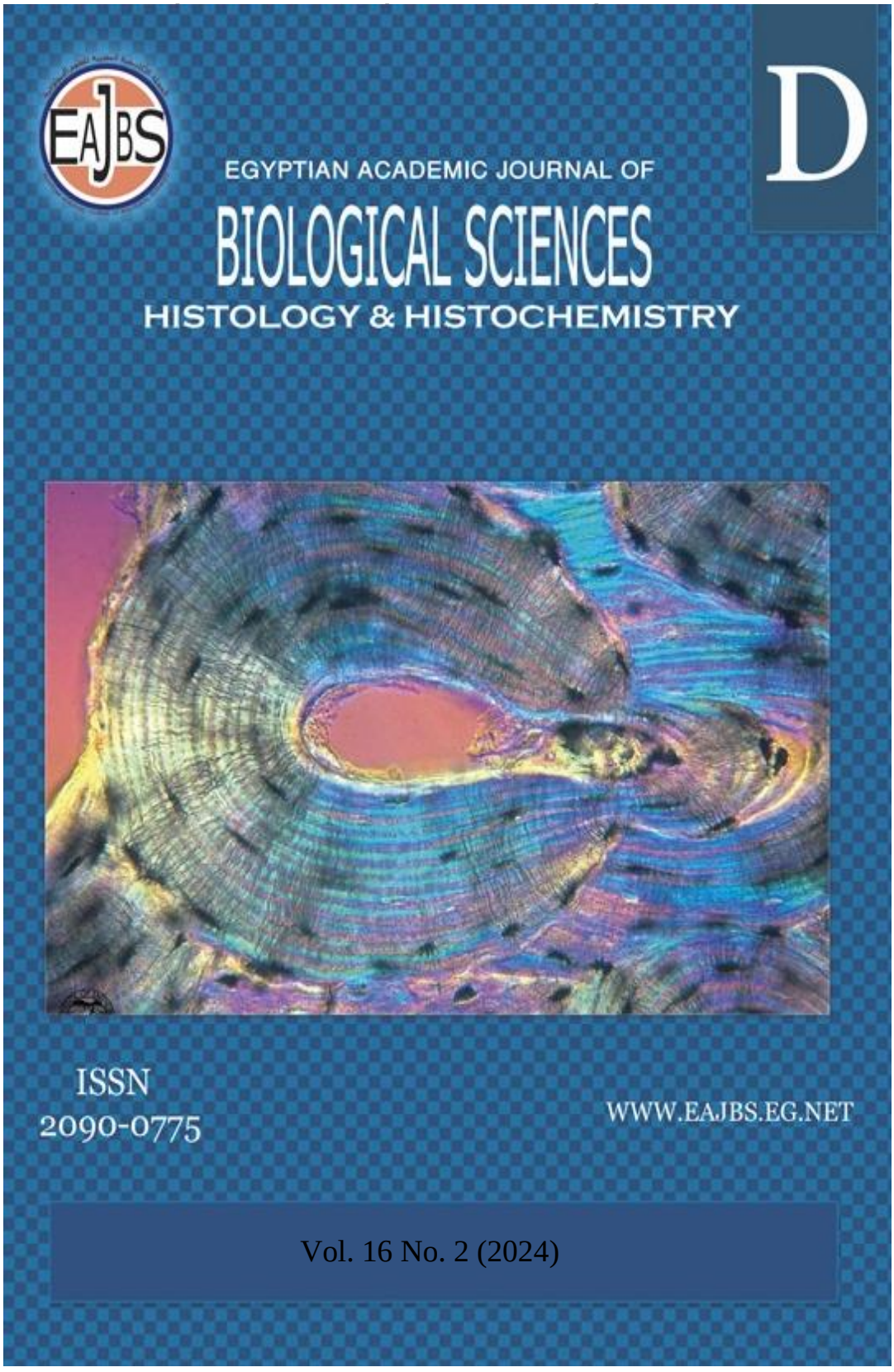

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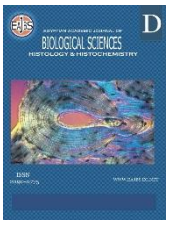

A Comprehensive Clinicopathological Analysis of Carcinoma of Endometrium: Histotype- Immunophenotype Correlation

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ABSTRACT

Background: High grade Endometrial carcinomas (ECs) include type II and grade 3 endometrioid carcinoma. Challenges include risk stratification on diagnostic biopsy and predictive biomarker assessment post- surgery for adjuvant treatment. Distinguishing grade 3 endometrioid carcinoma and type II EC is a challenge. We analyzed ER, PR, p53, Her2 expression in histotypes of EC and their utility to distinguish type I and II tumors. **Materials and methods:** A prospective study involving 106 cases of EC, categorized into low and high-grade ECs was conducted to analyze clinicopathological parameters and expression of several biomarkers, including estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor (HER2) and p53, using immunohistochemistry (IHC). The study specifically focused on differentiating grade 3 endometrioid carcinomas from non-endometrioid tumors. **Results:** ER was found to be sensitive and specific (90.42% & 80% respectively) in differentiating endometrioid carcinoma from serous carcinoma. PR was identified as specific in differentiating high-grade endometrioid carcinoma from serous carcinoma. p53 exhibited high sensitivity (100%) in differentiating high-grade endometrioid carcinoma from serous carcinoma and highly specific in differentiating endometrioid carcinoma from serous carcinoma, further emphasizing its importance as a diagnostic marker in distinguishing between these subtypes. **Conclusion:** The use of ER, PR, and p53 as a panel offers high sensitivity and specificity in EC diagnosis and classification, particularly in high-grade cases. Additionally, HER2 detection in type II ECs provides valuable information for targeted therapy selection, highlighting the importance of molecular profiling in guiding personalized treatment approaches for endometrial cancer.

