

Differential Expression of CD24 and its Significance in Normal Endometrium, Hyperplastic Lesions and Endometrial Carcinoma

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Abstract

Background: Endometrial carcinoma is considered the sixth most common cancer in women worldwide comprising 4% of all cancers in women. CD24 protein was originally reported to be expressed by pre-B lymphocytes. This protein had been recognized only as a cell marker for hematopoietic cell lineages. CD24 expression is considered as an important biomarker for diagnosis, disease progression and cancer-related death in many solid tumors, like breast, colonic and ovarian carcinomas.

Aim: The present work was conducted to study the immunohistochemical expression of CD24 in normal cyclic endometrium, hyperplastic endometrium and endometrial carcinoma, in order to demonstrate its expression pattern and to examine its association with various clinicopathological variables in endometrial carcinoma cases.

Material and Methods: The present study comprised 110 cases of endometrial tissue: 20 cases (18.2%) of cyclic endometrium, 30 cases (27.3%) of endometrial hyperplasia without atypia, 25 cases (22.7%) of atypical endometrial hyperplasia and 35 cases (31.8%) of endometrioid carcinoma. Immunohistochemistry was performed using the avidin biotin-peroxidase complex method.

Results: CD24 immunostaining was membranous and cytoplasmic. In the normal cyclic endometrium, membranous CD24 showed down-regulation in the proliferative phase and up-regulation in the secretory phase. Both membranous and cytoplasmic CD24 expression was statistically increase in endometrial carcinoma and reduced in hyperplastic lesion $p < 0.001$ and $p = 0.004$ respectively. Among cases of carcinoma, membranous CD24 expression was statistically significant with grade, stage and myometrial invasion ($p = 0.040$ & $p = 0.044$ and $p = 0.036$ respectively). Cytoplasmic expression showed significant association with grade only ($p = 0.037$). Membranous CD24 had 71.4% sensitivity and 76% specificity for differentiation of endometrioid carcinoma from atypical EH. Cytoplasmic CD24 had low sensitivity (40%) but high specificity (80%) for distinction of endometrioid carcinoma from atypical endometrial hyperplasia. Total CD24 had 77.2% sensitivity and 76% specificity for distinction of atypical endometrial hyperplasia from endometrioid carcinoma.

Conclusion: Membranous CD24 was expressed in a cyclic pattern in the normal endometrium. CD24 expression was increased in case of endometrial carcinoma than hyperplastic lesion. These results suggest that CD24 can be a useful as diagnostic marker in differentiation between endometrial hyperplasia and carcinoma. Also CD24 implicated in progression of endometrial carcinoma. Total CD24 expression (membranous and cytoplasmic) is more specific and more sensitive in differentiation of atypical endometrial hyperplasia from endometrioid carcinoma.

Key Words: CD24 – Normal cyclic endometrium – Endometrial hyperplasia – Endometrioid carcinoma – Immunohistochemistry

Introduction

WORLDWIDE endometrial carcinoma is considered the sixth most common cancer in women, comprising 4% of all cancers in women [1]. In developed countries it is the most common gynecologic malignancy and the second most common tumor in developing countries; also it is the seventh leading cause of death among women [2]. In Egypt, it represents 14.72% of female genital tract malignancies [3] and 0.62% of all female cancers [4].

CD24 protein was originally reported to be expressed at early stages of B lymphocytes maturation. It has been recognized only as a cell marker for hematopoietic cell lineages [5]. It is called the heat-stable protein due to its heat resistance [6]. CD24 is expressed on hematopoietic cells, including T and B cells [7,8]; neutrophils and eosinophils [9,10], dendritic cells and macrophages [7,11]. In many solid tumors, like breast, colonic and ovarian carcinomas, CD24 expression is considered as an important marker for diagnosis, disease progression and cancer-related death [12-17].

Few reports about CD24 expression in normal cyclic endometrium and other endometrial lesions were conducted, so this study is done to demon-

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strate the role of CD24 in normal cyclic endometrium, hyperplastic lesion and endometrial carcinoma of Egyptian females.

Material and Methods

Patients' and samples characteristics:

This study was conducted at Pathology Department, Minia University from August 2017 to May 2018.

This study included 110 cases of endometrial tissue: 20 cases (18.2%) of cyclic endometrium (10 cases were proliferative and 10 were secretory endometrium), 30 of endometrial hyperplasia cases (27.3%) of (EH) without atypia, 25 cases (22.7%) of atypical EH and 35 cases (31.8%) of Endometrioid Carcinoma (EC) diagnosed in Pathology Department, Minia University Hospital and Minia Oncology Center, Egypt during the period from April 2009 to March 2017.

Hematoxylin and eosin stained slides were reviewed to confirm the diagnoses of the cases. Cases were diagnosed according to WHO based classification [18], grouped into EH without atypia, EH with atypia, and endometrioid carcinoma. Grading of endometrioid carcinoma was done according to the FIGO criteria [18]. Staging of endometrioid carcinoma was done according to the FIGO 2009 staging criteria [19].

Results

The patient mean age \pm SD, median age and age range for each group included in this study was 37.2 ± 4.6 years, 38 years and 33-42 for cyclic endometrium, 61.9 ± 6.9 years, 62 years and 49-72 in EH without atypia, 60 ± 10.5 years, 61 years and 39-84 for atypical EH and 64.9 ± 6.7 years, 64 years and 49-80 for endometrioid EC.

