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OCT4, Ki-67 and VEGF as Prognostic Factors in Endometrial Carcinoma and Their Role in The Differentiation between Atypical Endometrial Hyperplasia and Grade 1 Endometrial Carcinoma: an Immunohistochemical study

Noha M. El -Anwar and Alaa I. Amer

Welcome letter from Editor-in-Chief

Welcome to the Int J Cancer and Biomedical Research (IJCBR)**!**

It is with great pleasure that I write this editorial to welcome you to the IJCBR. This journal provides a platform for publication of original and reviews research articles, short communications, letter to editor, thesis abstract, conference report, and case studies. These types of publication are directed at the interface of the fields of cancer and biomedical research.

The IJCBR relies on a distinguished expert of the Advisory and Editorial Board Members from the top international league covering in depth the related topics. They timely review all manuscripts and maintain highest standards of quality and scientific methodology and ethical concepts. Meanwhile, we take all possible means to keep the time of the publication process as short as possible.

I take this chance to welcome your contributions to the IJCBR and have every expectation that it will soon become one of the most respected journals in both the fields of cancer and biomedical research.

Mohamed L. Salem,

Editor in Chief

■■■ RESEARCH ARTICLE

OCT4, Ki-67 and VEGF as Prognostic Factors in Endometrial Carcinoma and Their Role in The Differentiation between Atypical Endometrial Hyperplasia and Grade 1 Endometrial Carcinoma: an Immunohistochemical study

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■■■ ABSTRACT

Background: Endometrial carcinoma develops through preliminary stages of endometrial hyperplasia. A population of epithelial stem cells was identified in the human endometrium, these cells may serve as a source of putative endometrial carcinoma. The most important member of cancer stem cell markers is OCT-4. Both tumor cell proliferation (Ki-67) and angiogenesis seem to be important for the development of aggressive tumors. **Aim of the Work:** This study aimed to evaluate the expression of OCT4, Ki-67 and VEGF in endometrial hyperplasia and endometrial carcinoma and to differentiate between grade 1 endometrial carcinoma and atypical endometrial hyperplasia. **Materials and Methods:** 60 cases of endometrial hyperplasia and 60 cases of endometrial carcinoma were stained by OCT4, Ki-67and VEGF immunohistochemical markers. **Results:** OCT-4 expression was significantly increased with advanced tumor grade and stage (P value = 0.001, 0.044) respectively. There was also significant association between Ki-67 expression and advanced tumor grade and stage (P value = 0.0016, 0.049) respectively. VEGF expression was significantly increased with advanced tumor grade and stage (P value = 0.001) for both. As regards the correlation of immunohistochemical results between atypical endometrial hyperplasia versus grade 1 endometrial carcinoma cases, Ki-67 was the marker with significant relation. **Conclusion:** OCT-4, Ki-67 and VEGF expressions showed a positive correlation with tumor aggressive pathological parameters. The Ki-67 labelling index can be used in differentiation between endometrial hyperplasia with atypia and grade 1 endometrial carcinoma.

Keywords: Endometrial, Carcinoma, Hyperplasia, Ki-67, OCT4, VEGF

■■■ ARTICLE INFO

INTRODUCTION

Worldwide, endometrial carcinoma is the sixth most common malignant tumor in women. It develops through preliminary stages of endometrial hyperplasia. Accurate diagnosis and optimal treatment of endometrial hyperplastic lesions are essential to prevent endometrial carcinoma development (Malpica et al., 2019).

It may be difficult, or sometimes even impossible, in a small biopsy specimen to distinguish between atypical endometrial hyperplasia and a grade 1 endometrial carcinoma as these two lesions don't have clearly defined boundaries. In patients with a diagnosis of atypical endometrial hyperplasia on an endometrial biopsy, there is a significant risk of finding a grade 1 endometrial carcinoma in a subsequent hysterectomy specimen (Yang et al., 2020).

Several histological features help to distinguish between atypical endometrial hyperplasia and grade 1 endometrial carcinoma. The desmoplastic stromal response, back-to-back glandular architecture with complete exclusion of stroma and bridging between adjacent glands

resulting in cribriform pattern and papillary architecture are in keeping with carcinoma. The differentiation between these two entities is important for surgical management or conservative treatment for patients wishing to preserve their childbearing potential (Murali et al.,2014).

A population of epithelial stem cells was identified recently in the human endometrium. The endometrial stem cell (ESC) associated proteins may contribute to tumorigenesis. OCT-4 is transcription factors that form a core regulatory network that determines endometrial stem cell (ESC) self-renewal and differentiation. In support of this hypothesis, clinical studies have shown that elevated expression of OCT-4 is associated with different tumors e.g. retinoblastoma, prostate cancer, metastatic germ cell tumors, and ovarian cancer (Lu et al., 2020).

