

HER2 Immunohistochemical Expression and p53 Patterns in Endometrial Carcinoma: Associations with Clinicopathological Features in Iraqi Women

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Abstract

Background: HER2 has emerged as a treatment-relevant biomarker in selected endometrial carcinomas. However, data on HER2 expression across histotypes, and its relationship with p53 status, are limited from the Middle East. We assessed HER2 and p53 immunohistochemistry in endometrial carcinomas from Iraq. **Methods:** This retrospective cross-sectional study, conducted at [n] centers in Karbala, Iraq, included 38 formalin-fixed paraffin-embedded endometrial carcinomas diagnosed between January 2024 and February 2025: 32 endometrioid carcinomas, four serous carcinomas and two carcinosarcomas. HER2 membranous staining was scored as 0, 1+, 2+ or 3+ according to [criteria]. p53 was classified as wild-type or abnormal (overexpression, null or cytoplasmic pattern). Proportions are reported with Wilson 95% confidence intervals (CIs). Group differences were tested with two-sided Fisher exact or Fisher-Freeman-Halton tests because of sparse cells. **Results:** HER2 was scored 0 in 25 cases (65.8%), 1+ in three (7.9%), 2+ in seven (18.4%) and 3+ in three (7.9%; 95% CI 2.7–20.8%). Ten tumors (26.3%; 95% CI 15.0–42.0%) showed HER2 2+ or 3+. Nineteen tumors (50.0%; 95% CI 34.8–65.2%) had abnormal p53 staining. The HER2 score distribution did not differ by p53 status ($P = 0.722$). All six non-endometrioid tumors were p53-abnormal, compared with 13 of 32 endometrioid tumors (40.6%; $P = 0.020$). HER2 2+/3+ was seen in three of four serous carcinomas, one of two carcinosarcomas and six of 32 endometrioid carcinomas (exploratory $P = 0.031$). **Conclusions:** In this Iraqi series, HER2 3+ staining was uncommon, while 2+ staining was more frequent. HER2 score was not associated with p53 status. Abnormal p53 was present in all non-endometrioid carcinomas and in a substantial proportion of endometrioid carcinomas. HER2 2+/3+ appeared more frequent in non-endometrioid histotypes, but the small subgroups mean larger studies with ISH confirmation are needed.

Keywords: Endometrial carcinoma; HER2; ERBB2; p53; TP53; Immunohistochemistry.

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INTRODUCTION

Endometrial cancer is one of the most common cancer-related causes of morbidity in women. Endometrial carcinoma is one of the most common cancers in women, with GLOBOCAN 2022 data reporting an estimated 420,000 new cases and 97,700 deaths due to the disease (1). The histotype, grading of the cancer, the extent of myometrial invasion, lymphovascular space invasion and staging of the disease are still critical factors in the diagnosis and risk assessment of endometrial cancer. However, the recent revisions to the International Federation of Gynecology and Obstetrics staging system (2023) and ESGO ESTRO ESP guidelines (2025) integrate molecular aspects (2, 3).

The molecular stratification by The Cancer Genome Atlas Consortium divides endometrial carcinomas into four groups: POLE ultramutated, mismatch repair-deficient or microsatellite instability-hypermutated, copy-number-low, and copy-number-high (4). Clinically practical surrogate algorithms are based on POLE sequencing, mismatch repair immunohistochemistry, and pattern-based p53 immunohistochemistry. In the PORTEC-3 cohort, the molecular class provides additional information regarding prognosis and possibly prediction of response to treatment (5). Professional guidelines recommend performing molecular testing in endometrial carcinomas where possible since the result may help in refining the risk and choosing appropriate treatment (3, 6).

Pattern-based p53 immunohistochemistry is a highly informative test since the correlation with TP53 mutation is high (7, 8). Diffuse and strong nuclear overexpression, complete absence in case of positive internal control, or definite cytoplasmic immunostaining are considered abnormal patterns; nuclear

heterogeneous immunostaining with varying intensity is wild type. The p53-abnormal group contains a large proportion of serous carcinomas, carcinosarcomas, and high-grade endometrioid carcinomas, and generally carries a poor prognosis; however, subclonal and tumors with multiple molecular classifiers should be interpreted cautiously (9, 10, 11, 12). Consequently, abnormal p53 staining should be considered a biomarker surrogate, not an established TP53 mutation, if sequencing was not performed.