Among EC group, the median age was 64 years old, 15/35 (42.9%) were <64 and 20/35 (57.1%) were >64 . Regarding tumor grade, 15/35 (42.8%) cases were grade 1, 10/35 (28.6%) cases were grade 2 and 10/35 (28.6%) cases were grade 3. Myometrial invasion $<1/2$ was present in 22/35 (62.9%) of cases, while 13/35 (37.1%) showed myometrial invasion $\geq 1/2$. Seventeen cases out of twenty two 17/22 (77.2%) were negative for LN metastasis and 5/22 (22.8%) were positive for LN metastasis.

Regarding FIGO stage of the tumor; 29/35 (82.9%) cases were stage I, 2/35 (5.7%) cases were stage II, 3/35 (8.5%) cases were stage III and 1/35 (2.8%) case was stage VI. These cases were cate-

gorized into two groups, one group included cases of stage I [29/35 (82.9%)] and the other group included stage II/III/IV cases [6/35 (17.1%)]. This was done for statistical purpose.

Immunohistochemistry method:

Tissue sections were prepared then deparaffinized and rehydrated through xylene and graded ethanol solutions and then slides incubated for 5 min with 3% hydrogen peroxide to block the endogenous peroxidase activity. Antigen retrieval was done according to manufacturer's instructions. Then slides incubated with the primary antibodies overnight in moist chamber. The used primary antibody for CD24 is (0.1 concentrated monoclonal mouse antibody at 1:100 concentration (clone SN3b Biocare Medical) then slides washed in PBS buffer. The avidin-biotin detection kit using diaminobenzidine (DAB) as chromogen were used to detect antibody reaction. Slides were counterstained with Mayers hematoxylin for 15 seconds, then dehydrated through graded alcohol solutions, cleared in xylene and coverslipped. Negative control was done by omitting the primary antibody. Ovarian serous carcinoma was used as positive control and tested on each run.

Immunohistochemical analysis:

CD24 antibody, positive staining was detected when a membranous and/or cytoplasmic staining was observed. CD24 expression was grouped into positive and negative cases according to [20]. Negative cases had to show definitely no immunoreactivity in any part of the section. All of the other cases, beginning with a weak but unequivocal staining of the cells, were defined as positive.

Statistical analysis:

Mean, standard deviation, frequency distribution and cross tabulation have been performed. Chi-square and Fisher exact test were used to compare categorical variables. Results were considered statistically significant when p -value <0.05 . Data were analyzed using the Statistical Package for Social Sciences (SPSS) Version 18 software. To determine the diagnostic efficacy of CD24, the numbers of True-Positive (TP), True-Negative (TN), False-Positive (FP), and False-Negative (FN) cases were determined for the marker. Accordingly, the sensitivity, specificity, Positive Predictive Values (PPV), Negative Predictive Values (NPV) and diagnostic accuracy of CD24 were calculated using MedCalc statistical software.

Immunoreactivity for CD24:

CD24 immunostaining was membranous and cytoplasmic; each subcellular localization was

separately evaluated and statistically analyzed. Then combined membranous and cytoplasmic CD24 immunostaining was evaluated.

1- Membranous CD24 expression in different histopathological groups:

As regard cyclic endometrium, 9/20 (45%) cases were positive. Among them, 2/10 cases of proliferative phase (20%) were positive. Conversely,

7/10 cases (70%) of secretory phase cases were positive and 3/10 cases (30%) were negative Fig. (1A,B). In EH without atypia and atypical EH cases, majority of cases 26/30 (86.6%), 19/25 (76%) respectively) were negative for the membranous CD24 immunoreactivity Fig. (2A,B). Positive membranous CD24 expression in endometrioid carcinoma cases was detected in 25/35 (71.4%) cases Fig. (3).

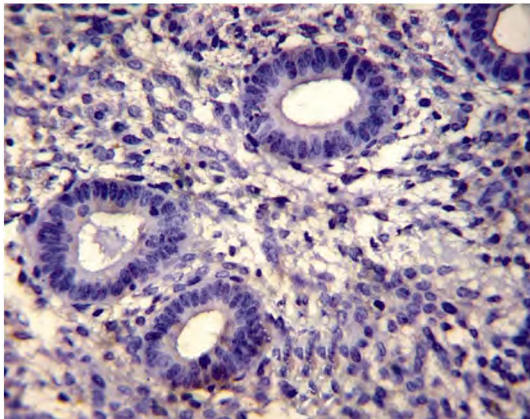


Fig. (1A): Proliferative endometrium showing negative CD24 expression (DAB chromogen, haematoxylin counter stain X400).

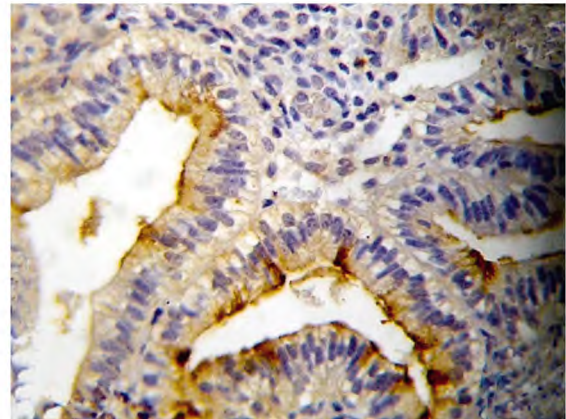


Fig. (1B): Secretory endometrium showing positive CD24 expression (DAB chromogen, haematoxylin counter stain X400).