The Ki-67 protein is a cellular marker for proliferation. Ki-67 is an antigen present in the nuclei of cells in all phases of the cell cycle as well as in mitosis, but quiescent cells in the G0 phase do not express it. Ki-67 is an excellent marker to determine the growth fraction of a cell population. The fraction of Ki-67 positive tumor cells (the Ki-67 labelling index) is often correlated with the clinical course of cancer. The best-studied examples in this context are carcinomas of the prostate, brain, breast and nephroblastoma (Li et al., 2013).

VEGF is important in carcinogenesis as it is responsible for the growth of the neoplasm and is also involved in the mechanism of metastasis. Angiogenesis is essential for tumor proliferation, progression and the formation of metastasis (Melincovici et al., 2018).

MATERIALS AND METHODS

This retrospective study included 60 cases of endometrial hyperplasia and 60 cases of endometrial carcinoma, total cases 120. Formalin-fixed paraffin-embedded blocks were provided from the Pathology Department, Tanta University. Inclusion criteria included all available cases of endometrial hyperplasia and primary endometrial carcinoma. Cases without a full clinicopathological sheet or bad quality of tissue blocks were excluded. This study was performed during the period from January 2017 to June 2020. All procedures performed in the current study (specifically involving human participants) were accepted from the Local Research Ethics Committee of Faculty of Medicine, Tanta University. Detailed clinicopathologic data of these patients were obtained from their medical reports. FIGO Grading and staging of endometrial carcinoma with subtyping of cases were according to the WHO classification 2014 (Kurman et al.,2014).

Immunohistochemical staining

Formalin-fixed paraffin-embedded tissue sections (4-µm) were mounted on to APES-coated glass slides (Chenglin, Shanghai, China). Slides were dewaxed in xylene twice for 10 min and rehydrated through graded ethanol. Antigen retrieval was performed in 0.01 mol/l citrate buffer (pH 6.0) by boiling for 10 min. Endogenous peroxidase activity was suppressed with 3% hydrogen peroxide for 10 min. After washing with phosphate-buffered saline (PBS), the slides were blocked with 5% BSA for 30 min at 37˚C. Sections were incubated with primary antibody to

- **OCT-4** antibody was an epitope-specific purified mouse monoclonal antibody raised against the OCT-4 antigen, (CA 95677) (Cell marque, CA, USA). It was received as 7 mL. Ready to use.
- **Ki-67** (Kit no. Cat. #RB-1510-R7 (7.0ml), labvision) (dilution 1:800)
- **VEGF** primary rabbit polyclonal antibody to human (Kit no. Cat. MA5-17041, Labvision), at 4˚C overnight in a humidified chamber. After washing three times with PBS, sections were incubated for 30 min with the secondary antibody (peroxidase goat anti-mouse IgG; dilution, 1:300; catalog no. 32230; Zymed, San Diego, CA, USA). After washing three times in PBS, 3,3'-diaminobenzidine (as chromogen) was used. Slides were counter-stained with hematoxylin for 1 min. Sections incubated without the primary antibody were used as negative controls.

Interpretation of OCT-4 immunostaining

OCT-4 staining was detected as brownish nuclear staining (Zhou et al., 2011) defined

immunohistochemical scoring for OCT-4 based on the percentage of immunopositive cells into negative (0), no staining in tumor cells; (+1) nuclear staining in more than 10% tumor cells; and (+2) nuclear staining in more than 50% tumor cells.

Ki-67 labelling index

The sections were examined at low magnifications (X40 and X100) to identify the area with the most intense staining. Only cells with a distinct nuclear Ki-67 staining were considered positive and the percentage of immunoreactive nuclei was calculated and scored as negative (no stained cells), low index (5% to 25%) and high index (more than 25 %) (Masjeed et al., 2017).

Interpretation of VEGF immunostaining

VEGF was detected as cytoplasmic brownish staining. Immunostaining score was classified according to the percentage and intensity of staining into negative, weak staining in less than 10% tumor cells. Moderate staining in 11 to 50%, and strong in more than 50% of tumor cells were considered as positive results, according to Guşet et al., 2010.

Statistical analysis

Statistical analysis was performed using a statistical package for the social sciences (SPSS version 23, IBM corp., Armonk, New York, USA). Categorical variables were expressed as frequencies and percentages, whereas mean± SD was used to express continuous variables. Chi-square (χ2) Test was performed for comparing categorical variables. Fisher's exact test was applied when one of the expected frequencies was up to 5. P values of less than 0.05 were considered statistically significant and those of less than 0.001 were considered highly significant.

RESULTS

1. Clinicopathologic Characteristics

This study was carried on 120 specimens of endometrial lesions, which were divided into (Group I: 60 cases of endometrial hyperplasia, Group II: 60 cases with endometrial carcinoma. The mean age of endometrial hyperplasia cases was 53.88 ± 12.36 years (range 32-84) and the mean age of endometrial carcinoma cases was

54.77 ± 9.81 years (range 35-81). One hundred cases complained of abnormal vaginal bleeding and discharge. Only two cases of endometrial carcinoma patients were presented with purulent vaginal discharge and a sense of pelvic pressure or discomfort. The remaining cases were found accidentally with other uterine lesions like fibroids.