INTRODUCTION

Endometrial carcinoma (EC) is most common neoplasm of female genital tract and ranks fourth most frequently diagnosed cancer in women globally. Each year, approximately 319,600 cases are diagnosed worldwide (Torre LA *et.al.,* 2015). In 1983, Bokhman classified EC into two major categories: type I and type II. Type I carcinomas are exemplified by endometrioid subtype, which is associated with unopposed estrogenic stimulation (Bokhman JV *et.al.,* 1983). Type II carcinomas, like serous carcinoma, are not typically related to estrogenic stimulation and are considered more aggressive with high grade features (Sherman ME *et.al.,* 1997).

Other type II tumors include clear cell carcinoma, dedifferentiated and undifferentiated carcinoma and carcinosarcoma (Kurman JR *et.al.,* 2019).

Risk stratification on diagnostic biopsy is crucial for guiding extent of surgery and determining optimal treatment. This involves identifying histologic subtype and grade of EC, and assessing clinicopathological parameters that influence prognosis and treatment (Huvila J *et.al.,*2021). High-grade ECs encompass all type II tumors and FIGO grade 3 endometrioid carcinomas. Grade 3 endometrioid tumors represent a heterogeneous group and molecular studies have suggested that grade 3 endometrioid carcinomas share similarities with type II tumors, indicating a potential overlap in their biological characteristics. This area of investigation highlights ongoing efforts to better understand molecular underpinnings of EC subtypes and refine current classification to improve risk stratification and treatment (Murali R *et.al.,* 2019).

The Cancer Genome Atlas (TCGA) has provided valuable insights into molecular landscape of ECs and classified into four distinct molecular subtypes: POLE mutant, Mismatch Repair Deficient (MMRd), No Specific Molecular Profile (NSMP) and P53 mutant groups. These subtypes exhibit differences in prognosis and response to treatment, emphasizing importance of molecular characterization in guiding therapy. While molecular analysis offers detailed classification of EC subtypes, it may not always be feasible in resource-poor settings. In such cases, IHC serves as a valuable alternative (Douglas A. Levine *et al.,* 2013). Distinguishing grade 3 endometrioid carcinoma and type II EC remains a common problem especially in resource poor setting where molecular analysis is far from reality. In such settings, immunohistochemical analysis is often useful. Although no one biomarker provides excellent statistical performance, a panel of IHC markers is often beneficial in difficult cases (Murali R *et.al.,* 2019). We analyzed expression patterns of selected biomarkers such as ER, PR, p53 and Her2 in different histotypes of EC and studied their utility to distinguish between type I and type II tumors.

Aim & objectives:

1. To analyze the histological and immunohistochemical features of ECs.
2. To assess the diagnostic utility of immunohistochemical biomarkers in precisely classifying ECs**.**
3. To evaluate the prognostic significance of immunohistochemical biomarkers in ECs.

MATERIALS AND METHODS

A prospective study of selected 106 cases of ECs was conducted including 66 type I (endometrioid) carcinoma and 40 type II (non-endometrioid) carcinomas. Cases were divided into low grade (grade 1 & 2) endometrioid carcinomas, high grade (grade 3) endometrioid carcinomas and non-endometrioid carcinomas. All non-endometrioid carcinomas are inherently high grade biologically. Study was conducted at Department of Pathology at The Gujarat Cancer and Research Institute, Ahmadabad during the period of two years from September 2019 to August 2021.

Inclusion Criteria:

- All patients with confirmed EC who underwent radical hysterectomy with or without diagnostic biopsy in our institute.
- Review cases of radical hysterectomy for EC who underwent surgery elsewhere.

Exclusion Criteria:

- Those who did not undergo radical hysterectomy following diagnostic biopsy
- Cases of high-grade ECs with inconclusive or ambiguous IHC, which further required other biomarker assay or molecular investigations.

All clinically relevant data including clinical history, radiological findings and biopsy reports were retrieved from patient's case file and electronic records. Slides and paraffin embedded tissue blocks of histopathologically diagnosed cases of EC were retrieved from archives of Department of Pathology. Clinicopathological parameters including age of patient, histologic type, grade and stage of tumor, extent of myometrial invasion, lower uterine segment (LUS) and cervical stromal involvement, lymphovascular invasion (LVI) and lymph node metastasis were collected and analyzed in different EC groups.

Immunohistochemistry (IHC):

Immunohistochemistry for estrogen receptor (ER), progesterone receptor (PR), Her2 and p53 was done on all 106 cases to study their expression in type I and type II tumors. Patterns of expression of biomarkers and their utility in diagnosis of high-grade tumors to differentiate between grade 3 endometrioid and non-endometrioid tumors were studied.