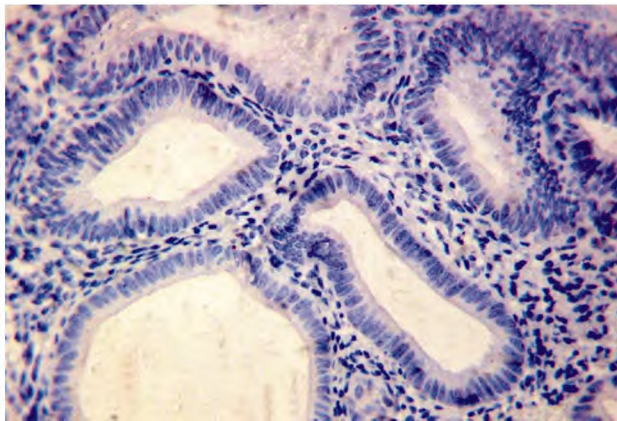


Fig. (2A): Endometrial hyperplasia without atypia showing negative CD24 expression (DAB chromogen, haematoxylin counter stain X400).

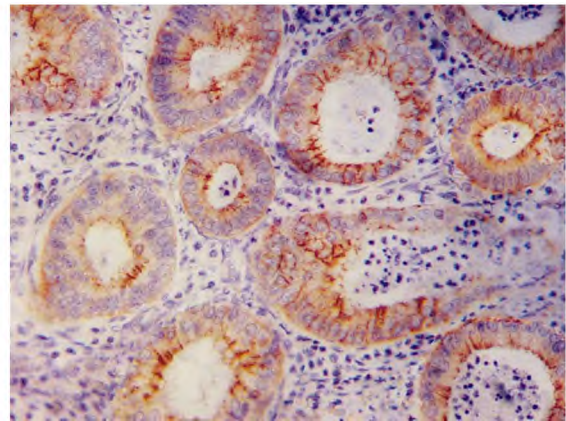


Fig. (2B): Atypical endometrial hyperplasia showing positive membranous CD24 expression (DAB chromogen, haematoxylin counter stain X400).

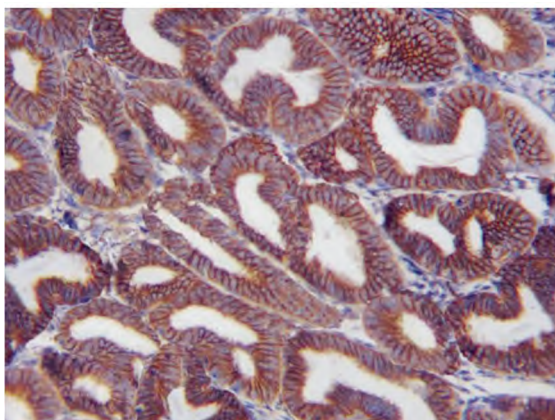


Fig. (3): Well differentiated endometrioid carcinoma showing positive membranous CD24 expression (DAB chromogen, haematoxylin counter stain X200).

Association between membranous CD24 expression and different histopathological groups:

Among the histopathological groups a statistically significant association was found in the membranous CD24 expression ($p < 0.001$). There was statistically significant increase in the expression of membranous CD24 in secretory phase cases as compared in proliferative phase cases ($p = 0.02$).

A statistically significant association in membranous CD24 immunostaining in cyclic endometrium cases as compared to its expression in EH without atypia, atypical EH cases and endometrioid carcinoma cases were found ($p = 0.04$, $p = 0.012$ and $p = 0.042$, respectively).

As regards cases of EH no statistically significant difference was found between expression among cases of EH without atypia and atypical EH cases ($p = 0.307$).

A statistically significant difference in the immunoexpression of membranous CD24 in endometrioid carcinoma cases as compared to its expression in EH without atypia and atypical EH cases was detected ($p < 0.001$ for both).

For membranous CD24 immunolocalization a statistically significant difference between atypical EH compared with well-differentiated endometrial carcinoma was found ($p = 0.023$).

The diagnostic validity of membranous CD24 immunoreactivity:

Sensitivity, specificity, PPV, NPV and accuracy of membranous CD24 in differentiating endometrioid carcinoma from atypical EH were shown in (Table 1). Membranous CD24 had 71.4% sensitivity and 76% specificity for differentiation of endometrioid carcinoma from atypical EH.

The diagnostic validity of membranous CD24 in distinction of well differentiated endometrioid carcinoma from atypical EH, had much lower sensitivity (46.7%).

Table (1): The diagnostic validity of membranous CD24 immunoreactivity.

Diagnosis	Sensitivity	Specificity	PPV	NPV	Accuracy
• Atypical EH Vs. carcinoma	71.4%	76%	80.6%	65.5%	73.3%
• Atypical EH Vs. well differentiated carcinoma	46.7%	76%	61.5%	65.5%	45%

PPV: Positive Predictive Value. NPV: Negative Predictive Value.

Association between membranous CD24 expression and clinicopathological variables of endometrioid carcinoma cases (Table 2):

There was statistically positive significant association between membranous CD24 immunostaining and tumor grades ($p = 0.040$). All cases of grade 3 were positive compared to (7/10) 70% and (8/15) 53.3% for grade 2 and grade 1, respectively.

Table (2): Association between membranous and cytoplasmic CD24 immunostaining and clinicopathological variables of endometrioid carcinoma cases.

