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Research Paper

The Role of COX2 Immunohistochemical Expression in Endometrial Endometrioid Carcinoma and Endometrial Hyperplasia and Its Correlation with Clinicopathological Parameters

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ABSTRACT:

BACKGROUND:

Uterine corpus tumour is one of the most prevalent gynecological malignancies. 75% of women are postmenopausal at time of diagnosis. Thus, Postmenopausal bleeding is the most common symptom, COX2, also known as prostaglandin H synthase, catalyzes the formation of prostaglandin from arachidonic acid, identified as a potential indicator of the neoplastic transformation of normal cells into tumours cell.

OBJECTIVE:

To evaluate the Cyclooxygenase-2(COX2) immunohistochemical expression in endometrial carcinoma and endometrial hyperplasia with and without atypia and its correlation with clinicopathological parameters.

PATIENTS AND METHODS:

Cross sectional study carried out in the Department of Pathology, Babylon Center during the period from December 2022 through December 2023 including (70) cases: (36) endometrial biopsy (34) hysterectomy specimens; patients age ranges from 31 to77 years. (20) cases as endometrial hyperplasia without atypia; (20) cases as atypical endometrial hyperplasia; (30) cases of endometrial carcinoma. Two sections of 5µm thickness were taken from each block, the first was stained with (H&E) stain for histopathological revision, the other section was stained for COX2 (IHC) antigen expression .

RESULTS:

Higher COX2 expression with strong cytoplasmic and membranous staining was found in endometrial carcinoma than endometrial hyperplasia which mostly show weak to negative staining, this expression increase with advanced stage and grade of endometrial carcinoma which mean that COX2 have a role in tumour progression from hyperplasia to carcinoma.

CONCLUSION:

- COX2 immunohistochemical expression increased in endometrial carcinoma, associated closely with parameters of tumour aggressiveness such as advanced tumour stage and grade, this makes the possibility of the use of COX2 inhibitors as adjunct endometrial carcinoma treatment and to decrease recurrence after treatment, also the possibility for it use in prevention of endometrial carcinoma in high risk groups.
- Endometrial carcinoma with strong positivity for COX-2 expression have a poor prognostic significance.

KEYWORDS: Cox2 enzyme, endometrial carcinoma, endometrial hyperplasia.

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INTRODUCTION:

Endometrial cancer is the sixth most common cancer in women and the fifteenth most common cancer worldwide [(1)]. Typically occurring in elderly women, 80% of them being postmenopausal at the time of diagnosis, most endometrial carcinomas occurring in women aged 40 years or younger are of endometrioid type, well to moderately differentiated, and early-stage disease. Conversely, tumours of elderly patients are more likely to be higher grade and to have more advanced disease at the time of diagnosis [(2)]. An early symptom of endometrial cancer is irregular or postmenopausal bleeding. Advanced endometrial cancer frequently has unfavourable outcomes, largely due to limited effective treatment options [(3)]. Different pathological types

of primary endometrial carcinomas including endometrioid endometrial carcinoma that closely resembling preexisting endometrial epithelium , clear cell carcinomas, uterine serous carcinomas (also referred to as uterine papillary serous carcinomas), undifferentiated carcinomas and carcinosarcomas (mixed epithelial-mesenchymal differentiation) [4] Cyclooxygenase-2 is an inducible enzyme, which catalyzes the first step in the prostanoids synthesis and is associated with inflammatory diseases as well as carcinogenesis by promoting angiogenesis and tissue invasion by tumours and apoptosis resistance [5].

Generally, the COX2 protein is only expressed in a limited number of cell types, whereas overexpressed in a variety of malignant tumours,suggesting its possible role in carcinogenesis,therefore, cox2 might be a potential factor affecting EC progression [6] .

METERIALS AND METHOD:

Primary antibody (COX2): Rabbit monoclonal antibody, for an enzyme called prostaglandin-endoperoxide synthase 2 (PTGS2), that involved in conversion of arachidonic acids to prostaglandins, COX-2 has cytoplasmic and membranous staining, 6ml; PathnSitu prediluted format ready to use; further dilution may lose the activity and may yield to suboptimal staining, Clone: EP293.

Positive immunohistochemical reaction for COX2 antibody is evaluated by the diffuse brown cytoplasmic and membranous staining.