Sections (3µm) of formalin-fixed, paraffin-embedded tissue were tested for presence of antigens using the Ventana benchmark XT autoimmunostainer using ultra view DAB detection kit. Sections were deparaffinised using EZ prep buffer and antigen retrieved by CC1 buffer at pH 9. All slides were incubated with ER (SP1, Ventana, ready to use), PR (1E2, Ventana ready to use), p53 (DO7, Dako, 1:50 dilution), Her2 (4B5, Ventana, ready to use) antibodies at 37 ° C for 16 min, 16 min, 40 min and 32 min respectively as per recommended guidelines. Enzyme conjugated secondary antibody was then added, we used HRP multimer- incubated for 8 mins, then applied with 3% H_2O_2 and DAB chromogen for 8 mins. Counterstaining with hematoxylin was done for 8 mins and decolourization with blueing reagent done for 4 mins. Finally mounted with DPX and then examined under microscope for immunoreactivity.

We used College of American Pathologists (CAP) "Template for Reporting Results of Biomarker Testing of Specimens from Patients with Carcinoma of the Endometrium" for reporting of IHC results (College of American Pathologists. 2023).

1. ER, PR and Her2 (ERB2) IHC:

There are no outcome-driven consensus opinions that have been developed for reporting of results of assays for ER, PR and Her2 for EC currently. CAP recommends using a similar format that is used for reporting results of immunohistochemical assays for ER, PR and Her2 for breast cancer (College of American Pathologists. 2020).

2. p53 IHC:

In normal endometrial glands, p53 expression is typically low, with nuclear staining in only a small percentage (1-5%) of cells (wild type). Three distinct staining patterns are considered diagnostic of abnormalities in p53 gene. Most cases are associated with overexpression with intense nuclear staining in over 90% of affected cells. Second shows complete absence of protein in all affected cells. Third is cytoplasmic staining. Low levels of expression in stroma or nonmalignant epithelium were used as internal control.

Statistical Analysis:

In this descriptive study, data collected were entered into a Microsoft Excel master sheet and analyzed using Statistical Package for Social Sciences (SPSS) version 20 software. Chi-square test was employed to assess association between various parameters. A value $P < 0.05$ was considered significant.

Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of ER, PR, HER2 and p53 in differentiating type I and type II tumors were studied. Association of ER, PR and p53 with different tumor groups and their utility to differentiate between tumor types were analysed. Association of ER, PR and p53 between

Type 1-LG & Type 1-HG, Type 1-LG &Type II and Type I-HG & Type II groups were analysed separately and p <0.05 was considered significant.All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards

RESULTS

A total of 106 cases of confirmed EC were included in our study who's pathological and immunohistochemical features were analyzed. Among 106 cases, 66 were type I (endometrioid) carcinomas and 40 were type II (non-endometrioid) carcinomas. Type I tumors were subdivided as grade 1& 2 low-grade (42 cases) and grade 3 high-grade (24 cases) tumors. Type II tumors were further classified as serous carcinoma, clear cell carcinoma, undifferentiated carcinoma, dedifferentiated carcinoma, carcinosarcoma and mixed carcinomas. Among type II tumors, serous carcinoma (25 out of 40 cases) was the predominant type (Table1).

Table 1: Pathological characteristics of endometrial carcinoma in 106 patients.

Characteristic		Value (N)
Histologic tumor type	Endometrioid carcinoma Grade 1	20
	Endometrioid carcinoma Grade 2	22
	Endometrioid carcinoma Grade 3	24
	Serous carcinoma	25
	Clear cell carcinoma	4
	Dedifferentiated carcinoma	1
	Undifferentiated carcinoma	2
	Carcinosarcoma	5
	Mixed carcinoma (Clear cell + Endometrioid)	2
	Mixed carcinoma (Serous + Endometrioid)	1
Myometrial invasion	<50%	45
	≥50%	61
LUS involvement		42
Cervical stromal involvement		11
LVI		22
Lymph node metastasis		8
FIGO Stage	Stage I Stage II	78 11
	Stage III Stage IV	15 2

LUS-Lower uterine segment; LVI-Lymphovascular invasion; FIGO-The International Federationof Gynecology and Obstetrics.

The mean age of patients was 59.4 years (range, 35-87 years). Mean age of type I tumors was 55 years and that of type II tumors was 62 years. Type II tumors occurred in older age compared to type I tumors and were common in post-menopausal women with post-menopausal bleeding as the common clinical presentation. Mean size of tumors was 5cm (range, 1.5 to 12cm). Grossly, they showed exophytic polypoidal to endophytic growth involving up to full thickness of uterine wall. Papillary serous and villoglandular carcinomas showed papillary friable superficial surface. Pathological characteristics of 106 cases are depicted in Table 1.

Low grade endometrioid carcinoma (Type I-LG) comprised of 42 cases of grade 1 (20/42) and grade 2 (22/42) endometrioid carcinomas. Tumors showed predominantly well-defined glandular architecture (37/42) and a few were of villoglandular variant (5/42). Squamous differentiation was seen in 10 cases and mucinous metaplasia in 3 cases. All Type I-LG were positive for ER and PR with diffuse and strong nuclear expression. One case showed mutant type p53 staining with nuclear overexpression and rest all were of wild type p53 phenotype (Fig. 1). None showed Her2 overexpression.