Clinicopathological variables	No.	Membranous expression		p-value	Cytoplasmic expression		p-value
		Negative (%)	Positive (%)		Negative	Positive	
<i>Age:</i>							
<64	15	3 (20)	12 (80)	0.091	7 (46.7)	8 (53.3)	0.156
>64	20	8 (40)	12 (60)		14 (70)	6 (30)	
<i>Grade:</i>							
Grade 1	15	7 (46.7)	8 (53.3)	0.040	13 (86.7)	2 (13.3)	0.037
Grade 2	10	3 (30)	7 (70)		5 (50)	5 (50)	
Grade 3	10	0	10 (100)		4 (40)	6 (60)	
<i>Myometrial invasion:</i>							
<1/2	22	9 (40.9)	13 (59.1)	0.036	16 (72.7)	6 (27.3)	0.116
>1/2	13	1 (7.7)	12 (92.3)		6 (46.2)	7 (53.8)	
<i>LN metastasis:</i>							
Negative	17	5 (29.4)	12 (70.6)	0.168	11 (64.7)	6 (35.3)	0.323
Positive	5	0	5 (100)		2 (40)	3 (60)	
<i>FIGO stage:</i>							
Stage I	29	10 (34.5)	19 (65.5)	0.044	18 (62.1)	11 (37.9)	0.447
Stage II-III-IV	6	0	6 (100)		3 (50)	3 (50)	

Test of significance: Chi-square test and fisher exact test, Significant p -value < 0.05 .

As regards myometrial invasion a statistically significant positive association between membranous CD24 immunostaining and myometrial invasion ($p=0.036$), the positivity was higher among cases had $>1/2$ myometrial invasion in which 12/13 cases (92.3%) that had $>1/2$ myometrial invasion were positive to membranous CD24 immunostaining. Statistically significant association was found between membranous CD24 immunostaining and FIGO stage of carcinoma ($p=0.044$). All advanced stage cases 6/6 (100%) showed positive expression compared to early stage cases that had positivity in 19/29 (65.5%) of cases.

No statistically significant association was found between membranous CD24 immunorexpression and patients' age and LN status ($p=0.091$ & $p=0.168$ respectively).

2- *Cytoplasmic CD24 expression in different histopathological groups:*

The present study demonstrated statistically significant difference between the expression of cytoplasmic CD24 among the different histopathological groups ($p=0.022$). No statistically significant difference was found among normal cyclic endometrium ($p=0.276$).

There was a statistically significance in the expression of cytoplasmic CD24 in cyclic endometrium cases as compared to its expression in EH without atypia cases ($p=0.011$). However no significant differences were detected between cases of cyclic endometrium and atypical EH cases or endometrioid carcinoma cases ($p=0.293$ and $p=0.847$, respectively).

As comparing the immunocytoplasmic expression of CD24 between cases of EH without atypia and endometrioid carcinoma cases a statistically significant increase in the expression was detected ($p=0.004$).

Between cases of atypical EH cases and EH without atypia cases or endometrioid carcinoma cases no statistically significant differences were detected ($p=0.140$ and $p=0.153$, respectively). Also there was no statistically significant difference was found between cytoplasmic CD24 expression in atypical EH compared with well-differentiated endometrial carcinoma Fig. (4A,B) ($p=1.00$).

The diagnostic validity of cytoplasmic CD24 immunoreactivity:

Sensitivity, specificity, PPV, NPV and accuracy of cytoplasmic CD24 in differentiating endometrioid carcinoma from atypical EH were shown in (Table 3).

Cytoplasmic CD24 had low sensitivity (40%) but high specificity (80%) for distinction of endometrioid carcinoma from atypical EH.

As regard CD24 positive cytoplasmic immunorexpression in diagnosis of well differentiated endometrioid carcinoma in comparison with atypical EH, cytoplasmic CD24 had much lower sensitivity (13.3%).

Table (3): The diagnostic validity of cytoplasmic CD24 immunoreactivity.

Diagnosis	Sensitivity	Specificity	PPV	NPV	Accuracy
• Atypical EH Vs. carcinoma	40%	80%	73.6%	48.7%	56.6%
• Atypical EH Vs. well differentiated carcinoma	13.3%	80%	28.5%	60.6%	36.6%

PPV : Positive Predictive Value.
NPV : Negative Predictive Value.

Association between cytoplasmic CD24 expression and clinicopathological variables of endometrioid carcinoma:

The association of cytoplasmic CD24 expression with clinicopathological variables of endometrioid carcinoma cases was summarized in (Table 2).

This study demonstrate statistically significant positive association between cytoplasmic CD24 immunostaining and tumor grades ($p=0.037$). The frequency rate of positive cytoplasmic CD24 expression was highest in grade 3 [6/10 (60%) compared to 2/15 (13.3%) and 5/10 (50%) for grade 1 and 2, respectively].

Regarding other clinicopathological variables and cytoplasmic CD24 expression no statistically significant associations were detected.

3- *Combined membranous and cytoplasmic CD24 expression in histopathological groups:*

In cyclic endometrium, 9/20 (45%) were totally negative for CD24, 5/20 cases (25%) were positive for both expressions and 6/20 cases (30%) were -veMEM/+veCYTO or +veMEM/-veCYTO.

In EH without atypia, most cases 26/30 (86.6%) were totally negative for CD24, while 2/30 cases (6.7%) were positive for both expressions and 2/30 cases (6.7%) were +veMEM/-veCYTO.

Among cases of atypical EH, most cases 19/25 (76%) were totally negative for CD24, and 1/25 (4%) was +veMEM/-veCYTO while both staining qualities showed a rate of concordance in 5/25 cases (20%).

Regarding endometrioid carcinoma cases, the total CD24 expression was seen in 77.1% of cases, 16/35 cases (45.7%) showed veMEM/+veCYTO or +veMEM/veCYTO patterns of expression and both staining qualities showed a rate of concordance in 31.4% of cases.

