Immunohistochemical Expressions of ER, PR and ALDH1 in Endometrial Hyperplasia and Carcinoma

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Abstract: Epithelial stem/progenitor cells are considered as cancer initiating cells which has been detected in human endometrium, may initiate and progress endometrial carcinoma. Targeting cancer-initiating cells would be necessary to improve cure rates. The relation of endometrial adenocarcinoma and stem cell markers expression has not been reported yet, in spite of ALDH1 expression is frequently altered in malignant tumors compared to their respective healthy tissues. We studied the endometrium of 54 cases; 44 complaining of peri/postmenopausal bleeding, 10 women in proliferative and secretory phases seeking for other gynecological causes. Immunohistochemical study of the endometrium for ER, PR and ALDH1in normal, hyperplastic and endometrial carcinoma showed that there was statistical significance relation (p<0.001) between ER, PR, ALDH1 epithelial expression and clinicopathological parameters; age, myometrial depth of invasion, also between ALDH1 expression in the stroma of endometrial carcinoma and the clinicopathological parameters. It is concluded that the decline of ER and PR and significant increase in ALDH1 expression may have relation with the tumorigenesis and endometrial cancer progression mainly in type II which might be related to poor prognosis.

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1.Introduction

One of the most common malignancies of the female genital system is endometrial adenocarcinoma (EC) [1,2]. Although the advances in methods for diagnosis and treatment; the prognosis of patients with endometrial adenocarcinoma still remains unfavorable. Epithelial stem/progenitor cells; the source of the putative cancer stem cells (CSCs) considered as cancer initiating cells (CIC) has been detected in human endometrium, which may initiate and maintain EC [3,4]. So targeting cancer-initiating cells would be necessary to improve cure rates. However studies on these cells of endometrioid adenocarcinoma are limited. Endometrial carcinomas are classified based on the etiology and clinical behavior into Type I and type II, the former ECs have an endometrioid histology and account for 70–85% of sporadic cases which have a favorable prognosis and are of a low grade often arise from endometrial hyperplasia in peri- and postmenopausal women which estrogen and progesterone receptors are commonly expressed [5,7]. On the other hand type II tumors where they tend to be composed of markedly atypical cells arise in a background of atrophic post-menopausal endometrium independent of estrogen, these tumors may be preceded by endometrial carcinoma in situ have poor prognosis, account for 10–20% of sporadic ECs and often have a serous papillary or clear cell histology [7,8]. The relation of endometrial adenocarcinoma prognosis and stem cell marker expression has not been reported yet, in spite of ALDH1 expression is frequently altered in malignant tumors compared to their respective healthy tissues [9-11].In early stages of stem cell differentiation; hematopoietic and neural stem cells show high activity aldehyde dehydrogenase 1"a predominant isoform of the ALDH family in mammals which oxidizes retinol to retinoic acid" [12,14], encoded by the ALDH1A1gene at chromosome 9q21 [15], belongs to the aldehyde dehydrogenase super family that is responsible for the oxidation of aldehydes to their corresponding carboxylic acids [16,17], responsible for tissue specific irreversible oxidation of retinal to the signalling molecule, retinoic acid (RA) [18]that works on retinoic acid receptors which affects cell differentiation, decreasing cell proliferation, tissue homeostasis as well as apoptosis in various cell types[19].

The aim of the study is to correlate the expression of ALDH1, ER and PR in endometrial hyperplasia as well as endometrial carcinoma and its clinical implication whenever possible.

2.Materialand Methods

A total number of 54 cases studied from Egyptian women. Cases were collected from El-Kasr El-Aini Hospital; Cairo University as well as Beni Sueif General Hospital. 44 women of the age group (43-67 y); 30 endometrial biopsies and 14 with total hysterectomies and bilateral salpingo-opherectomy

specimens, 9 cases of the latter with omentectomies, one of such had lymph nodes resection, complaining of peri/postmenopausal bleeding. Endometrial biopsies were also obtained from another 10 women of the age group (21-38y) in proliferative phase (5 cases) as well as secretory phase (5 cases) in non-pregnantstate, in outpatient clinic, seeking for other gynecological causes. They had regular menstrual cycles and had not used oral contraception in the two months preceding the biopsy. Informed consent was obtained from each woman. All the cases were studied by H&E for histopathological assessment and by ER, PR and ALDH1 for immunohistochemical study.