Human epidermal growth factor receptor 2 is a transmembrane receptor tyrosine kinase encoded by ERBB2. Overexpression or amplification activates proliferative pathways and identifies tumors potentially responsive to HER2-targeted therapy. Endometrial carcinomas pose several specific problems for HER2 evaluation, such as intratumoral heterogeneity, basolateral or incomplete membranous staining, antibody-specific problems, and lack of an accepted scoring system for all histotypes (13, 14). Large molecularly annotated series showed that HER2 amplification is rare in unselected endometrial carcinomas but prevalent in p53-abnormal and high-risk tumors (15, 16, 17, 18). Lower HER2 immunoreactivity levels are present in different histological and molecular groups (16, 19).

The significance of accurate HER2 assessment is growing. Trastuzumab addition to carboplatin and paclitaxel increased the progression-free and overall survival in advanced or recurrent HER2-positive uterine serous carcinoma (20, 21, 22). Trastuzumab deruxtecan was active in HER2-expressing solid tumors, and in uterine carcinosarcoma, with the strongest activity in tumors with higher HER2 expression (23, 24, 25). Little data are available concerning the expression

and distribution of HER2 and p53 abnormalities in Iraqi endometrial carcinoma. Therefore, we aimed to quantitatively assess HER2 immunohistochemical scores in a multicentre archival series of Karbala patients and its association with p53 staining pattern,

MATERIALS AND METHODS

Study design and setting

This retrospective multicenter cross-sectional study involved archival formalin-fixed paraffin-embedded endometrial carcinoma tissue, collected from the Karbala Teaching Centre of Histopathology, Imam Zain Al-Abideen Hospital, Safeer Al-Imam Al-Hussain Hospital, and Al-Kafeel Hospital in Karbala, Iraq. The sampling interval was January 2024 to February 2025. The paper was reported according to the appropriate principles of the STROBE statement for observational studies (26).

Forty blocks of endometrial carcinoma were selected. Two blocks had inadequate tissue processing for reliable morphology or immunohistochemistry, thus resulting in 38 blocks for evaluation. Seven blocks were harvested from dilation and curettage, and 31 blocks – from hysterectomy. The criterion for case selection was availability of adequate tumor and basic clinicopathologic information. Blocks with inadequate tumor, heavy artifact, or tissue quality making it impossible to interpret were excluded. No a priori sample-size calculation was provided; all available cases meeting eligibility criteria comprised the analytical sample.

Histopathologic review and data collection

Hematoxylin and eosin sections were evaluated to confirm the diagnosis and define histotype and grade. The sample included endometrioid carcinoma, serous carcinoma, and carcinosarcoma. Age, specimen type, histotype, tumor grade, pathologic T category,

histotype, grade, T category, and lymphovascular invasion. It was hypothesized that high HER2 score would be more prevalent in non-endometrioid and p53-abnormal tumors.

and lymphovascular invasion were abstracted from the pathology material and accompanying records. The only T category specified as T1c was united with other T1 tumors for summarizing purposes since there is no current T1c category of endometrial carcinoma TNM classification. The pathologic T category was unknown in seven curettage cases and recorded as Tx. The lymphovascular invasion parameter was considered as missing in the curettage samples since it was impossible to assess intact tumor vascular interfaces reliably.

Three serial sections, each 5 µm thick, were made from each block. Hematoxylin and eosin staining was done in one section; the others were applied for HER2 and p53 immunohistochemistry. The diagnostic slides and immunostains were evaluated by pathologist before inclusion into the final dataset.

Immunohistochemistry

Sections were mounted on positively charged slides and baked at 60 °C for 2 hours. They were deparaffinized in two changes of xylene and rehydrated through a graded ethanol series. Heat-induced epitope retrieval was performed in citrate buffer (pH 6.0) at 95–100 °C for 60 minutes. Endogenous peroxidase activity was blocked for 5 minutes. Ready-to-use primary antibodies against p53 (Bio SB, Santa Barbara, CA, USA) and HER2 (Dako, Agilent Technologies, Glostrup, Denmark) were applied for 60 minutes. Detection used a mouse linker and a horseradish peroxidase–labelled polymer, each applied for 15 minutes.

3,3'-Diaminobenzidine (DAB) chromogen was applied for 10 minutes. Slides were then counterstained with hematoxylin, dehydrated and coverslipped. Invasive breast carcinoma showing strong, complete membranous HER2 staining served as the positive HER2 control (**Figure 1**), while normal breast tissue served as the negative control.