2. Histopathological and immunohistochemical

According to WHO (2014) histopathological classification of endometrial hyperplasia cases were classified into (Kurman et al., 2014). Twenty-four cases of simple endometrial hyperplasia without atypia representing (40%) of endometrial hyperplasia cases, 16 cases of complex endometrial hyperplasia without atypia representing (26.7%) and 20 cases of atypical endometrial hyperplasia representing (33.3%) of endometrial hyperplasia cases.

Endometrial carcinoma cases were classified into 38 cases of endometrioid carcinoma representing (63.3%) of all endometrial carcinoma cases, 10 cases were clear cell endometrial carcinoma (16.7%), 10 cases of papillary serous endometrial carcinoma (16.7%) and 2 cases mucinous endometrial carcinoma (3.3%).

According to the FIGO grading system (2009) of endometrial carcinoma, the included 60 cases were graded as follows: seventeen cases of well-differentiated endometrial carcinoma representing 28.3% of the total number of studied endometrial carcinoma cases, 15 cases (28.3%) were moderately differentiated and 28 cases of poorly differentiated endometrial carcinoma representing 46.7% of the total number of the studied cases of endometrial carcinoma. Regarding myometrial invasion, 5 cases (8.3%) were limited to the endometrium without myometrial invasion. 15 cases (25%) invaded less than half the myometrium and 40 cases (66.7%) invaded more than half the myometrium.

According to FIGO committee (2009) of endometrial carcinoma, the included 60 cases of endometrial carcinoma were staged as follows: Stage IA which included 20 cases (57.1%). Stage IB included 15 cases (42.9 %.).

Table 1. The relation between OCT-4 expression and clinicopathologic parameters of endometrial carcinoma

				OCT – 4				Chi-square	
				Negative	Positive N=52 (86.7%)				
			Total N=60	0 N=8 (13.3%)	Total +ve	+1 N=9 (17.3%)	+2 N=43 (82.7%)	X²	P-value
Hisological type	Endometrioid	N	38	8	30	8	22	13.572	0.035*
		%	100.0%	21.1%	78.9.0%	26.7%	73.3%		
	Mucinous	N	2	0	2	1	1		
		%	100.0%	0%	100.0%	50.0%	50.0%		
	Papillary	N	10	0	10	0	10		
		%	100.0%	.0%	100.0%	.0%	100.0%		
	Clear	N	10	0	10	0	10		
		%	100.0%	.0%	100.0%	.0%	100.0%		
Grade	I	N	17	8	9	5	4	33.312	0.001*
		%	100.0%	47.1%	52.9%	55.6%	44.4%		
	II	N	15	0	15	3	12		
		%	100.0%	.0%	100.0%	20.0%	80.0%		
	III	N	28	0	28	1	27		
		%	100.0%	.0%	100.0%	3.6%	96.4%		
Staging	I	N	35	8	27	8	19	12.932	0.044*
		%	100.0%	22.9%	77.1%	29.6%	70.4%		
	II	N	15	0	15	1	14		
		%	100.0%	.0%	100.0%	6.7%	93.3%		
	III	N	9	0	9	0	9		
		%	100.0%	0%	100.0%	.0%	100.0%		
	IV	N	1	0	1	0	1		
		%	100.0%	.0%	100.0%	.0%	100.0%		
myometrial invasion	No	N	5	2	3	1	2	6.482	0.166
		%	100.0%	40.0%	60.0%	33.3%	66.7%		
	< 50	N	15	2	13	4	9		
		%	100.0%	13.3%	86.7%	30.8%	69.2%		
	> 50	N	40	4	36	4	32		
		%	100.0%	10.0%	90.0%	11.1%	88.9%		

* Significant (P value < 0.05).

Stage II: Fifteen cases representing 25% of endometrial carcinoma cases, included tumors invading cervical stroma, but not extending beyond the uterus. Stage III: Nine cases representing 15% of endometrial carcinoma cases. All of them were of stage IIIA, and they included tumor with invasion of serosa of the corpus. Stage IV: One case of uterine papillary serous carcinoma, representing 1.7% of endometrial carcinoma cases. It was of stage IVA, and they included tumor with invasion of bowel mucosa.

OCT4: OCT-4 expression was detected by nuclear staining in only 13 cases (21.7%) of endometrial hyperplasia, all of them were of atypical endometrial hyperplasia and in 86.7% of endometrial carcinoma cases. The statistical analysis showed a significant direct relation between OCT-4 expression and grade of endometrial carcinoma cases, depth of myometrial invasion and stage of endometrial carcinoma cases (Figure 1 and Tables 1,4,5).

Ki-67: Low index nuclear Ki-67 expression was detected as nuclear staining in 6 cases (10%) of endometrial hyperplasia, all of them were of atypical endometrial hyperplasia [all of these six cases were low index]. In the case of endometrial carcinoma patients, Ki-67 expression was detected in 90% of endometrial carcinoma cases. There was a statistically significant direct relation between Ki-67 expression and histopathologic types of endometrial carcinoma cases, grading and stages were statistically significant (Figure 2 and Tables 2,4,5).