Cases were examined by routine H&E and classified according to the International Federation of Gynecology and Obstetrics (FIGO), malignant tumors were classified into type I (endometrioid histology; grades 1,2) and Type II (serous papillary or clear cell histology; grade 3).

Immunohistochemical Staining:

5 µm sections of formalin-fixed paraffin-embedded tissues were mounted onto ChemMate capillarygap slides (Dako, Glostrup, Denmark), dried in a slide oven at 60°C for 1 h, deparaffinized with xylene, and rehydrated with ethanol to distilled water. The staining procedures were performed on an automated immunostainer (TechMate 1000; Dako) using the biotin–streptavidin detection system (ChemMate-HRP/DAB;Dako). The primary antibody was diluted in ChemMate diluent, and incubation performed overnight at 4°C. All following procedures were carried out at room temperature in accordance with the ChemMate protocol. Each TechMate holder included positive and negative control slide. The results of this analysis revealed that the optimal procedure was epitope retrieval in microwave heating/TEG buffer with anti-ER, anti PR and anti ALDH1 antibodies. We determined the nuclear expression for ER and PR while cytoplasmic expression for ALDH1.

Immunohistochemical Analysis:

Immunohistochemical staining for ER and PR was assessed using a semi quantitative score according to Remmele and Steger, comprising optical staining intensity (graded as 0 = no, 1 = weak, 2 = moderate, and 3 = strong staining) and the percentage of positively stained cells (0 = no, 1 = <10%, 2 = 11–50%, 3 = 51–80% and 4 =>81% cells). The final score was the sum of intensity and percentage scores. Tumors were scored as positive if more than 10% of cells were scored with an immunoreactive score (IRS) higher than 2 [20]. The staining intensity for ALDH1 was rated according to the following scale: 0 = no visible staining, 1 = faint staining, 2 = moderate staining and 3 = strong staining. Percentage of cells with positive ALDH1 was graded as 0%<10%, 10% to 25%, 25% to 50% and 50% to 75% or higher [21, 22]. The slides were reviewed blindly by two independent pathologists (D. and N.).

Statistical Analysis:

Data was statistically analyzed by the Statistical Package of Social Science Software program (SPSS), version 21, summarized using frequency and percentage for qualitative variables. Comparison between groups was done using chi square test as well as Fisher's exact test. *P* values less than 0.05 were considered statistically significant and less than 0.01 were considered highly significant.

3.Results

In this study 10 cases of normal endometrium ranged in age between 21-38y with mean age 32y, while 44 of the 54 cases ranged in age between 43-67y with mean age 53y, the latter had history of peri/post-menopausal vaginal bleeding. By routine H&E stains 28/54 cases (51.85%) were categorized as non-malignant endometrium [10 cases normal (proliferative phase or secretory phase), 12 cases of simple endometrial hyperplasia (SEH), 6 cases of atypical/complex endometrial hyperplasia (AEH)], while 26/54 cases (48.15%) were malignant [6 cases with carcinoma in situ (CIS), 20 cases endometrial carcinoma (EC) (16 cases were of Type I; grade II endometrial carcinoma and 4 cases type II; grade 3 endometrial carcinoma)]. That with lymph nodes showed negative metastasis, the omentum of such case was free of tumor deposits.

The expression of ER, PR incases with normal glandular epithelium was 8/10 (80%), 7/10 (70%) of cases receptively. On the other hand ER, PR were expressed in the glandular epithelium of 14/20 (70%) of cases with endometrial carcinoma (Figures a, b).As regards ALDH1 expression in the epithelium it was negative in normal proliferative as well as secretory endometrium whileit was expressed in hyperplastic endometrium, atypical complex, CIS cases as well as cases of endometrial carcinoma, the latter showed positive immunostaining expression in 9/20 cases of endometrial carcinoma (45%) shown in (figure c), results were summarized in(Tables 1,2).