Table (4): Combined membranous and cytoplasmic CD24 expression in different histopathological groups.

Histopathological groups	No.	Negative			Positive	
		-ve/-ve (%)	-ve/+ve or +ve/-ve (%)	+ve/+ve (%)		
Cyclic endometrium	20	9 (45)	6 (30)	5 (25)		
EH without atypia	30	26 (86.6)	2 (6.7)	2 (6.7)		
Atypical EH	25	19 (76)	1 (4)	5 (20)		
Endometrioid carcinoma	35	8 (22.9)	16 (45.7)	11 (31.4)		

ve/-ve : -veMEM/-veCYTO. +ve/-ve: +veMEM/-veCYTO.
 -ve/+ve: -veMEM/+veCYTO. +ve/+ve: +veMEM/+veCYTO.

Association between combined membranous and cytoplasmic CD24 immunostaining and histopathological groups:

The present study showed a statistically significant difference between combined membranous and cytoplasmic CD24 immunostaining among the histopathological groups ($p < 0.001$). Endometrioid carcinoma cases demonstrate the highest frequency rate of combined positive membranous and cytoplasmic expression.

A statistically significant increase in combined positive CD24 immunostaining in cyclic endometrium cases as compared to its expression in EH without atypia cases was found ($p = 0.007$).

However no statistically significant difference was found between cyclic endometrium cases and atypical EH cases or endometrioid carcinoma cases ($p = 0.283$ and $p = 0.225$, respectively).

The combined positive CD24 immunostaining in endometrioid carcinoma cases as compared to its expression in EH without atypia and atypical EH cases showed statistically significant increase in the expression ($p < 0.001$ and $p = 0.002$, respectively). Between cases of EH without atypia and atypical EH cases no statistically significant difference was detected ($p = 0.230$).

The diagnostic validity of total CD24 immunoreactivity:

Sensitivity, specificity, PPV, NPV and accuracy of total CD24 in differentiating endometrioid carcinoma from atypical EH were shown in (Table 5).

Total CD24 had 77.2% sensitivity and 76% specificity for distinction of atypical EH from endometrioid carcinoma. Regarding its diagnostic validity in distinction of well differentiated endometrioid carcinoma from atypical EH, total CD24 had 52.9% sensitivity and 76% specificity.

Table (5): The diagnostic validity of total CD24 immunoreactivity.

Diagnosis	Sensitivity	Specificity	PPV	NPV	Accuracy
• Atypical EH Vs. carcinoma	77.2%	76%	81.9%	70.3%	76.7%
• Atypical EH Vs. well differentiated carcinoma	52.9%	76%	60%	70.3%	70%

PPV : Positive Predictive Value.

NPV : Negative Predictive Value.

Association between combined membranous and cytoplasmic CD24 immunostaining and clinicopathological variables of endometrioid carcinoma cases (Table 6):

A statistically significant association was found between combined positive CD24 immunostaining and histological grades ($p = 0.035$). In grade 3, 6/10 (60%) showed combined positive membranous and cytoplasmic CD24 immunostaining compared to 2/15 (6.7%) and 4/10 (40%) for grade 1 and 2, respectively Fig. (5).

Table (6): Association between combined membranous and cytoplasmic CD24 immunostaining and clinicopathological variables of endometrioid carcinoma cases.

Clinicopathological variables	No.	Negative			p-value
		-ve/-ve (%)	-ve/+ve or +ve/-ve (%)	+ve/+ve (%)	
<i>Age:</i>					
<64	15	2 (13.3)	6 (40)	7 (46.7)	0.186
>64	20	8 (40)	7 (35)	5 (25)	
<i>Grade:</i>					
Grade 1	15	6 (40)	8 (53.3)	2 (6.7)	0.035
Grade 2	10	2 (20)	4 (40)	4 (40)	
Grade 3	10	0	4 (40)	6 (60)	
<i>Myometrial invasion:</i>					
<1/2	22	7 (31.8)	10 (45.5)	5 (22.7)	0.173
≥1/2	13	1 (7.6)	6 (46.2)	6 (46.2)	
<i>LN metastasis:</i>					
Negative	17	4 (23.5)	9 (52.9)	4 (23.5)	0.063
Positive	5	0	1 (20)	4 (80)	
<i>FIGO stage:</i>					
Stage I	29	8 (27.6)	14 (48.3)	7 (24.1)	0.183
Stage II-III-IV	6	0	2 (33.3)	4 (66.7)	

Test of significance: Chi-square test and f Significant p -value < 0.05
 -ve/-ve: -veMEM/-veCYTO. +ve/-ve: +veMEM/-veCYTO.
 -ve/+ve: -veMEM/+veCYTO. +ve/+ve: +veMEM/+veCYTO.

Association between combined positive CD24 immunostaining and LN status was non-significant ($p=0.063$), however, combined positive CD24 immunostaining was more frequently seen among cases positive for LN metastasis 4/5 (80%). Also No association was found between combined pos-

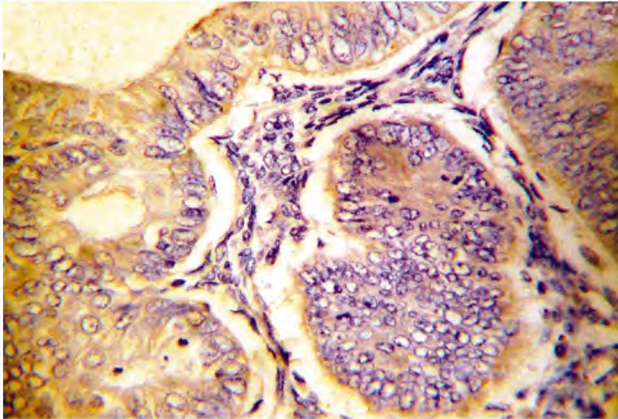


Fig. (4A): Atypical endometrial hyperplasia showing positive membranous CD24 expression (DAB chromogen, haematoxylin counter stain X400).