HER2 and p53 interpretation

HER2 was assessed by membranous tumor cell staining according to the predefined four-tier classification proposed by Vermij et al. (14). The score 0 corresponded to the absence of membranous staining or membranous staining in less than 10% of tumor cells. The score 1+ corresponded to faint membranous staining in at least 10%; the score 2+ corresponded to moderate membranous staining in at least 10%; the score 3+ corresponded to strong membranous staining in at least 10%. For the purpose of exploratory analysis, scores 2+ and 3+ were pooled as moderate to strong expression and compared with scores 0 and 1+. This classification describes only the staining intensity and does not represent the same as HER2 positivity since in situ hybridization was not done. More specifically, the score 2+ was still regarded as equivocal.

p53 staining was classified depending on the pattern but not on the simple presence of positive staining (7, 8, 10, 12). The wild-type pattern included heterogeneous nuclear staining with variable intensity and

distribution. The abnormal pattern included diffuse strong nuclear overexpression in more than 80% of tumor cells, complete absence of tumor cell nuclear staining with retention of staining in non-neoplastic internal control cells, or unequivocal cytoplasmic staining. As TP53 sequencing was not done, the manuscript uses the term p53-abnormal pattern without assuming immunohistochemistry equals to confirmed mutation. The numbers of individual sub patterns of abnormal pattern could not be retrieved for separate analysis.

Statistical analysis

Continuous age was described using mean, standard deviation, and range. Categorical variables were presented as number and percentage. Confidence intervals (95%) for the major biomarkers' percentages were computed using the Wilson method. The sparse contingency tables were analyzed using Fisher two-sided exact test for 2x2 tables and Fisher-Freeman-Halton two-sided exact test for other tables. In the first association analysis, the four-level HER2 scoring was associated with p53 pattern. In the second association analysis, p53 pattern was associated with clinicopathological groups. The comparison of moderate to strong HER2 staining among histotypes was an exploratory analysis, excluding cases with missing lymph vascular invasion. Multivariable modeling was not attempted due to insufficient sample and event sizes.

seven (18.4%) were curettage samples. Endometrioid carcinoma accounted for 32 cases (84.2%), serous carcinoma for four (10.5%), and carcinosarcoma for two (5.3%). Seventeen tumors were grade 1, 11 were grade 2, and 10 were grade 3. Twenty-four tumors were classified within T1, five as T2, and two as T3; T category was unavailable for seven

RESULTS

Cohort characteristics

The 38 patients had a mean age of 59.55 years with a standard deviation of 11.30 years and a range of 35 to 85 years. Eighteen patients (47.4%) were aged 60 years or younger and 20 (52.6%) were older than 60 years. Thirty-one specimens (81.6%) were hysterectomies and

curettage specimens. Lymphovascular invasion was present in 15 of 31 assessable cases (48.4%). Detailed clinicopathological characteristics are summarized in **Table 1**.

Representative histopathological features of the major histological types and tumor grades are shown in **Figure 2**.

Table 1: Clinicopathological Characteristics of the Study Cohort.

Characteristic	Overall (N = 38)
Age, years	
Mean $\pm$ SD	59.55 $\pm$ 11.30
Range	35–85
Age group, years	
≤ 60	18 (47.4)
> 60	20 (52.6)
Specimen type	
Dilation and curettage	7 (18.4)
Hysterectomy	31 (81.6)
Histological type	
Endometrioid carcinoma	32 (84.2)
Serous carcinoma	4 (10.5)
Carcinosarcoma	2 (5.3)
Tumor grade	
Grade 1	17 (44.7)
Grade 2	11 (28.9)
Grade 3	10 (26.3)
Pathologic T category	
T1	24 (77.4)
T2	5 (16.1)
T3	2 (6.5)
Unavailable (Tx)	7 (18.4)
Lymphovascular invasion	
Absent	16 (51.6)
Present	15 (48.4)
Unavailable	7 (18.4)
Values are n (%) unless otherwise specified. T category and lymphovascular invasion were recorded for 31 cases; percentages for their observed categories use 31 as the denominator. Unavailable cases are shown as 7/38 (18.4%). T1 includes source-recorded T1a (n = 12), T1b (n = 11), and one case labelled T1c. Percentages may differ from 100% by rounding.	

HER2 and p53 immunohistochemical findings

Twenty-five tumors (65.8%) were HER2 score 0, three (7.9%) were 1+, seven (18.4%) were 2+, and three (7.9%; 95% confidence interval 2.7% to 20.8%) were 3+. Thus, 13 tumors (34.2%; 95% confidence interval 21.2% to 50.1%) displayed some membranous HER2 staining and 10 (26.3%; 95% confidence interval 15.0% to 42.0%) had moderate-to-strong staining. The p53 pattern was abnormal in 19 tumors (50.0%; 95% confidence interval 34.8% to 65.2%) and wild type in 19.