There was statistical significance relation (p<0.001) between ER, PR, ALDH1 epithelial expression and clinicopathological parameters; age, myometrial depth of invasion, while no statistical significance (p>0.05) in relation of histologic type of EC 12/16 (75%) for ER, 11/16 (68.7%) for PR 7/16 (43%) of cases with Type I EC and, while, 2/4 (50%), 1/4(25%), 2/4(50%) for ER, PR and ALDH1 respectively in Type II EC (Tables 2,3). However, study revealed that cases of EC at which the stroma was negative in all cases for ER and PR they were positive for ALDH1 (figure d), (Table 4), in addition there was high significant correlation between

ALDH1 expression in the stroma of endometrial carcinoma and the clinicopathological parameters as it was positive in all 4 cases of type II, while in 9/16 cases of types I, 11 of the latter were of grade II.

Table 1: Immunostaining expression of ER, PR, ALDH1 in the glandular epithelium of studied cases

Immunostaining	ER				PR				ALDH1				*P* value
Endometrial changes	+VE		-VE		+VE		-VE		+VE		-VE		
	N	%	N	%	N	%	N	%	N	%	N	%	
Non-malignant	22	53.7	6	46.2	19	54.3	9	47.4	8	42.1	20	57.1	**0.003, HS**
Malignant	19	46.3	7	53.8	16	45.7	10	52.6	11	57.9	15	42.9	**0.08, NS**
Total	41	100.0	13	100.0	35	100.0	19	100.0	19	100.0	35	100.0	

Table 2: Correlation between immunostaining expression of ER, PR and ALDH1 in the glandular epithelium of different histologicalvariants

Immunostaining	ER		PR		ALDH1		*P* Value
Histological type	N	%	N	%	N	%	
Normal endometrium	8/10	80.0%	7/10	70.0%	0/10	0.0%	**0.001, HS**
Simple endometrial hyperplasia	10/12	83.3%	8/12	66.6%	5/12	41.6%	0.1
Atypical endometrial hyperplasia	4/6	66.6%	4/6	66.6%	3/6	50.0%	0.8
Carcinoma insitu	5/6	83.3%	4/6	66.6%	2/6	33.3%	0.2
Endometrial carcinoma	14/20	70.0%	12/20	60.0%	9/20	45.0%	0.3
Total	41/54	75.9%	35/54	64.8%	19/54	35.2%	**<0.001, HS**

Table 3: Correlation between the type of endometrial carcinoma and immunostaining expression of ER, PR, ALDH1 in the glandular epithelium

Immunostaining	ER				PR				ALDH1				*P* value
Type of Endometrial carcinoma	+VE		-VE		+VE		-VE		+VE		-VE		
	N	%	N	%	N	%	N	%	N	%	N	%	
Type I	12	85.7	4	66.7	11	91.7	5	62.5	7	77.8	9	81.8	0.3, NS
TypeII	2	14.3	2	33.3	1	8.3	3	37.5	2	22.2	2	18.2	0.7, NS
Total	14	100.0	6	100.0	12	100.0	8	100.0	9	100.0	11	100.0	

Table 4: Immunostaining expression of ER, PR, ALDH1 in the stroma and the type of endometrial carcinoma

Immunostaining	ER				PR				ALDH1				*P* value
EC	+VE		-VE		+VE		-VE		+VE		-VE		
	N	%	N	%	N	%	N	%	N	%	N	%	
Type I	0	0	16	80.0	0	0	16	80.0	9	69.2	7	100.0	**<0.001, HS**
TypeII	0	0	4	20.0	0	0	4	20.0	4	30.8	0	0.0	**0.003, HS**
Total	0	0	20	100.0	0	0	20	100.0	13	100.0	7	100.0	