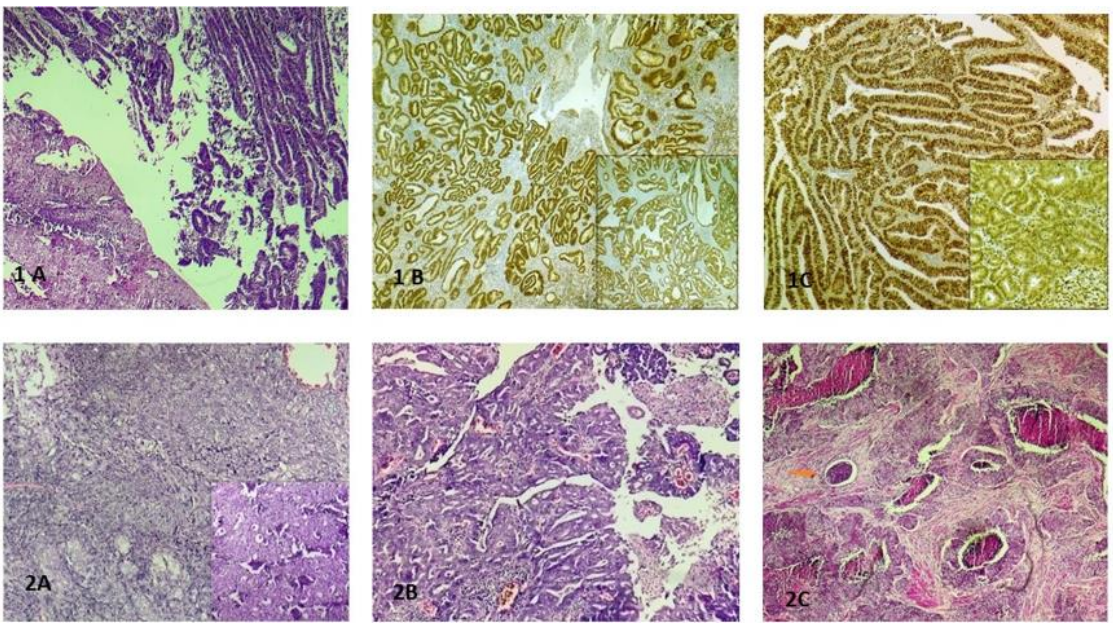

Fig.1 **A**: Villoglandular type endometrioid carcinoma with squamous differentiation (20X, H&E); **B**: Diffuse and strong ER & PR (Inset) in endometrioid carcinoma, G1 (20X, IHC), **C:** Mutant type p53 in endometrioid carcinoma, G1. Inset-Wild type p53 (20X, IHC), Fig.2 **A**: Solid pattern of endometrioid carcinoma, G3. Inset: Solid cribriform pattern (40X, H&E), **B**: Papillary pattern of endometrioid carcinoma, G3 (20X, H&E), **C:** Comedo necrosis with lymphovascular emboli (arrow) in endometrioid carcinoma, G3 (20X, H&E).

There were 24 cases of high grade endometrioid carcinoma (Type I-HG) which were of grade 3. Tumors were heterogenous and showed architecture varying from predominantly solid (Fig 2A) to cribriform (Fig 2A, inset), papillary (Fig 2B) and comedo necrosis (Fig 2C) patterns, individually and in varied combinations. Squamous differentiation was seen in 6 cases. Nuclear pleomorphism varied from mild to severe. Some cases showed extensive lymphovascular invasion (LVI). Immunohistochemically, tumors showed

varied positivity for ER and PR with 5 showing mutant type p53 expression and Her2 overexpression in one case. (Table 2).

Table 2: Expression of biomarkers in Type I and Type II tumors

Biomarkers	Type I-LG(42)	Type I-HG(24)	Type II(40)
ER, n (%)	42(100)	19(79.2)	10(25)
PR, n (%)	42(100)	17(70.8)	8(20)
p53, M,n (%)	1(2.4)	5(20.8)	33(82.5)
Her2, n (%)	0	1(4.2)	7(17.5)

ER-Estrogen receptor; PR-Progesterone receptor; M-Mutant type; Her2-Human epidermal growthfactor receptor 2; LG-low-grade endometrioid, HG-high-grade endometrioid.

There were 40 type II ECs in our study among which serous carcinoma (Fig. 3A) was the commonest (25/40) followed by carcinosarcoma (5/40) (Fig. 3B), clear cell (4/40) (Fig. 3C), mixed (3/40) (Fig. 3D), undifferentiated (2/40) (Fig. 3E) and dedifferentiated (1/40) (Fig. 3F) carcinomas. P53 expression was seen commonly (33/40, 82.5%) in type II carcinomas with ER and PR expressed in 10 and 8 type II carcinomas respectively. Her-2-neu overexpression was seen only in 7 type II carcinomas.