VEGF: VEGF expression was detected in 83.3% of endometrial carcinoma cases, while only 8 cases of endometrial hyperplasia cases showed

Table 2. The relation between Ki-67 expression and clinicopathologic parameters of endometrial carcinoma.

			Total N=60	Ki- 67 Negative N=6 (10%)	Positive N=54 (90%) Total +ve	Low index N=23 (42.6%)	High index N=31 (57.4%)	Chi-square X^2	P-value
Hisological type	Endometrioid	N	38	6	32	22	10	28.781	0.001*
		%	100.0%	15.8%	84.2%	68.7%	31.2%		
	Mucinous	N	2	0	2	1	1		
		%	100.0%	.0%	100.0%	50.0%	50.0%		
	Papillary	N	10	0	10	0	10		
		%	100.0%	.0%	100.0%	.0%	100.0%		
	Clear	N	10	0	10	0	10		
		%	100.0%	.0%	100.0%	.0%	100.0%		
Grade	I	N	17	4	13	9	4	14.302	0.006*
		%	100.0%	23.5%	76.5%	69.2%	30.8%		
	II	N	15	1	14	8	6		
		%	100.0%	6.7%	93.3%	57.1%	42.9%		
	III	N	28	1	27	6	21		
		%	100.0%	3.6%	96.4%	22.2%	77.8%		
Staging	I	N	35	5	30	17	13	12.574	0.049*
		%	100.0%	14.3%	85.7%	56.7%	43.3%		
	II	N	15	1	14	6	8		
		%	100.0%	6.7%	93.3%	42.9%	57.1%		
	III	N	9	0	9	0	9		
		%	100.0%	.0%	100.0%	.0%	100.0%		
	IV	N	1	0	1	0	1		
		%	100.0%	.0%	100.0%	.0%	100.0%		
myometrial invasion	No	N	5	1	4	2	2	2.302	0.680
		%	100.0%	20.0%	80.0%	50.0%	50.0%		
	< 50	N	15	1	14	4	10		
		%	100.0%	6.7%	93.3%	28.6%	71.4%		
	> 50	N	40	4	36	17	19		
		%	100.0%	10.0%	90.0%	47.2%	52.8%		

* Significant (P value < 0.05).

positive expression. The relation between VEGF and histopathologic types, grade, myometrial invasion and stages of endometrial carcinoma cases was statistically significant as in Table 3. As high expression of VEGF was associated with higher grade stages of endometrial carcinoma and myometrial invasion (Figure 3 and Tables 3,4,5).

Correlation between different immunomarkers expression in atypical endometrial hyperplasia versus grade 1 endometrial carcinoma cases: Among the studied markers, only Ki-67 showed a statistically significant relation between atypical endometrial hyperplasia and grade I endometrial carcinoma cases (P value=0.005). It was positive in (76.5%) of grade 1 endometrial carcinoma cases and (30%) of atypical endometrial hyperplasia cases (Table 5).

DISCUSSION

The present study included 120 selected cases of endometrial lesions classified histologically into two major groups, group I constituted of 60 cases of endometrial hyperplasia, and group II constituted of 60 cases of endometrial carcinoma. The present work aimed to study the relationship between OCT-4, Ki-67and VEGF immunohistochemical expression and clinicopathologic data of endometrial hyperplasia and carcinoma.

OCT-4 is a transcription factor that forms a core regulatory network that determines endometrial cancer stem cells (ESC) self-renewal and differentiation. These ESC-associated proteins may also contribute to tumorigenesis. OCT-4 expression was positive in (86.7%) of endometrial carcinoma cases, with

Table 3. The relation between VEGF and clinicopathologic parameters of endometrial carcinoma.

			Total N=60	VEGF				Chi-square	
				Negative	Positive N=50 (83.3%)				
				N=10 (16.7%)	Total +ve	Moderate N=16 (32%)	Strong N=34 (68%)	X^2	P-value
Hisological type	Endometrioid	N	38	10	28	15	13	23.983	0.001*
		%	100.0%	26.3%	73.7%	53.6%	46.4%		
	Mucinous	N	2	0	2	1	1		
		%	100.0%	.0%	100.0%	50.0%	50.0%		
	Papillary	N	10	0	10	0	10		
		%	100.0%	.0%	100.0%	.0%	100.0%		
	Clear	N	10	0	10	0	10		
		%	100.0%	.0%	100.0%	.0%	100.0%		
Grade	I	N	17	10	7	5	2	36.442	0.001*
		%	100.0%	58.8%	41.2%	71.4%	28.6%		
	II	N	15	0	15	6	9		
		%	100.0%	.0%	100.0%	40.0%	60.0%		
	III	N	28	0	28	5	23		
		%	100.0%	.0%	100.0%	17.9%	82.1%		
Staging	I	N	35	10	25	15	10	27.253	0.001*
		%	100.0%	28.6%	71.4%	60%	40%		
	II	N	15	0	15	1	14		
		%	100.0%	.0%	100.0%	6.7%	93.3%		
	III	N	9	0	9	0	9		
		%	100.0%	.0%	100.0%	.0%	100.0%		
	IV	N	1	0	1	0	1		
		%	100.0%	.0%	100.0%	.0%	100.0%		
myometrial invasion	No	N	5	2	3	1	2	16.102	0.003*
		%	100.0%	40.0%	60.0%	33.3%	66.7%		
	< 50	N	15	3	12	9	3		
		%	100.0%	20.0%	80.0%	75.0%	25.0%		
	> 50	N	40	5	35	6	29		
		%	100.0%	12.5%	87.5%	17.1%	82.9%		