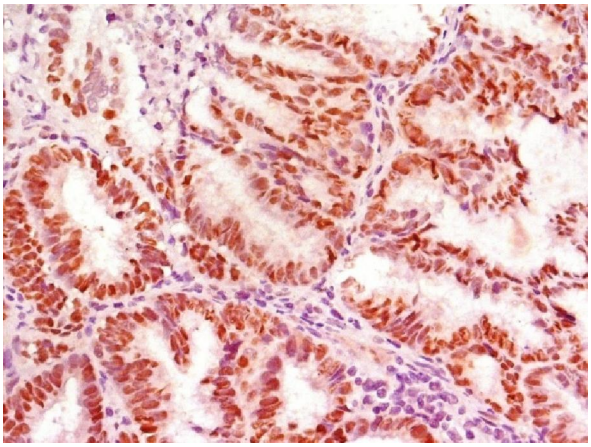

Figure a: Strong positive immunostaining nuclear reaction for ER of malignant epithelial cells of Type I endometrial carcinoma.

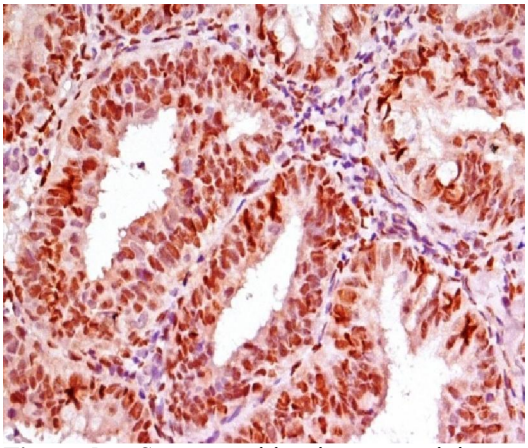

Figure b: Strong positive immunostaining nuclear reaction for PR of malignant epithelial cells of Type I endometrial carcinoma.

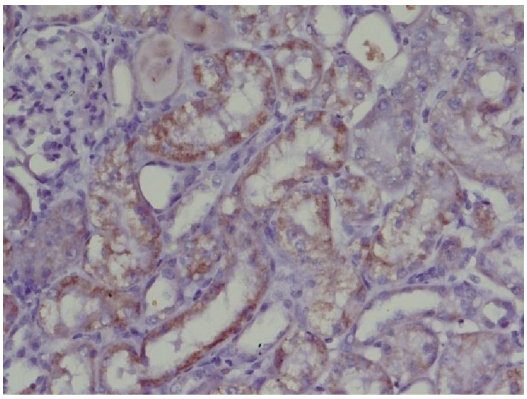

Figure c: Cytoplasmic immunostaining reaction for ALDH1 of malignant epithelial cells of Type I endometrial carcinoma.

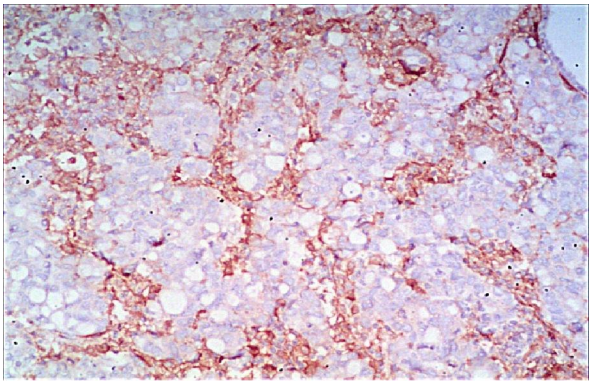

Figure d: Cytoplasmic immunostaining reaction for ALDH1 of stromal cells of Type II endometrial carcinoma.