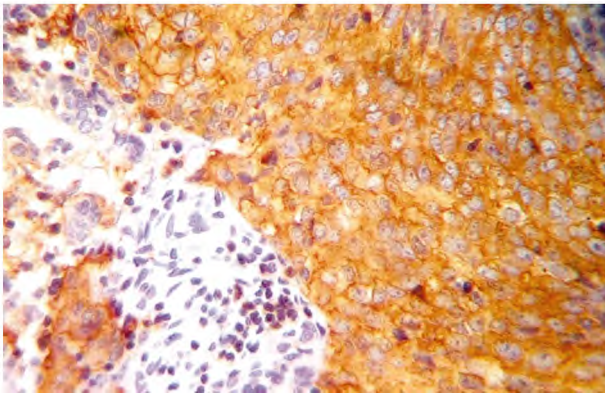


Fig. (5): Poorly differentiated endometrioid carcinoma showing positive membranous and cytoplasmic CD24 expression (DAB chromogen, haematoxylin counter stain X400).

Discussion

The endometrioid EC are indolent and low stage cancer. It commonly depends on hyperestrogenism and EH [21,22]. Both simple and complex hyperplasia without atypia is not considered pre-neoplastic forms [23]. Only atypical EH is clearly associated with the subsequent development of adenocarcinoma, so it is important to definitely differentiate between well-differentiated carcinomas and atypical EH [24].

In the present study, well differentiated endometrioid carcinoma representing 42.8%, this was in concordance with previous studies by Ohno et al.,

itive CD24 immunostaining and FIGO stage ($p=0.183$), however, most of advanced stage tumors 4/6 (66.7%) showed combined positive CD24 immunostaining. For patients' age or myometrial invasion no association were found in combined positive CD24 expression.

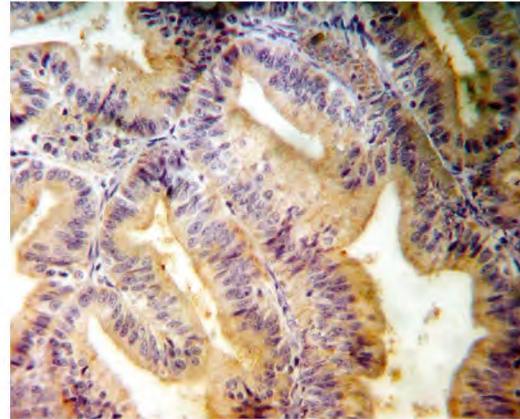


Fig. (4B): Well differentiated endometrioid carcinoma showing positive cytoplasmic CD24 expression (DAB chromogen, haematoxylin counter stain X400).

[25] and Markova et al., [26] who reported well differentiated endometrioid carcinoma ranging from 44.1%-48.3% among their series, while Younes et al., [27] reported that moderately differentiated endometrioid carcinoma represented 58.1% and in a study done by Abd El-Maqsoud and El-Gelany, [28] moderately differentiated endometrioid carcinoma and poorly differentiated endometrioid carcinoma (37.1%) was equally represented in their studied cases.

Regarding myometrial invasion, 62.9% cases showed myometrial invasion $<1/2$, while 37.1% showed myometrial invasion $\geq 1/2$, this was in line with Markova et al., [26] and Younes et al., [27].

For LN metastasis 77.2% of cases were negative and 22.8% were positive for LN metastasis. This finding in concordance with reported data by Markova et al., [26]. Moreover, much higher frequency of cases negative for nodal metastasis was reported (93.6% and 92.8%) by Ozkara and Corakci, [29] and Ohno et al., [25].

With respect to the stage of endometrioid carcinoma cases in this study, 82.9% were (stage I) and 17.1% were advanced stage (II/III/IV stages). These findings were consistent with other studies [26,27] who reported highest occurrence of localized disease ranging from 68.1%-81.6%, this confirming that endometrioid carcinoma often produces symptoms at relatively early stages, so this helping in early diagnosis of the disease.

CD24 protein was first reported to be expressed by pre-B lymphocytes, but it is lost during maturation to plasma cells [5]. CD24 immunorexpression have been reported to be an important marker for diagnosis, tumor progression and cancer-related death in many carcinomas [13-17].

In the current study, CD24 expression was observed in both membrane and cytoplasm of cells. This findings support earlier reports of membrane as well as cytoplasmic localization of CD24 in endometrial tissue [30-32]. As regard expression of membranous CD24 in cyclic endometrium positive membranous expression was detected in 45% of cases, the proliferative phase and secretory phase cases had 20% and 70% positivity, respectively. This was comparable to Kim et al., [31] and this difference was statistically significant as reported by Sundqvist et al., [32]. This up regulation of CD24 membranous expression from proliferative to secretory may suggest that CD expression has a role in differentiation or maturation of the endometrial glandular cells.