* Significant (P value<0.05).

significant direct relation with the histopathological types, as it was positive in (78.9%) of endometrioid carcinoma cases while positive in all cases of mucinous, papillary serous and clear types with the highest expression (score +2) in all cases of papillary serous, clear types and 73.3% and 50% of positive cases of endometroid and mucinous carcinoma types. However, Shorky et al., (2017) reported that OCT-4 expression in their study was positive in all cases of endometrial carcinoma with the highest expression in (37%) of cases of type 1 carcinoma, whereas type 2 showed low expression. These discrepancies can probably be explained by the variations in methodology, principally differences in the specificity of antibody and dilution used.

In a present study, the highest expression of OCT-4 was positive in (100%) of grade 3 endometrial carcinoma [Which included poorly differentiated endometrioid carcinoma, papillary serous type and clear cell type] with the highest expression (score +2) in (96.4%) of cases of grade 3. Also, in grade 2 endometrial carcinoma cases, it was positive (100%), while it was only 52.9% in grade 1 endometrial carcinoma. It was found that there was a significant direct relation between OCT-4 immunohistochemical expression and grading of endometrial carcinoma cases; as higher expression of OCT-4 was related to higher tumor grade.

These findings were parallel to those of Xie et al. (2019) who found a significant direct relation between OCT-4 expression and FIGO grading of endometrial carcinoma in their series as OCT-4 expression was positive in all cases of grade 3 and grade 2 endometrial carcinoma, while it

Table 4. Correlation of immunohistochemical results between the two studied groups (Endometrial hyperplasia and Endometrial carcinoma).

		Total	-ve	+ve	X^2	P-value
OCT-4	Endometrial hyperplasia	60	47 (78.3%)	13 (21.7%)	51.052	0.001*
	Endometrial carcinoma	60	8 (13.3%)	52 (86.7%)		
Ki- 67	Endometrial hyperplasia	60	54 (90%)	6 (10%)	76.801	0.001*
	Endometrial carcinoma	60	6 (10%)	54 (90%)		
VEGF	Endometrial hyperplasia	60	52 (86.7%)	8 (13.3%)	58.871	0.001*
	Endometrial carcinoma	60	10 (16.7%)	50 (83.3%)		

* Significant (P value < 0.05).

Table 5. Correlation of immunohistochemical results between atypical endometrial hyperplasia versus grade 1 endometrial carcinoma cases.

		Total	-ve	+ve	X^2	P-value
OCT-4	Atypical endometrial hyperplasia	20	7 (35%)	13 (65%)	0.552	0.457
	Grade 1 endometrial carcinoma	17	8 (47.1%)	9 (52.9%)		
Ki- 67	Atypical endometrial hyperplasia	20	14 (70%)	6 (30%)	7.942	0.005*
	Grade 1 endometrial carcinoma	17	4 (23.5%)	13 (76.5%)		
VEGF	Atypical endometrial hyperplasia	20	12 (60%)	8 (40%)	0.012	0.942
	Grade 1 endometrial carcinoma	17	10 (58.8%)	7 (41.2%)		

