Expression of hormone receptors; p53 and CD44 in endometrial carcinoma and their prognostic significance

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Abstract: Background: Endometrial cancer is the most frequently occurring female genital cancer. Traditional prognostic factors for the disease are histological type, grade, depth of myometrial invasion and tumor stage. The current diagnostic procedures are insufficient to identify endometrial cancer patients with poor prognosis. **Objective:** To evaluate the prognostic significance of immuno-histochemical markers (estrogen receptor "ER", progesterone receptor "PR", p53 and CD44) in endometrial carcinoma (EC) and correlate the results with known predictors of survival to avoid overtreatment of low-risk groups and to ensure adequate postoperative treatment for patients with highly aggressive tumors. **Materials and Methods:** The study was carried out on 80randomly selected endometrial carcinoma biopsies from archives of pathology records (15 curettage specimens and 65 hysterectomy specimens).Archival specimens included62endometrioid carcinomas (EMC)and 18endometrial serous carcinomas (ESC). Paraffin sections of 4–5 µm thickness were stained with H&E to confirm their histological diagnosis and grading. Immunohistochemical expression of hormone receptors (ER& PR), p53 and CD44were evaluated in all biopsies and correlated with known predictors of survival. **Results:** Hormone receptors ER and PR were more often positive in endometrioid than in serous tumors. Uterine endometrioid carcinomas showed significantly higher CD44 expression than did uterine endometrial serous carcinomas, the reverse was seen in p53 expression where ESC showed higher expression than EMC. **Conclusion:** Expression of hormone receptors (ER and PR) and CD44 were associated with low-grade and early stages of endometrioid carcinomas and they were mostly negative in aggressive endometrial serous carcinomas. Whereas, p53 overexpression was associated with high-grade and advanced stages of EMC and was also significantly higher in ESC. Thus ER, PR and CD44 high expressions could be considered as good prognostic markers, whereas p53 overexpression could be taken as a poor prognostic marker for endometrial carcinoma.

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Key words: Endometrial carcinoma, hormone receptors (ER and PR), p53, CD44, EMC, ESC, prognosis.

1. Introduction:

Endometrial cancer is the most common malignancy of the female genital tract, accounting for almost one half of all gynecologic cancers **(1).**

Endometrial cancers are classified into 2 broad histologic types, type I and type II. Type I consists of endometrioid carcinoma and its histologic variants; type II includes serous carcinoma, clear cell carcinoma, and carcinosarcoma. Endometrioid carcinoma (type I), accounts for about 80% to 85% of cases whereas endometrial serous carcinoma (type II), accounts for approximately 10% of cases. Clear cell carcinoma and other rare forms of endometrial carcinoma comprise the few remaining cases**(2).**

Although tumor histologic type, tumor grade, stage, depth of myometrial invasion, isthmus cervix extension and adnexal involvement are all established prognostic factors in cases of endometrial cancer **(3,4);** there is still need to define new prognostic indicators to anticipate the individual behavior of these tumors**(5).**

Immunohistochemical stains are extremely useful in resolving many of the problematic endometrial carcinoma cases. The knowledge in this area is constantly expanding and could be of an important prognostic significance **(6).**

Histologic subtypes and tumor differentiation may be important in determining the prognosis in early endometrial cancers. However, **Creasman.** [(7)] showed that hormone-receptor status (estrogen receptor "ER" and progesterone receptor "PR") also might be an important prognostic factor, particularly in early stage disease.

Ozsaran *et al.* [(8)] showed that p53 expression is more common in aggressive histologic subtypes than in endometrioid adenocarcinomas.Several studies support that ER, PR and p53 expression in primary tumors are independent prognostic markers**(9).**

CD44, a transmembrane receptor protein, belongs to the family of adhesion molecules, which are involved in cell–cell and cell–matrix interactions **(10).**CD44 binds to hyaluronic acid, which is a major component of the extracellular matrix, so it affects

tumor progression and metastasis **(11).** CD44 is one of the markers present on the surface of cancer stem cells, which are defined as a subset of tumor cells with the capacity to self-renew and give rise to the differentiated cells that comprise the bulk of the tumor **(12, 13).**There are limited and controversial reports about CD44 expression of various isoforms, and their prognostic value in cases of endometrial cancer **(14, 15).**

Thus, the main objective of this study was to evaluate the differences in immunohistochemical expression of ER, PR,p53 and CD44 in endometrial carcinoma and correlate the results with the known predictors of survival, such as histologic type, tumor grade, stage and depth of myometrial invasion to avoid overtreatment of low-risk groups and to ensure that patients with highly aggressive tumors receive adequate postoperative treatment.

2. Materials and methods:

Tissue specimens:

After the approval of the Research Ethics Committee of Tanta University, 80endometrial biopsies (15 curettage specimens and 65 hysterectomy specimen) of endometrial carcinoma were selected, from the files of Pathology Department (during period 2011-2015) referred from Obstetrics& Gynecology department, Faculty of Medicine, Tanta University. Complete history and clinicopathological features for each case were obtained from the pathology report and patient's accompanying clinical sheets. This study included: 62 endometrioid carcinomas and 18endometrial serous carcinomas.