The full HER2 score distribution did not differ between p53-abnormal and p53-wild-type tumors (Fisher-Freeman-Halton exact $P = 0.722$). Among p53-abnormal tumors, 11 of 19 were HER2 score 0, two were 1+, four were 2+, and two were 3+. Among p53-wild-type tumors, 14 of 19 were score 0, one was 1+, three were 2+, and one was 3+ (**Table 2 and represented Figure 3**). Moderate-to-strong HER2 staining occurred in six of 19 p53-abnormal tumors and four of 19 p53-wild-type tumors (Fisher exact $P = 0.714$).

Associations with clinicopathologic features

In addition, all four serous carcinomas and both carcinosarcomas had abnormal p53 staining, while among 32 endometrioid carcinomas only 13 (40.6%) were positive. The relationship between p53 staining pattern and histotype showed a marginal statistical

significance ($P = 0.020$). Abnormal p53 staining was more frequent in grade 3 tumors (eight of 10) than grade 1 (seven of 17) or grade 2 tumors (four of 11), however, exact statistical comparison between three groups failed to be statistically significant ($P = 0.115$). No associations were found between p53 staining pattern and age group or lymphovascular invasion; moreover, estimates for T category were extremely unstable due to the small sample sizes within several categories.

Moderate-to-strong HER2 staining was found in six out of 32 endometrioid carcinomas (18.8%, 95% confidence interval [CI] 8.9%-35.3%), in three out of four serous carcinomas (75.0%, 95% CI 30.1%-95.4%) and in one out of two carcinosarcomas (50.0%, 95% CI 9.5%-90.5%). Such exploratory comparison was statistically significant (exact $P = 0.031$). Score-specific counts of HER2 moderate-to-strong expression show that all three serous carcinomas with moderate or strong staining have 2+ score, while the single HER2-expressing carcinosarcoma was 3+. All three 3+ cases include two endometrioid carcinomas and one carcinosarcoma. Moderate-to-strong HER2 expression did not show any differences according to grade or presence of lymphovascular invasion (**see Table 3**).

Table 2: HER2 immunohistochemical scores according to p53 pattern

HER2 score	p53 abnormal, n (%)	p53 wild type, n (%)	Total, n (%)
0	11 (57.9)	14 (73.7)	25 (65.8)
1+	2 (10.5)	1 (5.3)	3 (7.9)
2+	4 (21.1)	3 (15.8)	7 (18.4)
3+	2 (10.5)	1 (5.3)	3 (7.9)
Total	19 (100.0)	19 (100.0)	38 (100.0)
Exact $P = 0.722$ for the four-level HER2 distribution by p53 pattern.			

Table 3: Biomarker Associations with Pathologic Features

Pathologic feature	Total N	p53 abnormal n (%)	Exact P	HER2 IHC 2+/3+ n (%)	Exact P
Histological type					
Endometrioid carcinoma	32	13 (40.6)	0.020	6 (18.8)	0.031
Serous carcinoma	4	4 (100.0)		3 (75.0)	
Carcinosarcoma	2	2 (100.0)		1 (50.0)	
Tumor grade					
Grade 1	17	7 (41.2)	0.115	4 (23.5)	0.577
Grade 2	11	4 (36.4)		2 (18.2)	
Grade 3	10	8 (80.0)		4 (40.0)	
Lymphovascular invasion					
Absent	16	7 (43.8)	0.479	5 (31.3)	1.000
Present	15	9 (60.0)		4 (26.7)	
Values are n (%) within each category; Total N gives the number assessable for the feature. Exact P values compare all categories within each feature: two-sided Fisher–Freeman–Halton tests for histological type and grade, and two-sided Fisher exact tests for lymphovascular invasion.					