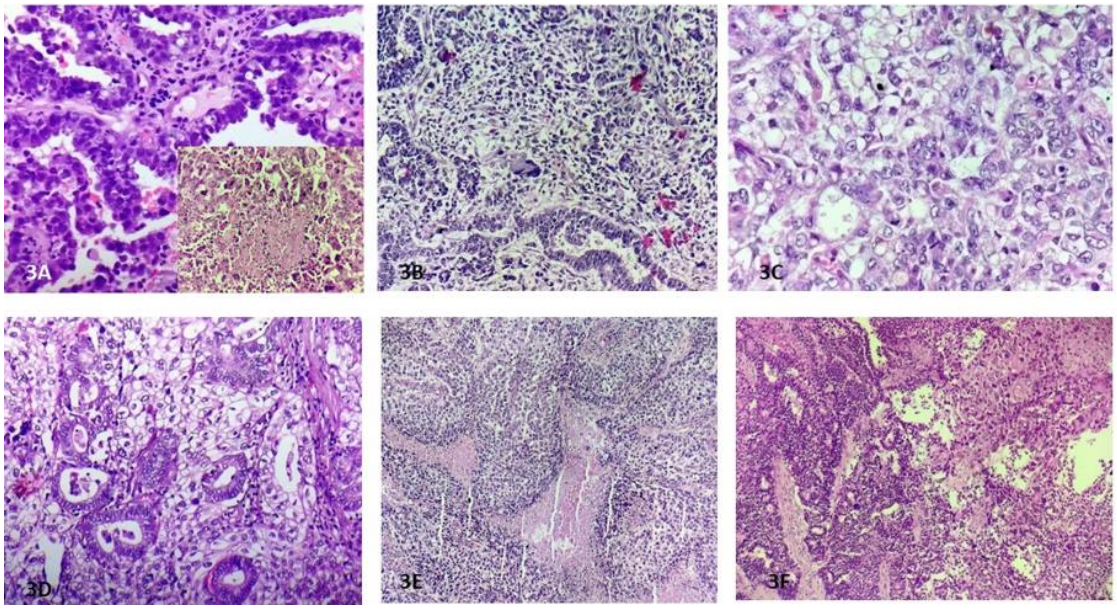

Fig 3A: Serous carcinoma, papillary pattern (40X, H&E). Inset- Pleomorphic nuclei in serous carcinoma (40X, H&E), Fig 3B: Biphasic morphology of carcinosarcoma (40X, H&E), Fig 3C: Clear cell carcinoma, solid pattern (40X, H&E), Fig 3D: Mixed endometrioid + clear cell carcinoma (40X, H&E), Fig 3E: Discohesive small cells in undifferentiated carcinoma (20X, H&E), Fig 3F: Dedifferentiated carcinoma with focal rhabdoid morphology (20X, H&E).

Various pathological variables like extent of myometrial invasion, involvement of LUS and cervical stroma, LVI, International Federation of Gynaecology and Obstertrics (FIGO) stage and lymph node status were compared between low-grade endometrioid carcinoma, high-grade endometrioid carcinoma and type II tumors. (Table 3**)** We found myometrial invasion involving more than 50% thickness of uterine wall was more common in high-grade endometrioid carcinomas compared to low-grade endometrioid carcinomas (p value=0.02) and involvement of LUS in type II tumors compared to high-grade endometrioid carcinomas (p value=0.03).

Advanced tumor stage (FIGO stage III/IV) was common in high-grade endometrioid and type II tumors compared to low-grade endometrioid carcinomas (p value= 0.002 and 0.001 respectively), whereas there was no statistical difference between high-grade endometrioid and type II tumors (p value=0.56). We also noted that lymph node metastasis was more common in type II tumors compared to endometrioid carcinomas of both grades (p value=0.001). We did not find statistically significant difference between high-grade endometrioid and type II tumors in the extent of myometrial invasion, involvement of cervical stroma, LVI and tumor stage (Table 3).

Table 3: Correlation of pathological variables between different tumor groups.

Variables	Number (%)		Tumor groups Comparison	p value
Myometrial Invasion ≥50%	Type I-LG (42)	20(47.6)	Type I- LG vs Type I-HG	**0.02***
	Type I-HG(24)	17(70.8)	Type I- LG vs Type II	0.16
	Type II(40)	24(60)	Type I- HG vs Type II	0.28
Involvement of LUS	Type I-LG(42)	19(45.2)	Type I- LG vs Type I- HG	**0.006***
	Type I-HG(24)	7(29.2)	Type I- LG vs Type II	0.46
	Type II(40)	16(40)	Type I- HG vs Type II	**0.03***
Involvement of cervical stroma	Type I-LG(42)	5(11.9)	Type I- LG vs Type I- HG	0.31
	Type I-HG(24)	2(8.3)	Type I- LG vs Type II	0.70
	Type II(40)	4(10)	Type I- HG vs Type II	0.50
LVI	Type I-LG(42)	6(14.3)	Type I- LG vs Type I- HG	**0.001***
	Type I-HG(24)	8(33.3)	Type I- LG vs Type II	0.11
	Type II(40)	8(20)	Type I- HG vs Type II	0.06
FIGO Stage (III/IV)	Type I-LG(42)	3(7.1)	Type I- LG vs Type I- HG	**0.002***
	Type I-HG(24)	5(20.8)	Type I- LG vs Type II	**0.001***
	Type II(40)	9(22.5)	Type I- HG vs Type II	0.56
Lymph node Metastasis	Type I-LG(42)	2(4.8)	Type I- LG vs Type I- HG	**0.02***
	Type I-HG(24)	0	Type I- LG vs Type II	**0.001***
	Type II(40)	6(15)	Type I- HG vs Type II	**0.001***
ER IHC, positive	Type I-LG(42)	42(100)	Type I- LG vs Type I- HG	**0.001***
	Type I-HG(24)	19(79.2)	Type I- LG vs Type II	**0.001***
	Type II(40)	10(25)	Type I- HG vs Type II	**0.001***
PR IHC, positive	Type I-LG(42)	42(100)	Type I- LG vs Type I- HG	**0.001***
	Type I-HG(24)	17(70.8)	Type I- LG vs Type II	**0.001***
	Type II(40)	8(20)	Type I- HG vs Type II	**0.001***
p53 IHC, Mutant type	Type I-LG(42)	1(2.4)	Type I- LG vs Type I- HG	**0.001***
	Type I-HG(24)	5(20.8)	Type I- LG vs Type II	**0.001***
	Type II(40)	33(82.5)	Type I- HG vs Type II	0.25