* Significant (P value < 0.05).
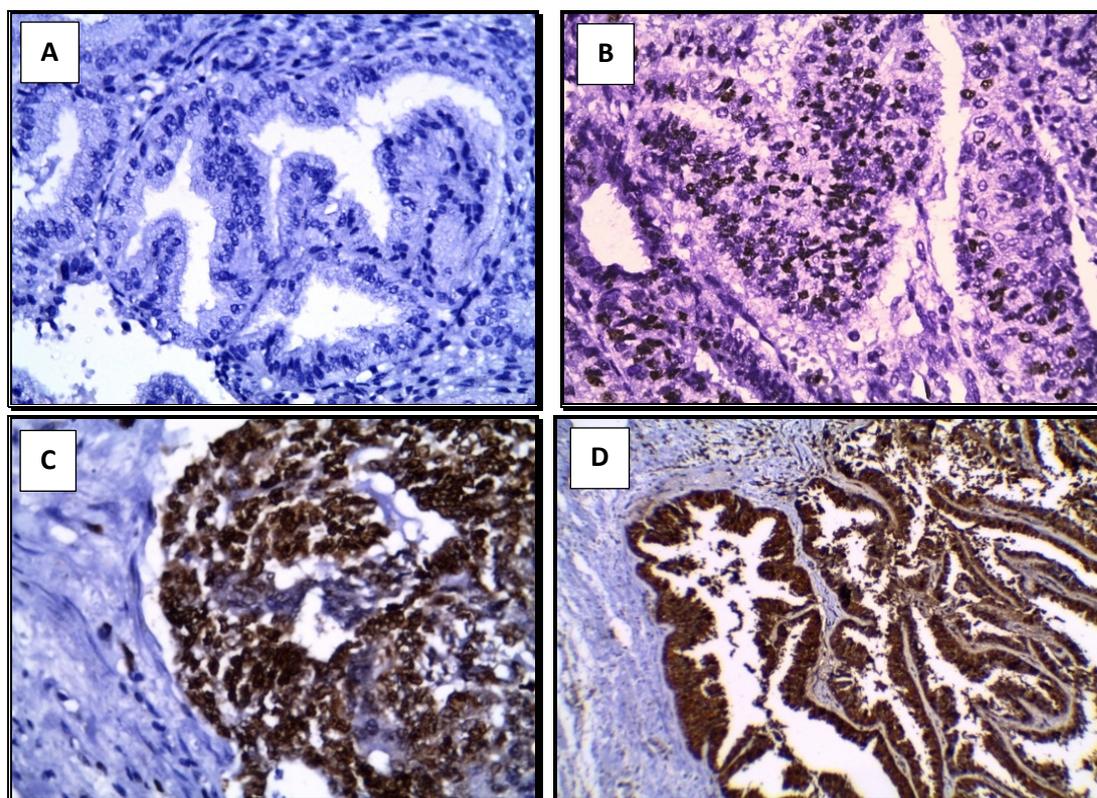
Figure 1. OCT4 Immunohistochemical expression   A: case of complex endometrial hyperplasia showing score (0) nuclear expression of OCT-4 (X 400). B: Conventional endometrioid carcinoma (grade 2) showing score (+1) nuclear expression of OCT-4 (X 400). C: Conventional endometrioid carcinoma (grade 3), showing score (+2) nuclear expression of OCT-4 (X 400). D: A case of uterine papillary serous type, showing score (+2) nuclear expression of OCT-4 (X 200).
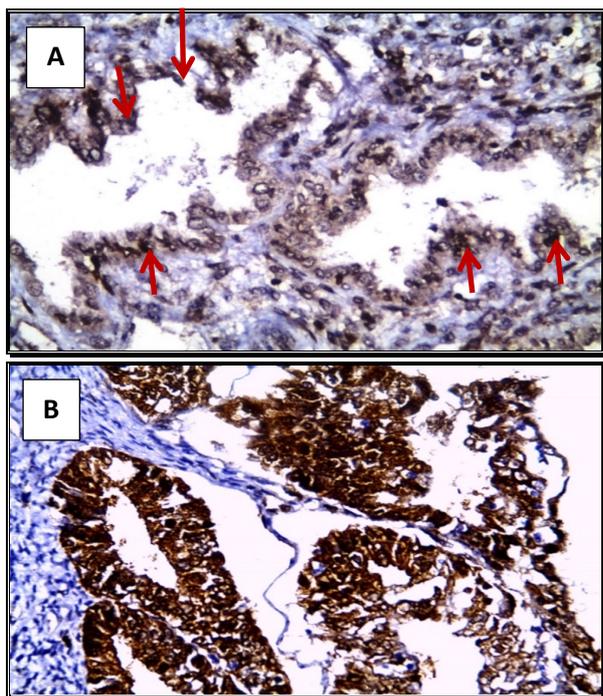
Figure 2. KI-67 Immunohistochemical expression A: a case of atypical endometrial hyperplasia showing low index nuclear expression of Ki-67 (X 400). B: Conventional endometrioid carcinoma (grade 1), showing a high index nuclear expression of Ki-67 (X 400).
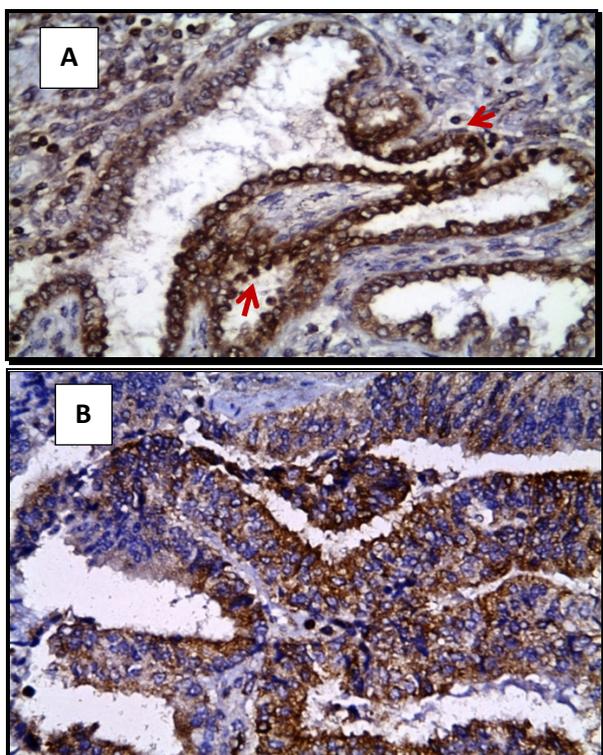