Paraffin sections of 4–5 μm thickness were stained with H&E to confirm their histological diagnosis. Pathological stage and histological type were determined according to 1988 International Federation of Gynecology and Obstetrics (FIGO) criteria. Grading was performed both on the curettage and hysterectomy specimens according to World Health Organization (WHO) classification, based on percentage of solid growth and nuclear atypia. Endometrial carcinoma having 5% or less solid growth are designated as grade 1, grade 2 those with 6% to 50% solid growth and grade 3those with more than 50% solid growth. Non endometrioid tumors were all considered as high grade **(16).**

Features of endometrioid adenocarcinoma included a background of hyperplasia, squamous metaplasia, cribriform growth pattern and usually lower-grade nuclei. Features favoring serous carcinoma included a background of atrophy or endometrial polyp, ragged luminal borders, slit-like spaces within solid sheets of tumor cells and the coexistence of high-grade nuclei with a papillary or glandular growth pattern**(2).**

Immunohistochemical staining:

Immunohistochemistry was performed on formalin-fixed paraffin-embedded4 mm thickness sections mounted on positively charged slides. Tissue sections were deparaffinized and rehydrated in graded alcohols to distilled water, next they were incubated in 3% hydrogen peroxide for 10 min to block the endogenous peroxidase. Slides were immersed in acetic acid and heated in microwave at 95˚ C for 30 min for antigen retrieval then left to cool down at room temperature and rinsed with phosphate buffered saline (PBS) then they were incubated overnight at room temperature with primary antibodies.For detection of ER and PR, the tissue sections were incubated with monoclonal mouse antibodies (Dako M7047), diluted 1:50 and (Dako M3569), diluted 1:150 respectively **(17).**The antibody used for the detection of p53 was a mouse, antihuman monoclonal antibody, clones D07 (DAKO, Nutley, NJ), diluted 1:50**(18).** The primary antibody used for the detection of CD44 was a mouse monoclonal antibody (DAKO, Denmark; no. M7082),diluted 1:40and then with secondary antibody (Dako EnVision K4007 detection system)for 30 min and 3-3'diaminobenzidine (DAB) as chromogen **(19).**

The slides of positive and negative controls were included in each run. Positive controls were human ductal carcinoma of breast for ER, PR and p53and normal human tonsil sections for CD44. Negative controls were prepared by excluding the primary antibody and replacing it with phosphate buffer solution (PBS).

Interpretation and assessment of immunohistochemical staining of the studied markers:

ER, PR and p53 showed nuclear staining; while CD44 showed mainly membranous expression. Ten randomly chosen fields of each slide were scored for the evaluation of ER, PR, p53 and CD44.

The evaluation of ER and PR was performed according to the method described by **Carcangiu *et al.*[20]** based on the percentage of stained cells and the intensity of nuclear stain. The percentage of positive cells was graded as follows: **1**: 0 to 25% of the nuclei stained; **2**: 26 to 75% of nuclei stained; **3**: more than 75% of the nuclei stained. The staining intensity was scored as follows: 1: absent or weak; 2:moderate; and 3: strong. The sum of both parameters gave the immunohistochemical score (IHS). Tumors were divided into three categories depending on the IHS. Immunohistochemical scoreI corresponded to a score of 0-2, IHS II corresponded to a score of 3-4, and IHS III corresponded to a score of 5 or 6. Immunohistochemical score I tumors were considered as immunonegative, whereas IHS II and III tumors were considered as immunopositive.

Nuclear staining of p53 was scored as 0 to 2 (0= negative; 1=focal/patchy; 2=strong/diffuse ">70% tumor cells") **(21).**

Percentages of CD44expression in cancer cells (mainly membranous staining) were scored as: 0 for 0–5 %, 1 >5–25 %, 2 > 25–75 % and 3 >75 % of cells **(19).**

Table (1): Distribution of immunohistochemical characteristics of 15 curettage specimens of endometrial cancer.

Variables	ER N (%)			PR N (%)			p53 N (%)			CD44 N (%)			
	I	II	III	I	II	III	0	1	2	0	1	2	3
Histological types(N=15)													
EMC (N=12) 80%	1(8.3)	3(25)	8(66.7)	--------	4(33.3)	8(66.7)	10(83.3)	--	2(16.7)	2(16.7)	2(16.7)	5(41.6)	3(25)
ESC (N=3) 20%	3(100)	--------	--------	3(100)	-------	-------	------	--	3(100)	2(66.7)	1(33.3)	----	------
P value EMC versus ESC	<0.001			<0.001			0.003			0.046			
Grading of EC													
Grade 1 (N=3)	----	-------	3(100)	------	----	3(100)	3(100)	--	----	------	------	2(66.7)	1(33.3)
Grade 2(N=7)	-----	3(42.9)	4(57.1)	-----	2(28.6)	5(71.4)	6(85.7)	--	1(14.3)	2(28.6)	2(28.6)	2(28.6)	1(14.2)
Grade 3(N=5)	4(80)	-----	1(20)	3(60)	2(40)	-------	1(20)	-	4(80)	2(40)	1(20)	1(20)	1(20)
***P* value**	0.008			<0.001			0.015			0.351*			