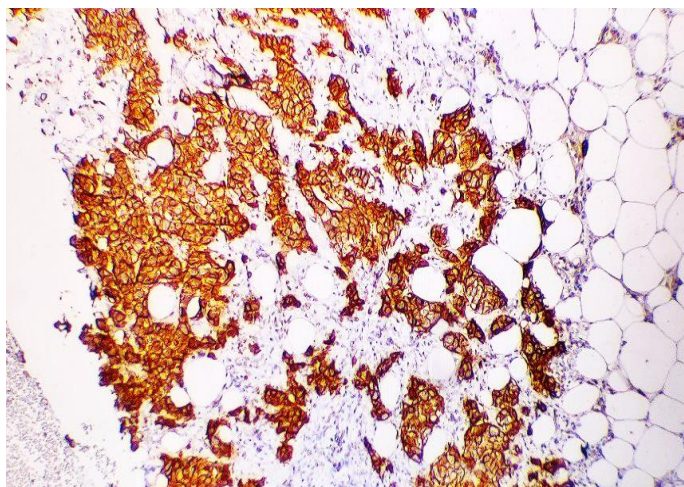
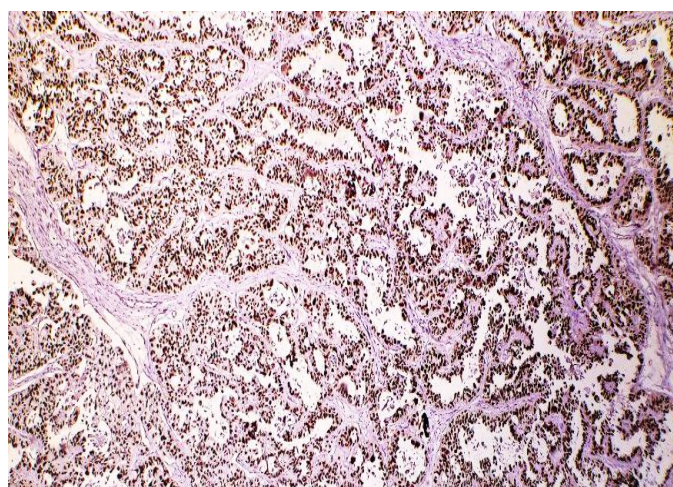
A**B**

Figure 1: Positive control for HER2 immunohistochemistry. Invasive ductal carcinoma of the breast showing strong, complete membranous HER2 immunoreactivity in tumor cells (HER2 IHC, ×100X).