Figure 3. VEGF Immunohistochemical expression A: A case of atypical endometrial hyperplasia showing strong cytoplasmic expression for VEGF (X 400). B: A case of conventional endometrioid carcinoma (grade 3), showing strong VEGF expression (X 400).

was only 50% in grade 1 endometrial carcinoma. On the other hand, Pityński et al. (2015) reported non-significant relation between OCT-4 expressions and grade 1 and 2 endometrial carcinomas compared to grade 3 carcinomas.

OCT-4 was positive in 60% of cases that did not show myometrial invasion, 86.7% of cases with invasion less than half the myometrium, and 90% of cases with invasion more than half the myometrial invasion. Although the relation between OCT4 and depth invasion did not reach a statistically significant relation, OCT4 was found to increase with the level of myometrial invasion, as it was positive in (90%) of cases with myometrial invasion >50%.

OCT-4 was expressed in 77.1% of endometrial carcinoma stage I cases, and 100% positive in stages II, III and stage IV cases. The relation between OCT-4 immunohistochemical expression and stage of endometrial carcinoma reached significant value with a direct relationship. These results indicate that elevated expression of OCT-4 is positively associated with late-stage progression and worse prognosis of endometrial cancer patients. Xie et al. (2019) reported that OCT-4 is an important transcription factor that contributes to both tumor metastasis and chemoresistance. Kutilin et al. (2019) also, has been reported that poorly differentiated tumors exhibit highly activated embryonic stem cell signals with accumulating evidence that the activation of key transcription factors like OCT4 is important for tumor oncogenesis, progression and metastasis.

OCT4 was positive in only 13 cases (21.7%) of endometrial hyperplasia, all of them were of atypical endometrial hyperplasia and in (86.7%) of endometrial carcinoma cases. There was a significant difference between expression of OCT4 in endometrial hyperplasia and endometrial carcinoma cases but no significant difference could be found between atypical hyperplasia and carcinoma. Although the expression of OCT 4 might be an indicator for the progression of endometrial hyperplasia to carcinoma it could not be used as a diagnostic tool to differentiate between atypical endometrial hyperplasia and carcinoma.

Shorky et al. (2017) reported the expression of OCT4 in nine cases (30%) of endometrial hyperplasia cases out of total of 30 cases: two (22.2%), four (44.4%), and three (33.3%) cases of simple endometrial hyperplasia, atypical endometrial hyperplasia and complex endometrial hyperplasia, respectively. This discrepancy in results to our study could be attributed to a small number of cases in their study.

Ki-67 is a cellular marker for proliferation and it is an excellent marker to determine the growth fraction of a given cell population (Rossi et al., 2020).Regarding endometrial carcinoma cases, Ki-67 expression was positive in 90 %. It was found that there was a significant relation between Ki-67 expression and the histopathologic types of studied cases of endometrial carcinoma. It was positive in 84.2% of endometrioid carcinoma cases and positive in all cases (100%) of mucinous type, papillary serous type and clear cell type of endometrial carcinoma with a high labelling index. There was a difference between the present study & the results of Yu et al. (2015) regarding Ki-67 percentage as it was positive in (64.2%) of endometrial carcinoma cases, may be due to the absence of aggressive types (uterine papillary serous carcinoma and clear cell carcinoma) in their study, which were completely positive for Ki-67 in our study.

In the present study, there was a significant direct relation between Ki-67 expression and the grade of endometrial carcinoma cases, as Ki-67 positive expression was lowest in grade 1 (76.5%), while it represented 93.3% and 96.4% in grade 2 & grade 3 cases respectively with high labelling index in 77.8% and 42.9% of grades 3 and 2, respectively.

A progressive increase of Ki-67 in grade 3 may be linked to tumor aggressiveness and loss of differentiation of endometrial cancer. This was in coincidence with the results of Gülseren et al., (2020). On the other hand, Yang et al., (2016), found an insignificant direct relation between the grade of endometrial carcinoma cases and Ki-67 expression in their studied cases.

In relation to the depth of myometrial invasion, Ki-67 was positive in 80% of cases without myometrial invasion, 93.3% of cases with invasion less than half of the myometrium and 90% of cases with invasion more than half of the myometrium. Although the relation between Ki-67 and depth of myometrial invasion did not reach a statistical significance Ki-67 was found to be significantly related to stage.