N= Number, ER=Oestrogen Receptor, PR = Progesterone Receptor, EMC= Endometrioid carcinoma, ESC= Endometrial serous carcinoma.
ER; PR (1 =IHS I= negative- II&III= IHS II&III= positive).
p53(0= negative; 1=focal/patchy; 2=strong/diffuse).
CD44 (0 = negative for 0–5 %, 1 for 5–25 %, 2 for 25–75 % and 3 for >75 % cells).
*= non-significant, *P* value ≤0.05 significant.

Statistical Analysis

Statistical analysis was performed with statistical package for the social sciences software (SPSS, version 20; Chicago, Illinois, USA). $P \leq 0.05$ was considered statistically significant.

3. Results:

The clinicopathologic data of the studied cases were obtained from pathological archives and patients accompanying clinical sheets. The median age of the studied patients was 50.4 years (range 32-74 years) including 17 premenopausal and 63 post menopausal cases.

Three out of the 17 premenopausal cases in the present study were symptomless. These were 2 endometrial biopsies taken by curettage for exploration of infertility and1 hysterectomy specimen for uterine prolapse. The rest of premenopausal cases (14) were **7** from curettage specimens and **7**hysterectomy specimens presented clinically with menometrorrhagia. The 63 post menopausal cases included 6 from curettage specimens and 57hysterectomy specimens presented clinically with post menopausal uterine bleeding.

Curettage from15EC cases were submitted for immunohistochemical analysis and for grading as it reflects mostly the part of tumor protruding in the uterine cavity not deeper parts of the tumor. The results of the four investigated biomarkers (ER, PR, p53& CD44) were summarized in **Table 1**. However, the results of these biomarkers in 65 hysterectomy specimens of endometrial cancer were summarized in **Table 2**.

Three of four investigated biomarkers (ER, PR& p53) showed mainly nuclear staining. Loss of either ER or PR expression (as opposed to positive expression in normal endometrium) was more pronounced in ESC both in curettage (100%) and hysterectomy (86.7%-80% respectively) and was associated with high grade in curettage specimens (80%, 60% respectively). Also in hysterectomy specimens loss of either ER or PR expression was associated with high grade (65.3%, 60.9% respectively), advanced FIGO stage (82.4%-76.4 %respectively) and deep myometrial invasion (55.2%-51.7% respectively). ER or PR expression was significantly inversely, correlated, with grade, stage and myometrial invasion **(Figs. 1& 2).**

Strong diffuse expression of p53 was demonstrated in 31.2% of cases: from both curettage (5/15) and hysterectomy (20/65). Only two hysterectomy cases of EMC grade 2 and 3 showed focal p53 expression. On the other hand, p53 loss was recorded in 66.2%, from both curettage (10/15) and hysterectomy (43/65). Immunostaining ofp53was mostly strong and diffuse in ESC cases both in curettage (100%) and hysterectomy (86.7%). Also most cases of high grade endometrioid carcinoma showed high expression of p53.Thus high expression of p53 in hysterectomy specimens was significantly correlated, with advanced FIGO stage (70.6%) and deep myometrial invasion (55.2%) **(Fig.3).**

CD44expression was mainly membranous in tumor cells **(Fig.4).** CD44 like ER and PR was more expressed in EMC than in ESC specimens, both from curettage (83.3%) and hysterectomy (72%). Loss of CD44 was detected in 16.7% of EMC and 66.7% of ESC in curettage and in 28% of EMC and 73.3% of ESC in hysterectomy. CD44 immuno-expression was inversely correlated, with high grade (26%), high stage (29.4%) and myometrial invasion (34.5%).

Thus loss of ER/PR, negative expression of CD44 and overexpression of p53, all were associated with bad prognosis, high grade, stage and deep myometrial invasion.

Table (2): Distribution of immunohistochemical characteristics of 65 hysterectomy specimens of endometrial cancer.