This study showed a statistically significant increase in the expression of membranous CD24 with the progression from EH without atypia, 13.4%, to atypical EH, 24%, to endometrioid carcinoma, 71.4%. This correlated with the study reported by Kim et al., [31] who reported that membranous CD24 expression significantly enhanced along different lesions; EH without atypia, 13.5%, atypical EH, 33.3% and endometrioid carcinoma, 64.6%. This finding suggests that CD24 expression may be linked with progression from precancerous lesion to carcinomatous lesion. A similar was documented in previous studies on colon cancer [33], and ovary, as Aktas et al., [34] and Moulla et al., [35] detected that the CD24 staining was significantly increased in ovarian carcinomas and borderline tumors compared to normal ovaries and cystadenomas. Also, this change of membranous CD24 expression in hyperplasia and carcinoma, in which there is lowering of expression in the hyperplastic lesions, followed by a remarkable increasing in endometrioid carcinoma, suggesting that membranous CD24 expression may be used as a diagnostic tool for the differential diagnosis between endometrioid carcinoma and atypical EH, which is a common diagnostic challenge, especially in the D & C Biopses.

In the current study, the positive expression rates of membranous CD24 were significantly lowered in either EH without atypia or atypical EH lesions than those of the cyclic endometrium. Moreover, no significant difference was found

between EH without atypia and atypical EH cases. These findings were consistent with Kim et al., [31].

On studying the association of membranous CD24 expression and different clinicopathological variables in endometrioid carcinoma cases, this study showed that positive significant association between membranous CD24 immunostaining and histological grades. This was in accordance with Karahan et al., [30] and Kim et al., [31].

Regarding CD24 immunostaining and myometrial invasion and FIGO stage as well, the present study demonstrates a significant positive association between them implying the role of CD24 in cancer progression. On contrary, Karahan et al., [30], and Kim et al., [31] did not found such association. These differences are related to the use of different scoring method and different antibodies.

Concerning LN status in the studied cases, no significant association was found between membranous CD24 immunostaining and LN status, although, all cases positive for LN metastasis were positive for membranous CD24 immunostaining versus 70.6% positivity for cases negative for LN metastasis. This was in accordance to Karahan et al., [30] and Kim et al., [31] who reported no significant association between membranous CD24 expression and LN metastasis. Similarly this finding reported in breast carcinoma [13] and ovarian carcinoma [36].

On studying cytoplasmic CD24 immunorexpression in cyclic endometrium; positive CD24 expression was detected in 35% of cases. These findings were in line with Kim et al., [31] who reported expression in 44.4% in their series.

No significant difference was detected between the expression of cytoplasmic CD24 and normal cyclic endometrium. This was comparable with Kim et al., [31] who found 41.7% and 47.8% positivity for proliferative phase and secretory phase cases, respectively.

A significant increase in cytoplasmic CD24 expression with lesion progression was detected in this study where the expression rates were 6.7%, 20%, 40% for EH without atypia, atypical EH and endometrioid carcinoma, respectively. Such finding demonstrated also by Kim et al., [31] where cytoplasmic CD24 expression was seen in 5.8%, 29.2%, 39% for EH without atypia, atypical EH and endometrioid carcinoma, respectively. Karahan et al., [30] reported lower rate of expression (28.9%) in endometrioid carcinoma cases. This different

rate of expression may be attributed to different clone used by that study (Ab2, clone 24 C02).

Concerning cytoplasmic CD24, in this study, the positive rates of cytoplasmic CD24 immunorexpression in various types of hyperplastic lesions were also lower than those of the cyclic endometrium and endometrioid carcinoma. There was a significant increase in the expression of cytoplasmic CD24 in EH without atypia cases as compared to its expression in cyclic endometrium and endometrioid carcinoma cases. But no significant difference was found between cyclic endometrium and atypical EH cases or cyclic endometrium and endometrioid carcinoma cases as well. This was in line with findings of Kim et al., [31].

Similarly, no significant differences were found between atypical EH cases and EH without atypia cases or endometrioid carcinoma cases regarding cytoplasmic CD24 expression. This was in concordance with Kim et al., [31].

Among cases of endometrial carcinoma this study demonstrates a positive significant association between tumor grade and cytoplasmic localization of CD24. However no significant associations were detected with other clinicopathological variables. Similar findings were reported by Karahan et al., [30] and Kim et al., [31].

In the current study, total positive CD24 expression was detected in 77.1% of endometrioid carcinoma. This correlated with the study performed by Karahan et al., [30] who reported total positive CD24 expression in 77.3% of their endometrioid carcinoma cases.

Based on previous reports and in the light of our findings, the intracellular localization of CD24 in cancer cells has been reported to express different biological features of cancer behavior. Membranous or cytoplasmic CD24 overexpression was closely related to adverse factors. However, the significance of CD24 overexpression was not consistent among carcinomas arising in different organs [15]. As in breast carcinoma, Kristiansen et al., [20] and Athanassiadou et al., [13] found no association between membranous CD24 immunoreactivity and clinicopathological parameters. While Bircan et al., [37] reported that membranous immunostaining was significantly positively correlated with tumor grade and there was no such an association with the cytoplasmic staining. In ovarian carcinoma, Kristiansen et al., [38] and Surowiak et al., [36] found no association for either membranous or cytoplasmic CD24 immunoreactivity with any of clinicopathological variables. But Moulla et al., [35],

reported that high-grade carcinomas and carcinomas with metastases to the omentum had considerably higher CD24 expression.

Accumulative data from this study and those of the literature [30,31] detected the positive correlation between adverse factors and membranous CD24 expression and the positive relationship with lesion progression for membranous CD24 immunorexpression and combined positive CD24 immunostaining as well. This supports the view that membranous CD24 expression and combined positive CD24 immunostaining as well may suggest a more aggressive phenotype in endometrioid carcinoma. In addition, the significant difference in membranous CD24 expression between endometrioid carcinoma and atypical EH is useful for the differential diagnosis between endometrioid carcinoma and atypical EH.