As dealing with the staging of endometrial carcinoma cases, the present study proved that there was a significant direct relation between Ki-67 expression and stage as Ki-67 expression was positive in 100% of stage IV cases and stage III cases, 93.3% of stage II cases and 85.7 % of stage I cases. So, those cancers with the greatest cell proliferation had the poorest clinical outcome and are more likely to be associated with disease metastasis and recurrence. This was in agreement with the findings of Furau et al., (2019), who found also a positive relation between Ki-67 expression on one hand with stage and depth of myometrial invasion.

In the present study, 6 out of 60 cases of endometrial hyperplasia were positive for Ki-67 immunoreactivity, representing 10% (all of these six cases were atypical hyperplasia with low labelling index). This can be explained by the increase in the malignant potential of atypical endometrial hyperplasia cases. This is in agreement with the findings of Zimik et al. (2020) who did not find expression of Ki-67 in nearly all their studied cases of endometrial hyperplasia without atypia.

Ki-67 expression in endometrial carcinoma cases (90%) was significantly higher than that of endometrial hyperplasia cases (10%). Also, there was a significantly higher Ki-67 expression in grade1 endometrial carcinoma cases (76.5%) than that of atypical endometrial hyperplasia cases (30%). This finding was in coincidence with that of Shalini et al., (2019) who found that there was a significant positive relationship between Ki-67 expression in endometrial hyperplasia and endometrial carcinoma. So, the Ki-67 labelling index could be used in differentiation between endometrial hyperplasia with atypia and grade 1 endometrial carcinoma. Further studies on a larger number of cases are needed to reach a cut-off value for Ki 67 in the diagnosis of

endometrial carcinoma and differentiation between atypical endometrial hyperplasia and grade 1 endometrial carcinoma.

Both tumor cell proliferation (Ki-67) and angiogenesis seem to be important for the development of aggressive tumors and to carry an independent significance for the tumor growth potential. Hence, the present research attempted to examine the expression of VEGF in endometrial hyperplasia and endometrial carcinoma, to characterize its relationship with a set of clinicopathologic characteristics.

Most of the endometrial carcinoma cases (83.3%) showed VEGF positive expression, with significant relation with histopathological types of endometrial carcinoma cases. VEGF expression was highest in grade 2 and grade 3 endometrial carcinoma (100%) and decreased expression in grade 1 (41.2%) cases. It was found that there was a significant direct relation between VEGF expression and grade of endometrial carcinoma. This was in agreement with the results of Wang et al. (2014).

Strong positivity for VEGF in grade 2 and grade 3 carcinoma could be explained that VEGF plays a role in tumor progression. This was in coincidence with Zinovkin et al. (2019), who found low VEGF expression in well-differentiated endometrial carcinoma cases in comparison to poorly differentiated cases.

VEGF had a significant relationship with a depth of myometrial invasion, as it was positive in 87.5% of cases with an invasion with more than half the myometrium, 80% of cases that show invasion less than half of the myometrial, and 60% of cases without myometrial invasion. As regards the stage of endometrial carcinoma, VEGF was expressed in all cases of stage IV, stage III and stage II (100%), while it was found in (71.4%) of stage I tumours with significant relation with the stage. This finding was in coincidence with Sunita et al. (2018) who reported that VEGF expression occurred more often in endometrial cancers with deep myometrial invasion.

These results emphasize the role of VEGF in carcinogenesis as it is a potent pro-angiogenic factor and also a key mediator of neovascularization, a process that is mandatory

in the pathogenesis of malignant tumors. its overexpression in tumor cells stimulates tumor growth and metastasis in cancer (Guşet et al., 2010; Sunita et al., 2018).

In the present study 52 out of 60 cases of endometrial hyperplasia didn't show positivity for VEGF, while only 8 cases of atypical endometrial hyperplasia were positive for VEGF with a strong expression representing (13.3%) of cases. There was a significant higher VEGF expression in endometrial carcinoma cases (83.3%) than that of endometrial hyperplasia cases (13.3%). This finding was in coincidence with that of Hassan and Ibrahim, (2020) who found that there is a higher expression of VEGF in endometrial carcinoma to hyperplasia with a significant relationship. But, there was weak cytoplasmic positive staining in atypical endometrial hyperplasia while in benign endometrial hyperplasia, the expression was negative.

These results emphasize that high expression of VEGF seems to have a role in the progression of endometrial hyperplasia into endometrial carcinoma. However, as regards the relation of VEGF expression in atypical endometrial hyperplasia versus grade 1 endometrial carcinoma; it was insignificant which explain that VEGF could not be used as a diagnostic tool in differentiation between them.

CONCLUSION

OCT-4, Ki-67 and VEGF expression showed a positive correlation with tumor aggressive pathological parameters. The Ki-67 labelling index can be used in differentiation between endometrial hyperplasia with atypia and grade 1 endometrial carcinoma. Although OCT-4 and VEGF expressed in endometrial cancer more than hyperplasia, they could not be used as diagnostic tools to differentiate between atypical endometrial hyperplasia and carcinoma. However, it indicates that they have a role in malignant transformation.

CONFLICTS OF INTEREST

All authors have approved this article and declare no conflicts of interest.

FUND

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