4.Discussion

Abnormal uterine bleeding is a common clinical presentation for endometrial hyperplasia as well as for endometrial carcinoma, most common in women who are postmenopausal and with increasing age in premenopausal period. In this study 10 cases of normal endometrium ranged in age between 21-38y with mean age 32y,with increasing the age of the patients the study has shown 44/ 54 cases ranged in age between 43-67y with mean age 53y, the latter had history of peri/post-menopausal vaginal bleeding. Routine histological examination of the endometrium has revealed. 28/54 cases (51.85%) were non-malignant endometrium [normal (proliferative phase or secretory phase), SEH, AEH/complex], while 26 /54 of the cases(48.15%) were either CIS or EC, the incidence of EC among patients with atypical endometrial hyperplasia has been shown to increase with age ranged from 17 to 52 %,adding that atypical endometrial hyperplasia have coexistent endometrial carcinoma in cases. As the risk factors for endometrial hyperplasia are the same as those for endometrial involving exposure of the endometrium to continuous estrogen unopposed by a progesterone [23]. The expression of ER, PR respectively in the current study was 8/10 (80%), 7/10 (70%) of cases with normal endometrium,83%,66.6% for SEH, 66.6% for both ER, PR in cases of AEH/complex this might be due to the limited number of cases of the latter (6 cases). However the expression of ER is increased to 83.3% for ER while for PR there were no great changes than cases with SEH and AEH/complex. On the other hand ER, PR were expressed in the epithelium of 14/20 (70%) of cases with endometrial carcinoma. Results were matched with recent study stated that ER in the normal group, hyperplasia, endometrial cancer tissues were: 87.50% (28/32), 75.31% (61/81), 63.64 % (63/99), endometrial cancer tissues was lower than the normal group, matching with the present study at which ER were positively expressed in 53.7% while 46.3% in malignant cases (CIS and EC) [24].As regards ALDH1 expression in the epithelium it was negative in normal proliferative as well as secretory endometrium while the expression in hyperplastic endometrium, atypical complex, CIS cases as well as cases of endometrial carcinoma, the latter showed positive immunostaining expression in 9/20 cases(45%),this might be because that heterogeneous cell populations of tumors are derived from a single clone although tumorigenic potential are limited to a small population among tumor cells [25]. There was statistical significance relation (p<0.001) between ER, PR, ALDH1 epithelial expression and clinicopathological parameters; age, myometrial depth of invasion, while no statistical significance (p>0.05) in relation of histologic type of EC (12/16 (75%) for ER 11/16 (68.7%) for PR 7/16 (43%) of cases with Type I EC and, while 2/4 (50%), 1/4(25%), 2/4(50%) for ER, PR and ALDH1 respectively in Type II EC. furthermore; a study evaluated the clinical implication of ALDH1 expression in 98 cases of endometrioid adenocarcinoma. The characteristics of patients, such as age and stage, in the current study were similar to studies by Rahadiani*et al.* and Stein *et al.*[26,27] indicating that the results obtained from the current study could be applicable to endometrioid adenocarcinoma worldwide. Another study showed that a high level of ALDH1 expression was correlated with T category, lymphatic invasion, resistance to chemotherapy, recurrence, and prognosis of patients adding to that patients with higher ALDH1 expression had poorer prognoses than those with lower expression, which is independent poor prognostic factor [24],noting that poor prognosis was associated with a high percentage of ALDH1-expressing cells in most types of epithelial tumors,

such as breast, lung, pancreatic, bladder, ovarian and prostate [25,26].Although, the present study revealed that cases of EC where the stroma was negative in all cases for ER and PR they were positive in for ALDH1 in addition there was high significant correlation between ALDH1 expression in the stroma of endometrial carcinoma and the clinicopathological parameters as it was positive in all 4 cases of type II, while in 9/16 cases of type I. Thus, ALDH1 might be a common marker for CIC among cancers of various organs including endometrial carcinoma.

Conclusion

The expression of ALDH1 is noted in atypical endometrial hyperplasia as well as endometrial cancer, suggested that ALDH1 may have role in endometrial cancer tumorigenesis. The decline of ER and PR and significant increase in ALDH1 expression may have relation with the tumorigenesis and endometrial cancer progression mainly in type II which might be related to poor prognosis at which ALDH1 is expressed more in the stroma of endometrial carcinoma. So more light should be shed on the role of the stromal cells and ALDH1 in tumor progression and type of therapy.

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