*- p value <0.05 is statistically significant

LUS-Lower uterine segment; LVI-Lymphovascular invasion; FIGO-The International Federation of Gynecology and Obstetrics; ER-Estrogen receptor, IHC-Immunohistochemistry; PR- Progesterone receptor, LG-low grade; HG-high grade

All cases of low-grade endometrioid carcinomas were positive for ER and showed diffuse, strong nuclear expression in almost all cases. High grade endometrioid carcinomas showed a positive rate of 79.25 for ER with 10-90% (19/24) cells exhibiting moderate to strong staining. Five out of 25 (20%) serous carcinomas showed focal, weak to moderate staining with ER with an exception of one case showing ER positivity in 90% of cells. ER was found to be a more sensitive (92.42%) biomarker with high PPV in

differentiating endometrioid carcinoma from serous carcinoma. However, it was found to be relatively less sensitive (79.17%) in differentiating high- grade endometrioid carcinoma from serous carcinoma which poses a real diagnostic challenge at times. ER was equally specific (80%) to differentiate serous carcinoma from endometrioid and high-grade endometrioid carcinomas (Table 4).

Table 4: Sensitivity, Specificity, PPV & NPV of ER, PR and p53.

Statistics	ER		PR		P53	
	HG Endo vs Ser	Endo vs Ser	HG Endo vs Ser	Endo vs Ser	HG Endo vs Ser	Endo vs Ser
Sensitivity	79.17%	92.42%	70.83%	89.39%	100.00%	100.00%
Specificity	80.00%	80.00%	88.00%	88.00%	79.17%	90.91%
PPV	79.17%	92.42%	85.00%	95.16%	83.33%	80.65%
NPV	80.00%	80.00%	75.86%	75.86%	100.00%	100.00%
Accuracy	79.59%	89.01%	79.59%	89.01%	88.80%	93.41%

PPV-Positive predictive value; NPV-Negative predictive value; HG Endo- Endometrioidcarcinoma, high grade; Ser-Serous carcinoma; Endo-Endometrioid carcinoma

The results of PR correlated with that of ER with respect to low-grade endometrioid carcinoma. All cases were positive and showed strong and diffuse nuclear expression in nearly all cases. Among high-grade endometrioid carcinomas, 17 out of 24 cases (70.8%) were positive and showed moderate to strong expression in 10-90% of cells. In serous carcinoma, PR was positive in 3 out of 25 cases (12%) showing weak staining. Only one case showed staining in 90% of cells, but other histomorphological features favored serous carcinoma. The sensitivity of PR was less in differentiating endometrioid carcinoma and serous carcinoma (89.39%) and high-grade endometrioid from serous carcinoma (70.83%) compared to ER. But it was found to be more specific (88%) than ER in differentiating endometrioid and high-grade endometrioid from serous carcinoma (Table 4).

Mutant type p53 phenotype was seen in 39 cases in our study which comprised of 25 serous carcinomas (25/25 cases), one low-grade endometrioid carcinoma (1/42), five high-grade endometrioid carcinomas (5/24) and 8 other type II tumors. In our study, p53 was found to be 100% sensitive in differentiating endometrioid, including high-grade endometrioid carcinoma from serous carcinoma. It was highly specific (90.91%) in differentiating endometrioid carcinoma from serous carcinoma, but was found to be relatively less specific (79.17%) in differentiating high- grade endometrioid from serous carcinoma, as some high-grade endometrioid carcinomas also displayed p53 mutation type phenotype (5 cases) on IHC. p53 IHC had high NPV (100%), meaning presence of wild type p53 phenotype nearly rules out the possibility of serous carcinoma (Table 4).

We found that association of ER and PR was statistically significant between all three groups and hence can be used to differentiate Type I (almost always positive) from Type II (most commonly negative) tumors and also Type I-LG (always positive) from Type I-HG tumors (most commonly positive). With respect to p53 IHC, we found statistical significance between Type I-LG & Type I-HG tumors and between Type I-LG & Type II tumors, but there was no statistical significance between

Type I-HG & Type II tumors (p value=0.25), as even Type I-HG tumors showed p53 like Type II tumors (Table 3).

DISCUSSION

Endometrial carcinoma (EC) is second most common carcinoma of female genital tract and sixth most frequently diagnosed cancer in women worldwide. ECs are broadly categorized into two major categories: type I and type II based on clinical and molecular features. Type I tumors are associated with estrogen stimulation, often manifesting as low-grade lesions in perimenopausal women, presenting as low-stage tumors and tend to be clinically indolent. In contrast, type II carcinomas are not related to estrogen stimulation and are characterized by non-endometrioid histology, high-grade features and aggressive behavior. Serous carcinoma is commonest subtype and prototype of type II tumors (Wei JJ *et.al.,* 2013). Endometrioid carcinoma is the prototype of type I tumors, whereas type II tumors encompass a diverse range of histological subtypes, including serous carcinoma, clear cell carcinoma, carcinosarcoma, mixed carcinomas, and undifferentiated and dedifferentiated carcinomas according to International Society of Gynecological Pathologists (ISGYP) and World Health Organization (WHO) classification (WHO classification of tumors. Female genital tumors, 5th Edition. 2020).