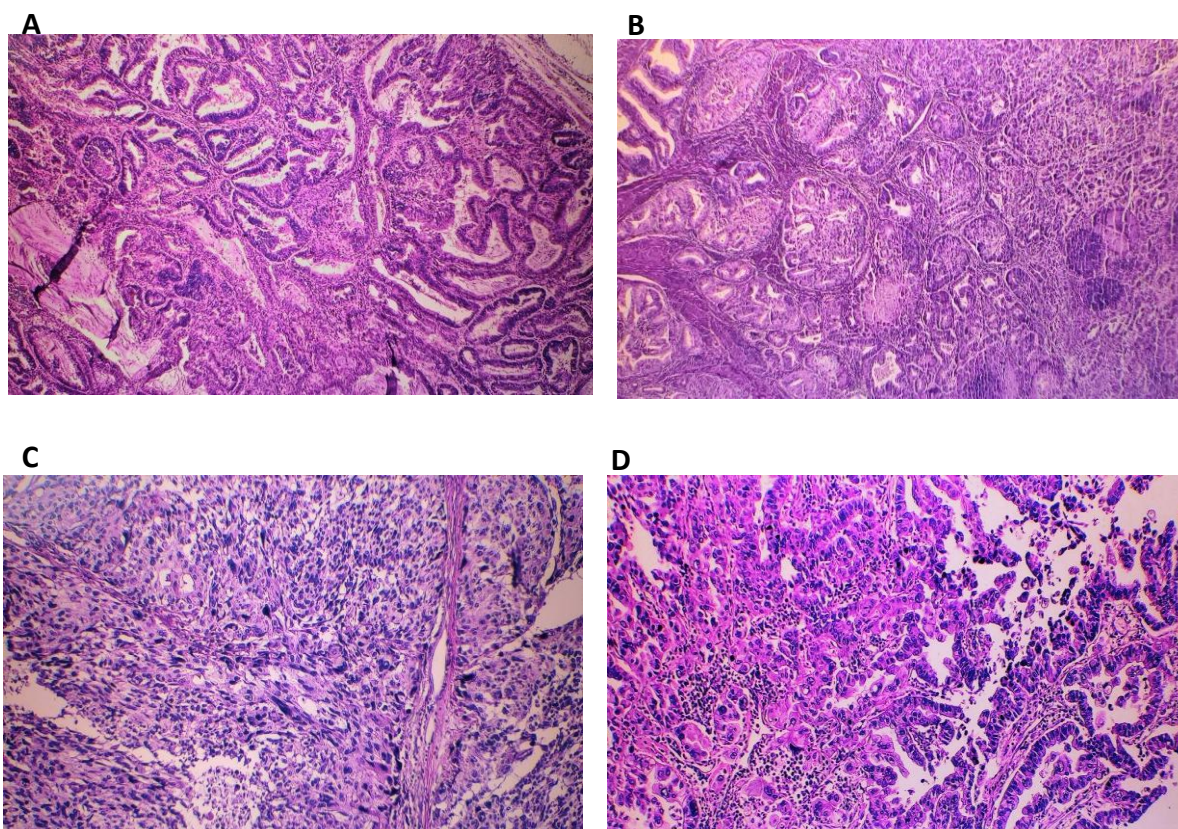


Figure 2: Representative histopathological features of endometrial carcinoma. (A) Grade 1 endometrioid carcinoma; (B) Grade 2 endometrioid carcinoma; (C) Grade 3 endometrioid carcinoma; and (D) serous carcinoma. Hematoxylin and eosin (H&E) staining, original magnification $\times 100X$.

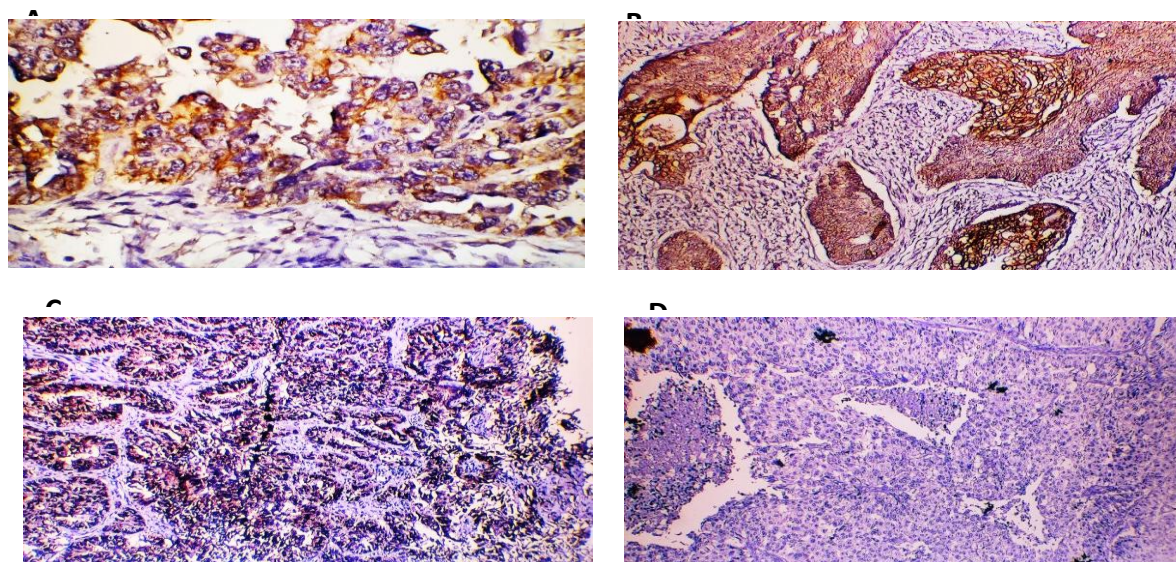


Figure 3. Representative HER2 and p53 immunohistochemical staining patterns in endometrial carcinoma. (A) HER2 score 2+ showing moderate membranous staining in serous carcinoma; (B) HER2 score 3+ showing strong membranous staining in endometrioid carcinoma; (C) p53-abnormal overexpression pattern showing diffuse strong nuclear staining; (D) p53-abnormal null pattern showing complete absence of tumor nuclear staining with preserved internal control staining. Original magnification $\times 100X$.

DISCUSSION

This study offers an initial description of HER2 and p53 immunohistochemical profiles in endometrial carcinoma tissue samples obtained from four pathology departments in Karbala. There are three relevant findings here. First, strong HER2 expression was found in three out of 38 tumors, while seven tumors were equivocal at 2+. Second, no difference was found in the HER2 four-level score distribution between p53-abnormal and p53-wild-type tumors. Third, all six non-endometrioid tumors had abnormal p53, and there was a higher incidence of moderate or strong HER2 expression in serous carcinoma and carcinosarcoma compared with endometrioid carcinoma. This is consistent with biology, but remains exploratory due to the limited number of cases in non-endometrioid subtypes.