Variables	ER N (%)			PR N (%)			p53 N (%)			CD44 N (%)			
	I	II	III	I	II	III	0	1	2	0	1	2	3
Histological types(N=65)													
EMC (N=50) 76%	8(16)	12(24)	30(60)	7(14)	12(24)	31(62)	41(82)	2(4)	7(14)	14(28)	8(16)	16(32)	12(24)
ESC (N=15) 24%	13(86.7)	2(13.3)	-----	12(80)	2(13.3)	1(6.7)	2(13.3)	------	13(86.7)	11(73.3)	3(20)	1(6.7)	------
***P* value EMC versus ESC**	<0.001												
Grading of EC													
Grade 1 (N=18)	-----	1(5.6)	17(94.4)	-----	2(11.1)	16(88.9)	18(100)	-----	-----	3(16.7)	2(11.1)	6(33.3)	7(38.9)
Grade 2 (N=24)	6(25)	8(33.3)	10(41.7)	5(20.8)	7(29.2)	12(50)	22(91.6)	1(4.2)	1(4.2)	5(20.8)	6(25)	8(33.3)	5(20.8)
Grade 3 (N=23)	15(65.3)	5(21.7)	3(13)	14(60.9)	5(21.7)	4(17.4)	3(13)	1(4.4)	19(82.6)	17 (74)	3(13)	3(13)	-----
***P* value**	<0.001												
FIGO stage													
I (N=36)	4(11.1)	10(27.8)	22(61.1)	3(8.3)	9(25)	24(66.7)	30(83.3)	2(5.6)	4(11.1)	8 (22.2)	5 (13.9)	14 38.9)	9 (25)
II (N=12)	3(25)	2(16.7)	7(58.3)	3(25)	3(25)	6(50)	8(66.7)	-----	4(33.3)	5(41.6)	2 (16.7)	2(16.7)	3(25)
III (N=17)	14(82.4)	2(11.8)	1(5.8)	13(76.4)	2(11.8)	2(5.8)	5(29.4)	-----	12(70.6)	12(70.6)	4(23.5)	1(5.9)	-------
***P* value**	<0.001												
Myometrial infiltration													
<50% (N=36)	5(13.9)	5(13.9)	26(72.2)	4(11.1)	6(16.7)	26(72.2)	30(83.3)	2(5.6)	4(11.1)	6(16.7)	5(13.9)	13(36.1)	12(33.3)
>50%(N=29)	16(55.2)	9(31)	4(13.8)	15(51.7)	8(27.6)	6(20.7)	13(44.8)	-----	16(55.2)	19(65.5)	6(20.7)	4(13.8)	------
***P* value**	<0.001												

N= Number, ER=Oestrogen Receptor, PR = Progesterone Receptor, EMC= Endometrioid carcinoma, ESC= Endometrial serous carcinoma.ER; PR (1 =IHS I= negative- II&III= IHS II&III= positive).
p53 (0= negative; 1=focal/patchy; 2=strong/diffuse).CD44 (0 = negative for 0–5 %, 1 for 5–25 %, 2 for 25–75 % and 3 for >75 % cells).
P value ≤0.05 significant.