In our study we encountered 66 cases of type I carcinoma out of 106 ECs accounting for 62.26% and 40 (37.74%) type II carcinomas which was in concordance with other studies (Wang, Y *et.al.,* 2023). We found 61 (61/106, 57.55%) tumors showing >50% myometrial invasion which was more than other study. LVI was found in 22 (22/106, 20.75%) carcinomas which was lower than other study (Devereaux, K.A *et.al., 2022*). Among type II carcinomas commonest was serous carcinomas (25/40, 62.5%), followed by carcinosarcomas (5/40, 12.5%), clear cell carcinoma (4/40, 10%), mixed carcinomas (3/40, 7.5%), undifferentiated (2/40, 5%) and dedifferentiated (1/40, 2.5%) which correlates with other studies (Hashmi AA *et.al.,* 2020).

We studied pathological characteristics of 106 cases of EC with respect to myometrial invasion, involvement of cervical stroma, LVI, FIGO stage and lymph node metastasis. All these variables were compared between low-grade endometrioid carcinoma (42 cases), high-grade endometrioid carcinoma (24 cases) and type II (40 cases) tumors to find their association in different tumor groups. In our study, myometrial invasion involving >50% thickness of uterine wall was seen in 61 cases. Highest incidence was seen in high-grade endometrioid carcinomas (70.8%) compared to type II tumors (60%) and low-grade endometrioid carcinomas (47.6%). Association in high-grade endometrioid carcinoma was statistically significant compared to low-grade endometrioid carcinoma (p value= 0.02). There was no difference in involvement of cervical stroma among three groups. Advanced stage (FIGO stage III/IV) was seen most commonly in type II tumors (22.5%) and high-grade endometrioid carcinomas (20.8%) compared to low grade endometrioid carcinomas (7.1%). Association was statistically significant when compared between them. Lymph node involvement was common in type II tumors compared to endometrioid carcinomas. LVI was seen frequently in high-grade endometrioid carcinomas (33.3%) and type II tumors (20%) compared to low-grade endometrioid carcinomas (14.3%). These findings show that, type II tumors and high-grade endometrioid carcinomas behave more aggressively and have high risk for recurrence due to high incidence of LVI and lymph node metastasis. Hence these tumors should be extensively staged and treated with combination of chemotherapeutic drugs. These tumors are potential candidates for molecular analysis and targeted therapy, field for future research.

Low-grade ECs showed strong and diffuse nuclear expression of ER and PR, with 100% immunoreactivity. In high-grade ECs, the immunoreactivity reduced, with 79.2% and 70.8% of cases showing reactivity for ER and PR respectively. The number of positive cells and staining intensity decreased as the grade of the tumor advanced. Even in high-grade endometrioid carcinomas, the staining for ER and PR was greater compared to serous carcinoma. Only 20% and 12% of serous carcinoma cases showed immunoreactivity for ER and PR respectively, with weak and focal staining. The overall positive rate of ER and PR in endometrioid carcinomas in the study was 92.4% and 89.4% respectively. In contrast, the positive rate in type II tumors (presumably serous carcinoma) was much lower, at 25% and 20% respectively. These findings corroborate with existing literature, indicating that hormone receptor expression, particularly ER and PR, can serve as useful markers for distinguishing between endometrioid and serous carcinomas, with endometrioid carcinomas generally showing higher expression levels compared to serous carcinomas (Salama A *et.al.,* 2019; Masjeed NMA *et.al.,* 2017; Yasuda M *et.al.,* 2014).

TP53 mutation status is a crucial molecular factor that predicts prognosis in ECs, with the presence of p53 mutation associated with an unfavorable outcome. Aberrant or mutant p53 expression is a hallmark of serous carcinoma and aids in distinguishing between serous and endometrioid carcinoma. p53 IHC is described as a quick, easy, inexpensive, and accurate surrogate for TP53 mutation analysis (Kobel M *et.al,* 2019; Garg K *et.al,* 2010). In the present study, the overall rate of p53 mutation type staining was 36.8%, with a higher prevalence in type II tumors (82.5%) compared to endometrioid carcinomas (9.1%). A small percentage of low-grade endometrioid carcinomas also showed p53 mutation type staining, suggesting a potentially more aggressive behavior, although there's insufficient evidence to predict their biological behavior (Fadare O *et.al.,* 2017). Some of high-grade endometrioid carcinomas (5 cases, 20.8%) showed p53 mutation type expression overlapping with serous carcinoma. High-grade endometrioid carcinomas with mutation type p53 expression have a worse prognosis compared to those without such expression. Mutation type p53 immunostaining can serve as an indicator of the TCGA-based molecular subtype of endometrioid carcinoma, with the worst prognosis when incorporated into a diagnostic algorithm (Vermij L *et.al.,* 2020; Buza N *et.al.,* 2021).