The overall frequency of HER2 expression in this sample was 34.2%, with 7.9% having 3+. Such a comparison should be made with great caution due to different antibodies used, methods of HER2 detection, the size of the area sampled, scoring criteria, composition of the cohort by histotype and, last but not least, lack of in situ hybridization. In the population-based sample of 1,239 endometrial carcinomas, Aro et al found HER2 expression in 21.3% but 3+ expression only in 1.0% of tumors; in all 3+ tumors, amplification was found, while only 20% of 2+ tumors were amplified (15). In the high-risk cohort of recurrent or metastatic patients studied by van Dijk et al, 17.2% were HER2-low and 7.9% were HER2-positive based on immunohistochemistry and dual in situ hybridization (16). HER2 frequency in the 3+ category in the current small series is comparable to the latter high-risk sample; however, in situ hybridization was not done in

our case, which prevents us from applying the same definition of HER2 positivity.

Histotype composition can explain some of the differences. The large sample sizes of ERBB2 amplifications in serous carcinoma, carcinosarcoma, clear cell carcinoma and high-grade endometrioid carcinoma have been consistently reported (15, 16, 17, 20, 22). Also, Ross et al showed that HER2-amplified endometrial carcinomas are phenotypically and molecularly diverse, not limited to classic serous morphology (17). In our sample, 3 out of 4 serous carcinomas were 2+ in HER2 expression, one of two carcinosarcomas was 3+, and 6 out of 32 endometrioid carcinomas were 2+ or 3+. Such a composition may not be considered stable estimates due to wide confidence intervals; however, it demonstrates the reasons why restricting testing to histotypes is insufficient to cover potentially relevant expression.

Distinguishing protein expression from gene amplification is crucial. HER2 IHC 3+ commonly correlates with ERBB2 amplification, but the link is far from perfect and assay dependent; HER2 IHC 2+ is deliberately ambiguous, and in situ hybridization must be performed as a reflex test if the traditional category of positive HER2 is desired (13, 14, 15, 18). There can be partial staining along the basolateral borders and substantial heterogeneity in endometrial cancers. Multi-institutional studies demonstrated only modest-to-substantial agreement between observers even after introduction of a specific training module for scoring (13). The present scoring scheme required more than 10% of tumor cells and did not record any heterogeneity analysis, repeat scoring, or reflex FISH. Consequently, the 26.3% prevalence of 2+ or 3+ staining is an estimate of immunophenotype, not the

prevalence of actionable ERBB2 amplification.

Abnormal p53 staining was seen in half of the tumors. All serous carcinomas and carcinosarcomas had an abnormal p53 pattern, while 40.6% of endometrioid carcinomas did so. The pattern is consistent with the molecular paradigm according to which p53-abnormal cancers belong to the group of copy-number-high and aggressive cancers (4, 5, 12). An abnormal p53 pattern in an endometrioid cancer is not an absolute contradiction. The p53-abnormal cancers may preserve endometrioid phenotype at the high grade and there are proven molecularly defined clinically significant subsets of p53-abnormal endometrioid tumors (5, 12). In the present study, eight of 10 high-grade tumors had abnormal p53 pattern, but grade distribution by histotype was not available and could not be used to assess its influence on the relatively high rate of p53-abnormality in endometrioid tumors.

p53 immunohistochemistry is a highly sensitive, but not 100% perfect marker of TP53 mutational status. Singh and coauthors described a good correlation between optimized p53 IHC and sequencing in biopsy samples of endometrial carcinomas (8). Further work in the area focused on negative, cytoplasmic, and subclonal staining pattern, the need for an internal control, and the influence of POLE or mismatch repair-deficient multiple classifiers on the results (9, 10, 11, 12). The present dataset included an aggregated abnormal staining group without TP53 sequencing, mismatch repair markers, or POLE testing. Therefore, it is proper to describe an abnormal p53 staining and not mutated p53 and improper to suggest a complete molecular class. Further regional studies should use the full algorithm and record

the subpattern and percent of subclonal staining.

In contrast to our hypothesis, HER2 score was not correlated with p53 pattern. It contrasts with the findings in large cohorts in which the majority of HER2 amplified cancers belong to the p53-abnormal molecular group (15, 16, 17, 18). There are at least three possible reasons for the apparent discrepancy. First, the HER2 staining was measured without confirmation of amplification in this study. Second, the number of tumors in this study is small, and some clinically relevant differences may be missed. Finally, the p53-wild type category was not subdivided into the mismatch repair deficient, POLE-mutated, and no-specific-molecular-profile groups. The current result should be considered as the lack of significant association, but not the proof of the biological independence of HER2 and p53.