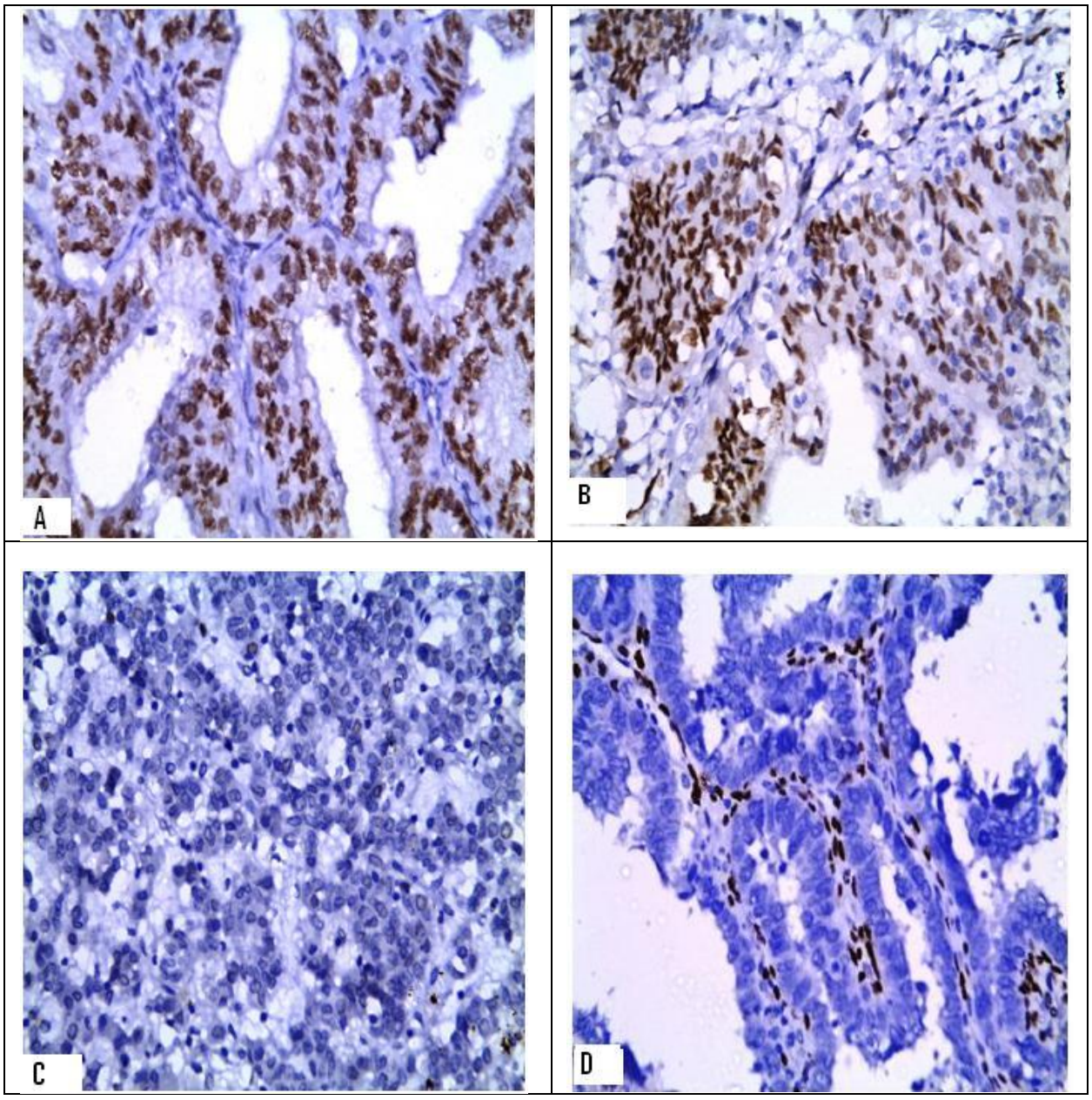

Fig. (1): Immunohistochemical expression of ER, x400: (A) Well-differentiated endometrioid carcinoma showing strong nuclear expression (IHS III); (B) Moderately differentiated endometrioid carcinoma showing moderate expression (IHS II); (C) Poorly-differentiated endometrioid carcinoma showing negative expression (HIS I); (D) Endometrial serous carcinoma showing negative expression with positive stromal expression (HIS I).