We analyzed statistical significance to find association of ER, PR and p53 with low-grade endometrioid carcinoma, high-grade endometrioid carcinoma and type II tumors. We found association of ER and PR with both grades of endometrioid carcinomas to be statistically significant when compared with type II tumors. Their association with low-grade endometrioid carcinomas was also found to be significant when compared with high-grade endometrioid carcinomas. Number of positive cases reduced as the grade of endometrioid carcinoma advanced. While all grade 1 and 2 endometrioid carcinomas were positive for both ER and PR, only 79.2% and 70.8% of grade 3 endometrioid carcinomas were positive for ER and PR respectively. p53 is a reliable marker to distinguish low-grade endometrioid carcinoma from serous carcinoma (p value= 0.001) but this is not true when it comes to high-grade endometrioid carcinomas (p value=0.25) as some of the grade 3 endometrioid carcinomas also displayed p53 mutation type phenotype like most of serous carcinoma. In our study, we also found the association to be statistically significant (p value=0.001) between high-grade and low-grade endometrioid carcinomas, as p53 mutation was detected in 20.8% of high-grade tumors as compared to only 2.4%

of low-grade tumors.

We analyzed sensitivity, specificity, positive predictive value and negative predictive value of ER, PR and p53 to validate their utility in distinguishing between endometrioid and serous carcinomas and most importantly between high-grade endometrioid and serous carcinomas as high- grade tumors with ambiguous morphology are a diagnostic challenge. We found ER to be a more sensitive marker and PR to be a more specific marker to distinguish endometrioid carcinoma and high-grade endometrioid carcinoma from serous carcinoma. Sensitivity of ER and PR to distinguish between high-grade endometrioid carcinoma and serous carcinoma reduced compared to that between endometrioid and serous carcinoma. This was because some high grade endometrioid carcinomas were negative for ER (20.8%) and PR (29.2%) like most serous carcinomas.

p53 IHC is described as an accurate surrogate test to detect TP53 mutation, with a sensitivity of 100% in the study, making it a reliable tool to diagnose serous carcinoma. But, p53 IHC is less reliable in distinguishing serous carcinoma from high-grade endometrioid carcinoma as some high-grade endometrioid carcinomas also displayed mutation-type immunophenotype for p53 (20.8%), reducing its specificity as a marker for serous carcinoma. An abnormal pattern of p53 expression is observed in approximately 80% to 90% of serous carcinoma cases. However, a caveat is noted regarding the reliance on p53 expression alone, as some endometrioid adenocarcinomas (~10%), particularly FIGO grade 3 cases, may also exhibit aberrant p53 expression. In cases with ambiguous morphology where a definitive histologic subtype cannot be established, aberrant p53 expression has been correlated with adverse clinical outcomes (Garg K *et.al.,* 2010; Ragni N *et.al.,* 2005).

This study highlights challenges in distinguishing between endometrioid and serous carcinomas solely based on individual immunohistochemistry (IHC) markers. It emphasizes importance of using a panel of IHC markers along with careful attention to morphological details to arrive at a diagnosis, particularly in difficult cases. Approximately 25% to 30% of endometrial serous carcinomas exhibit HER2 overexpression or amplification. Patients with HER2-positive tumors have been shown to derive significant survival benefits from targeted HER2 therapy, particularly with the use of trastuzumab. The National Comprehensive Cancer Network (NCCN) Uterine Neoplasm Guidelines have endorsed the addition of trastuzumab to standard chemotherapy as the preferred regimen for the treatment of HER2-positive tumors in advanced or recurrent endometrial serous carcinoma (Quddus MR *et.al.,* 2020; Vermij L *et.al.,* 2020). In our study HER2 IHC was performed on 106 cases of endometrial carcinoma. A 3+ score, indicating strong HER2 expression, was observed in 7.5% of cases which included serous carcinomas, a grade 3 endometrioid carcinoma, and a carcinosarcoma. Overall, HER2 IHC can provide valuable information in identifying HER2-positive endometrial carcinomas, particularly in the context of treatment decisions and prognosis assessment, in conjunction with other diagnostic markers and clinical factors.

Limitations of the Study:

1. Small sample size especially with respect to high grade endometrial carcinoma was a major limitation as expression of immunohistochemical markers is highly heterogenous among and within tumors with significant overlap between grade 3 endometrioid and type II tumors, emphasizing need to study on a big sample size.
2. Some high- grade endometrial carcinomas having ambiguous immunohistochemical findings were omitted from the study. Such cases require further marker study or molecular analysis for categorization of the tumor.

3. Limited IHC panel due to resource constraints was a major drawback of our study.
4. Lack of correlation with molecular findings.
5. Follow-up details of the patient's status were not adequately available for statistical analysis and most patients were lost to follow-up.

Conclusion:

Although there are many immunomarkers that have been reported to have utility in differential diagnosis of ECs, our experience is that most of difficult cases can be accurately classified based on 3 immunomarkers (p53, ER and PR) along with close attention to morphologic details. When used as a panel, specificity and sensitivity for differential diagnosis of EC can be significantly improved. P53 IHC is an accurate, quick, easy and inexpensive surrogate test to detect TP53 mutation status in high-grade ECs. Her2 IHC is a reliable test to detect Her2 amplification and may be recommended to perform on all endometrial serous carcinomas and mixed carcinomas having serous component to target them for trastuzumab therapy.

Declarations:

Ethics Approval: Institutional (The Gujarat Cancer and Research Institute) approval was taken. (**IRC/23/2019 dated 14/11/2019).**

Conflict of Interest: The authors declare no conflict of interest.

Author contribution: Each author took part in the design of the study, contributed to data collection, and participated in writing the manuscript. The manuscript is neither being published nor being considered for publication elsewhere until a decision is reached by this journal. The authors declared no conflict of interest.

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