This difference becomes clinically important in the therapeutic context. In cases of advanced or recurrent HER2-positive uterine serous carcinoma, the randomized phase II study conducted by Fader et al demonstrated that the addition of trastuzumab to carboplatin and paclitaxel increases the progression-free survival, with further analysis suggesting also overall-survival improvement (21). In the DESTINY-PanTumor02 study, trastuzumab deruxtecan had efficacy in various HER2-expressing solid tumors, including endometrial cancer, with more benefit in IHC 3+ tumors compared with IHC 2+ tumors (23, 25). The STATICE trial identified activity in previously treated uterine carcinosarcoma among prespecified HER2 expression groups (24). While these studies encourage testing in specific circumstances, they do not validate treating any IHC 1+ or 2+ endometrial carcinomas as HER2-positive. All the factors discussed above should remain relevant.

In the context of limited pathology resources, the staged testing approach appears to be a reasonable option. HER2 IHC will allow selecting score 0 and 1+ tumors and further testing the score 2+ tumors using reflexive dual-color in situ hybridization (FISH). Any strong 3+ staining should be confirmed with the indication of the percentage of the tumor area, completeness or basolateral character of the membrane staining, and confirmation requirements according to the selected therapy. While p53 staining pattern may help in detecting aggressive tumor biology and increasing testing cohort size, it should not serve as a reason for exclusion from HER2 testing because HER2 can be expressed independently of the p53 abnormalities or serous category (15, 16). Whole-slide analysis should be considered in the case of heterogeneity of HER2 staining.

There are some strengths of this study which are relevant to its setting. They include the use of whole sections from various Karbala services, inclusion of both biopsy and hysterectomy samples, preservation of complete HER2 score distribution and co-testing with p53 and pathologic features. Exact tests and confidence intervals appear to provide more accurate description of sparse tables. Representative micrographs were used for documentation of staining patterns.

There are many limitations like: The sample size is small and mostly represented by

CONCLUSIONS

In this 38-case Iraqi endometrial carcinoma series, HER2 IHC 3+ staining occurred in 7.9%, while 18.4% of tumors were equivocal at 2+. HER2 score distribution was not associated with p53 immunohistochemical pattern. Abnormal p53 staining was concentrated in non-endometrioid tumors, and

endometrioid carcinomas with only four serous carcinomas and two carcinosarcomas. The case selection is retrospective and potentially biased. Information on T category and lymphovascular invasion is not available in curettage samples. HER2 2+ and 3+ tumors are not subjected to further ISH, CISH, or DISH testing, nor is there any ERBB2 mutation analysis. The p53 result is unconfirmed by sequencing and not combined with the mismatch repair or POLE test. The antibody clone and catalogue information are not available in the thesis. Interobserver agreement is not evaluated. No data on recurrence, therapy, and survival were available. Various secondary analyses have been carried out without correction and thus the observed associations between nominal histotypes may be due to chance alone.

Future Iraqi studies, which should be much larger and prospective, require standardized preanalytic procedures, the recording of used antibodies and the platforms, the involvement of two independent observers or a consensus scoring, and reflex HER2 amplification testing of all 2+ results. An oversampling of serous carcinomas, carcinosarcomas, clear cell carcinomas, and high grade endometrioid carcinomas will increase precision. Complete molecular characterization with POLE sequencing, mismatch repair proteins, and p53 immunohistochemistry will allow to examine directly whether HER2 amplification is increased in p53 abnormalities.

at least moderate HER2 staining was more frequent in serous carcinoma and carcinosarcoma, but both findings were based on very small subgroups. The data support standardized HER2 reporting and reflex in situ hybridization for 2+ tumors, followed by larger studies using complete molecular

classification. They do not establish HER2 amplification, treatment eligibility, or prognosis.

DECLARATION

ETHICAL APPROVAL

The study was approved by the ethical committee from the Iraqi Board for Medical Specializations in Histopathology with approval ethical# IBMS-SUMS00325. The study adhered to the ethical principles of the Helsinki Declaration. As per the current study, since it is a retrospective study involving only the existing anonymous archived formalin fixed paraffin embedded tissue samples, the requirement for informed consent was waived by the IRB.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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DATA AVAILABILITY

The data set that analyzed and generated through the study are available from the corresponding author upon reasonable request.

AUTHOR CONTRIBUTIONS

H.M.A.; study design, data analyses, conceptualization, histopathological examination, manuscript drafting. M.F.; slides staining, data collection. S.J.; manuscript revision, statistical analyses and supervision. All authors reviewed and approved the final version of the manuscript

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