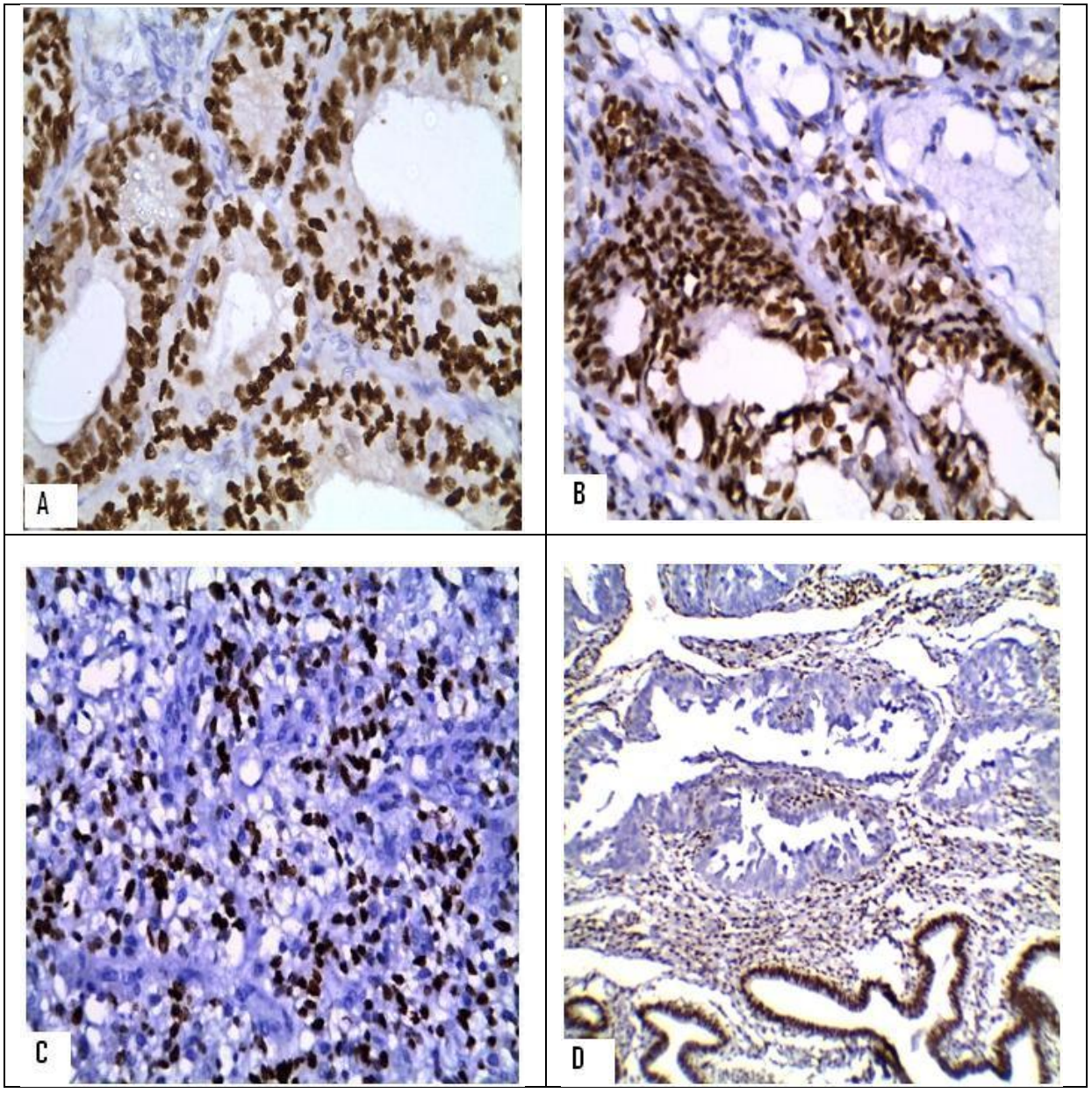

Fig.(2): Immunohistochemical expression of PR: (A) Well-differentiated endometrioid carcinoma showing strong nuclear expression (IHS III), x400; (B) Moderately differentiated endometrioid carcinoma showing strong expression (IHS III), x400; (C) Poorly differentiated endometrioid carcinoma showing moderate expression (IHS II), x400; (D) Endometrial serous carcinoma showing negative expression (IHS I) and nearby normal endometrium (glands &stroma) showing strong expression, x200. (D) Endometrial serous carcinoma –ve(HIS I). (x400)

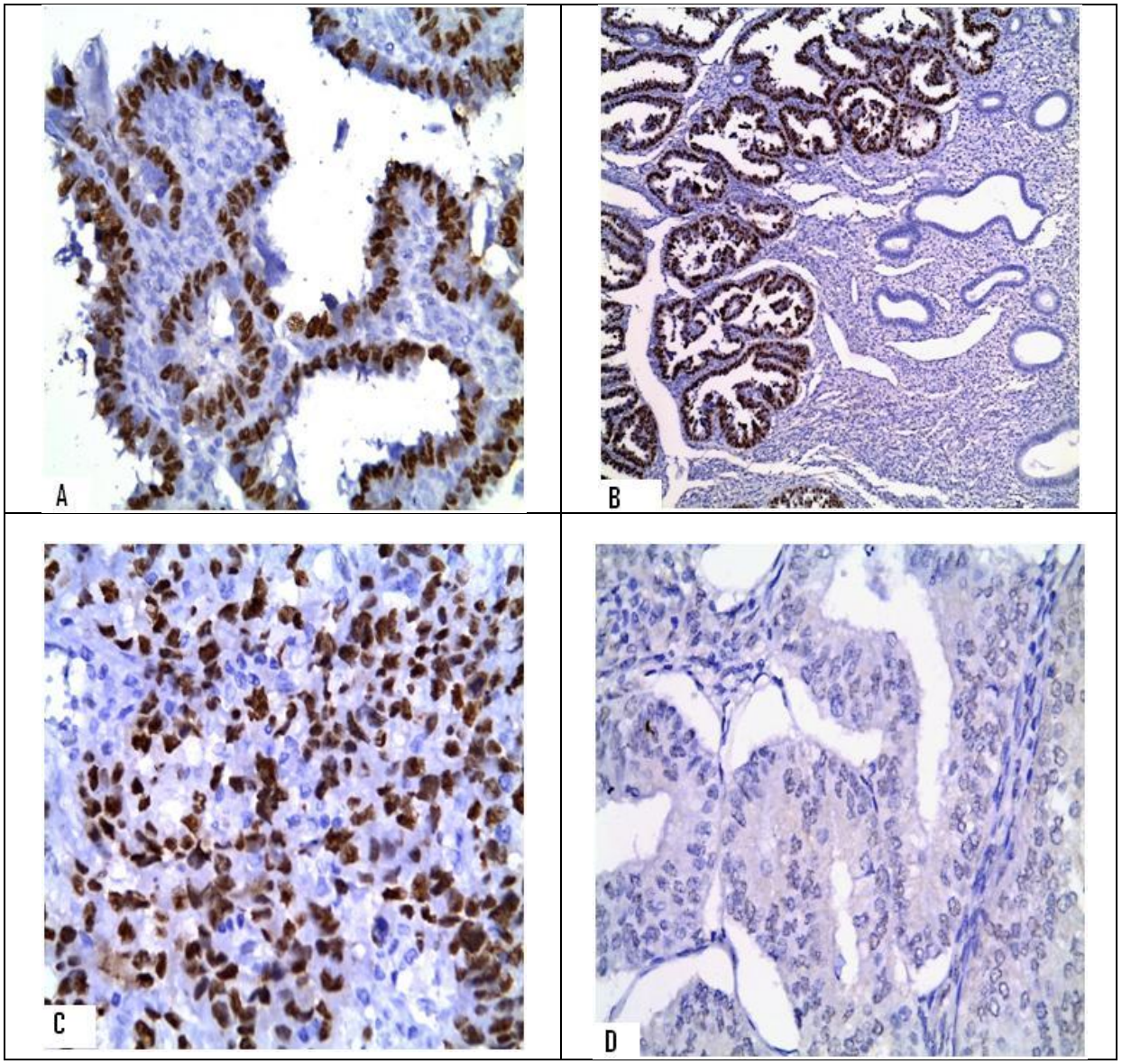

Fig.(3): Immunohistochemical expression of p53: (A) Endometrial serous carcinoma showing strong/diffuse nuclear expression, x400; (B) Another endometrial serous carcinoma showing strong/diffuse expression and normal endometrium showing negative expression, x200; (C) Poorly differentiated endometrioid carcinoma showing strong/diffuse expression, x400; (D)Well differentiated endometrioid carcinoma showing negative expression, x400.