To the best of our knowledge, this is the first study that evaluated the sensitivity and specificity of CD24 expression in differentiating atypical EH from endometrioid carcinoma and atypical EH from well-differentiated endometrioid carcinoma as well. We found that membranous CD24 expression had more sensitivity and specificity than cytoplasmic CD24 expression which was not sensitive but was specific for such distinction. However, total positive CD24 expression is more sensitive (77.2%) than positive membranous or cytoplasmic expression alone (71.4% and 40%, respectively) in distinction of endometrioid carcinoma from atypical EH. Furthermore, total positive CD24 expression is more sensitive (52.9%) than membranous or cytoplasmic expression alone (46.7% and 13.3%) in distinction of well differentiated endometrioid carcinoma from atypical EH.

This study demonstrates that CD24 is expressed in normal cyclic endometrium, hyperplastic lesion and endometrial carcinoma. The up regulation of membranous CD24 expression from proliferative to secretory endometrium indicates that CD24 has a role in differentiation of the endometrial glands. Enhanced membranous CD24 expression can be used as a diagnostic marker in distinction of well differentiated endometrioid carcinoma from atypical EH however total CD24 (membranes and cytoplasmic) expression is more sensitive in this Challenge. CD24 overexpression is involved in progression of endometrial carcinoma.

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التعبير التفاضلي ل سي دي ٢٤ وأهميته في بطانة الرحم الطبيعية وبطانة الرحم المفرطة وسرطان بطانة الرحم

الخلفية: يعتبر سرطان بطانة الرحم سادس أكثر أنواع السرطانات شيوعاً بين النساء في جميع أنحاء العالم، حيث يمثل ٤٪ من جميع أنواع السرطان لدى النساء بروتين سي دي ٢٤ في الأصل يتم التعبير عنه بواسطة الخلايا اللمفاوية قبل خلايا البى. تم التعرف على هذا البروتين فقط كعلامة لسلاسل الخلية المكونة للدم. يعتبر سي دي ٢٤ علامة بيولوجية مهمة للتشخيص وتطور المرض والوفاة المرتبطة بالسرطان في العديد من الأورام الصلبة، مثل سرطان الثدي وسرطان القولون والمبيض.

الهدف: أجرى هذا العمل لدراسة التعبير المناعي ل سي دي أربعة وعشرون في بطانة الرحم العادية، بطانة الرحم المفرطة وسرطان بطانة الرحم، من أجل إظهار نمط تعبيره ودراسة إرتباطه بالعديد من المتغيرات الإكلينيكية في حالات سرطان بطانة الرحم.

المواد والطرق: تضمنت هذه الدراسة ١١٠ حالات من أنسجة بطانة الرحم: ٢٠ حالة من بطانة الرحم العادية، ٣٠ حالة من تضخم بطانة الرحم النمطية، ٢٥ حالة من تضخم بطانة الرحم الغير نمطية و٣٥ حالة من سرطان بطانة الرحم. تم إجراء الكيمياء المناعية باستخدام طريقة الأفيدين البيوتين بيروكسيديز.

النتائج: التعبير المناعي ل سي دي أربعة وعشرون كان غشائى وسيتوبلازمى. في بطانة الرحم الطبيعية الدورية، في المرحلة التكاثرية كان هناك إنخفاض في التعبير الغشائى ل سي دي أربعة وعشرون أما في المرحلة الإفرازية. فقد أظهر زيادة في التعبير الغشائى له. تم زيادة إحصائية لكلا من سي دي أربعة وعشرون الغشائى والسيتوبلازمى في سرطان بطانة الرحم وإنخفاض كلا منهما في تضخم بطانة الرحم. من بين حالات السرطان، كان التعبير الغشائى ل سي دي أربعة وعشرون ذو دلالة إحصائية مع درجة ومرحلة الورم والغزو العضلى. وأظهر التعبير السيتوبلازمى إرتباط كبير مع درجة الورم فقط. كان للتعبير الغشائى ل سي دي أربعة وعشرون حساسية ٧١.٤٪ وخصوصية ٧٦٪ للتمايز سرطان بطانة الرحم من تضخم بطانة الرحم غير النمطية. أما التعبير السيتوبلازمى فقد كان له حساسية منخفضة (٤٠٪) ولكن خصوصية عالية (٨٠٪) للتمييز بين سرطان بطانة الرحم من تضخم بطانة الرحم غير نمطية. كان إجمالي التعبير ل سي دي أربعة وعشرون ٧٧.٢٪ حساسية و٧٦٪ خصوصية للتمييز بين تضخم بطانة الرحم غير نمطية من سرطان بطانة الرحم.

الخلاصة: التعبير الغشائى للسي دي أربعة وعشرون يتم في نمط دورى في بطانة الرحم الطبيعية. تمت زيادة التعبير للسي دي أربعة وعشرون في حالة سرطان بطانة الرحم عنه في. تضخم بطانة الرحم الغير نمطية هذه النتائج تشير إلى أن سي دي أربعة وعشرون يمكن أن يستخدم كعلامة تشخيصية مفيدة في التمايز بين تضخم بطانة الرحم الغير نمطى والسرطان. أيضاً سي دي أربعة وعشرون له دور في تطور سرطان بطانة الرحم. مجموع تعبير سي دي أربعة وعشرون (الغشائى والسيتوبلازمى) هو أكثر تحديداً وأكثر حساسية في التمايز من تضخم بطانة الرحم غير نمطية من سرطان بطانة الرحم.