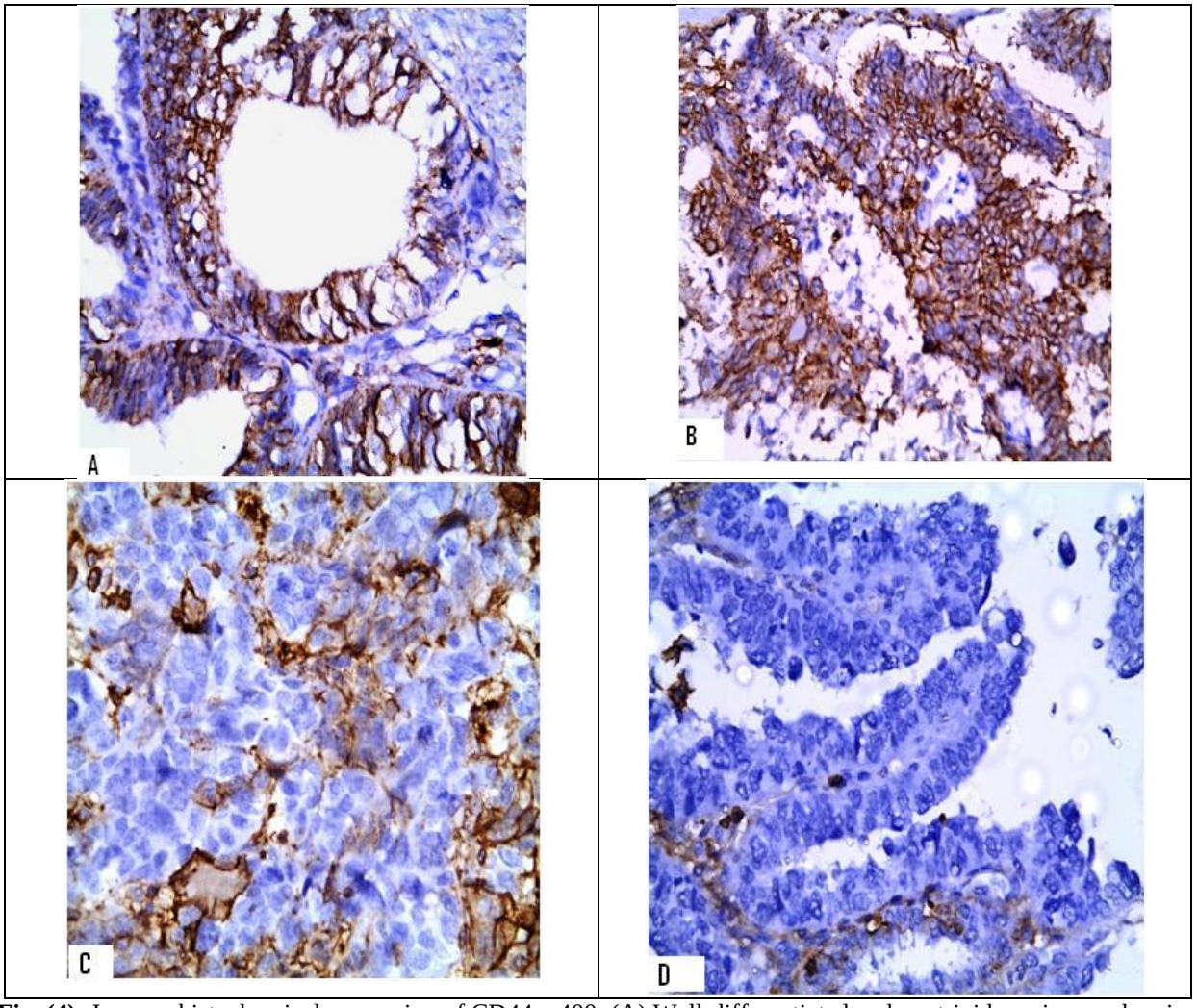

Fig. (4): Immunohistochemical expression of CD44, x400: (A) Well-differentiated endometrioid carcinoma showing strong membranous expression (score 3); (B) Moderately-differentiated endometrioid carcinoma showing strong expression (score 3); (C) Poorly-differentiated endometrioid carcinoma showing moderate expression (score 2); (D) Endometrial serous carcinoma showing negative expression (score 0).

4. Discussion

Endometrial cancer is currently the fourth most common cancer in women in developed countries and the most common among cancers of the female reproductive tract **(1, 22).** Menometrorrhagia was the most common clinical finding in premenopausal cases (14/17) in the present study as had been previously reported by **Hacker [23].**

Endometrial carcinoma is a disease of the elderly**(18).**In the present study 78.7% of patients were postmenopausal (63/80) complaining of uterine bleeding.

We have chosen evaluation of immunohistochemical expression of hormone receptors, p53 and CD44 in our study to carefully diagnose the status of EC and its progression.

In current study, ER and PR expressionswere prevalent in grades 1 and 2 EMCs, but they were generallynegative or weaker in serous and grade 3 EMCs. This coincided with the previous work of **Darvishian *et al.*[24]**. Also, **Wei *et al.*[2]** used hormone receptors to differentiate between endometrioid and serous carcinomas and generally showed strong diffuse expression in approximately 80% of low grades (grades 1 and 2) endometrioid tumors. They reported that the degree of immunoreactivity in high grade (grade 3) endometrioid tumors was markedly less, ranging from 15% to 50% and the frequencies overlap with those seen in ESC (5%–54%).

Therefore, the absence of ER and PR expression may be an important finding in the process of advancement of endometrial carcinogenesis as had been previously reported by **Li *et al.* [25]**.

In the present study overexpression of p53 was seen in more than 88% of ESCs, in contrast, to less than 18% of EMCs.p53 expression was mostly absent in grade 1 to grade 2 ECs tumors , whereas it was stronglypositive (82%) in grade 3 cases. The previous findings were in agreement with those previously reported by **Sherman *et al.*[26]** and **Lax *et al.*[27]**. They added that p53 immuno-histochemistry is therefore not recommended to differentiate grade 3 EMC from ESC.

The present study showed that p53 positivity in EC was 16.6% in early FIGO I stage and 70% in advanced FIGO III stage. This is similar to previous researchers **(18).**

Kounelis *et al.*[18] showed that the overall rate of p53 positivity in their study was 49%, with two thirds of cases were of either high grade or advanced stage (Stage III and IV), which is consistent with previous reports that described p53 positivity as approximately 10 to 15% in early- and 40 to 52% in advanced-stage EC **(28,29).** Also, as in previous studies, p53 positivity was significantly higher (76.2%) in endometrial serous than in endometrioid carcinomas **(30-32).**

We observed significantly stronger CD44 expression in endometrioid than in endometrial serous carcinoma both in hysterectomy and curettage specimens. We found negative CD44 expression in 72%ESCcases compared to 25% in EMC. Thus decrease CD44 in ESC could be related to poor prognosis with a stronger tendency for deep myometrial invasion. The biologic aggressiveness of this tumor type may, in part, be related to its lack of CD44 expression. Since, CD44 belongs to the family of adhesion molecules, binds to hyaluronic acid **(10)** and is involved in cell–cell and cell–matrix interactions, thus affecting tumor progression and metastasis **(11).**Our findings corresponded to those of **Hosford *et al.*[33]** where they found that 81 % of papillary serous carcinoma specimens did not express CD44 at all.

In the current study CD44 expression was significantly ($p<0.001$) weakin high-grade cancers in hysterectomy samples, but did not significantly ($p=0.351$) differ according to the grade in curettage specimens which may be attributed to less number of samples .In contrast, **Hoshimoto *et al.*[34]** found that overexpression of CD44v3 significantly correlated with higher grade.

However, **Stokes *et al.* [35]** found that the standard CD44 (sCD44) expression and depth of invasion were inversely correlated, which is similar to our findings. On the other hand, **Leblanc *et al.*[36]** reported CD44 expression to increase with depth of myometrial invasion and suggested that alterations of CD44 concentration could mainly be due to local invasion. Such inconsistent results may be due to different methodology and patient's number.

In most reports, CD44, and some of its variants were expressed significantly more in EC especially in early-stage disease. However, CD44 expression decreased as the disease became invasive and progressive **(5, 37).**Our results tended to support this hypothesis. Nevertheless, in some other reports, expression of CD44, CD44v3 and v6 increased with cancer stage **(34, 36, 38)** or showed no correlation **(39).**

In conclusion, we found in our series that high expression of the hormone receptor (ER&PR) and CD44 correlated well with low tumor grade, stage and myometrial invasion in EMC and was significantly negative or weak in aggressive ESC. Whereas, p53 overexpression was associated with high-grade, advanced-stage, and deep myometrial invasion in EMC and was also significantly higher in aggressive ESC. Thus ER, PR and CD44 high expressions could be considered as a good prognostic markers and p53 overexpression could be taken as a poor prognostic marker for EC.

It is recommended that if curettage was done for EC cases before surgical operation, it is better to do immunohistochemical stain using ER, PR and p53and CD44 to predict aggressiveness of the tumor. This is in-order to inform the surgeon to determine the appropriate surgical decision and the accompanying possible postoperative treatment required whether chemotherapy or radiation. Such procedure is important to avoid overtreatment of low-risk groups and to ensure adequate postoperative treatment for patients with highly aggressive tumors.

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