RESULTS:

The score calculation: No staining was scored as 0, negative; 1, 1–25 % positive cells; 2, 26–50 % positive cells; 3, 51-75 % positive cells; 4, 76-100%. Immunostaining intensity was evaluated and rated as follows: 0, negative; 1, weakly positive; 2, moderately positive; 3, strongly positive. Then for each case, a combined score was evaluated by multiplying the score for extent by the score for intensity so the combined immunoreactivity score (IRS) ranged from 0-12. [8]

Table1: Distribution of patients according to age (n=70).

Age (years)	Number	%
30-40 years	7	10.0%
41-50 years	18	25.7%
51-60 years	24	34.3%
61-70 years	13	18.6%
>70 years	8	11.4%
Total	70	100.0%

Table 2: Distribution of patients according to the type of vaginal bleeding.

Clinical presentation	Number	%
Postmenopausal bleeding	28	40.0%
Heavy menstrual bleeding	42	60.0%
Total	70	100.0%

Table 3: Distribution of patients according to type of biopsy (N=70).

Type of biopsy	Number	%
Total abdominal hysterectomy	34	48.6%
Dilation and curettage	36	51.4%
Total	70	100.0%

Table 4: Distribution of patients with endometrial carcinoma according to staging and grading of tumour (N=30).

Study variables	Number	%
Staging		
Stage 1a	12	40.0%
Stage 1b	9	30.0%
Stage 2	7	23.3%
Stage 3	2	6.7%
Total	30	100.0%
Grading		
Grade 1	14	46.7%
Grade 2	12	40.0%
Grade 3	4	13.3%
Total	30	100.0%

Table 5: Significant elevation in age of patients with endometrial carcinoma.

Histopathological diagnosis	N	Mean ± SD	F-test	P- value
Endometrial carcinoma	30	57.97 ± 12.72	6.466	0.003
Atypical endometrial hyperplasia	20	53.90 ± 9.43		
Endometrial hyperplasia without atypia	20	47.05 ± 7.39		

Table 6: Significant association between histopathological diagnosis with clinical presentation and type of biopsy among study patients.

Study variables	Histopathological diagnosis			P value
	Endometrial carcinoma	Atypical endometrial hyperplasia	Endometrial hyperplasia without atypia	
Clinical presentation				**0.002**
Postmenopausal bleeding	18 (60.0)	8 (40.0)	2 (10.0)	
Heavy menstrual bleeding	12 (40.0)	12 (60.0)	18 (90.0)	
Total	30 (100.0)	20 (100.0)	20 (100.0)	
Type of biopsy				**<0.001**
Total abdominal hysterectomy	30 (100.0)	4 (20.0)	0 (0.0)	
Dilation and curettage	0 (0.0)	16 (80.0)	20 (100.0)	

Table 7: Significant association between diagnosis and Cyclooxygenase-2 (COX2) immunohistochemical expression.

Cyclooxygenase-2 (COX2) immunohistochemical expression	Histopathological diagnosis			P value
	Endometrial carcinoma	Atypical endometrial hyperplasia	Endometrial hyperplasia without atypia	
Negative (no expression)	8 (26.7)	7 (35.0)	16 (80.0)	<0.001
Weak expression (+)	6 (20.0)	11 (55.0)	4 (20.0)	
Moderate expression (++)	10 (33.3)	2 (10.0)	0 (0.0)	
Strong expression (+++)	6 (20.0)	0 (0.0)	0 (0.0)	
Total	30 (100.0)	20 (100.0)	20 (100.0)	

Table 8: Significant association between staging of endometrial carcinoma and Cyclooxygenase-2 (COX2) immunohistochemical expression. All patients with stage 3 presented with strong expression (+++).

Cyclooxygenase-2 (COX2) immunohistochemical expression	Staging of endometrial carcinoma				P value
	Stage 1b		Stage 2	Stage 3	
Negative (no expression)	5 (41.7)	2 (22.2)	1 (14.3)	0 (0.0)	0.03
Weak expression (+)	5 (41.7)	1 (11.1)	0 (0.0)	0 (0.0)	
Moderate expression (++)	2 (16.7)	4 (44.4)	4 (57.1)	0 (0.0)	
Strong expression (+++)	0 (0.0)	2 (22.2)	2 (28.6)	2 (100.0)	
Total	12 (100.0)	9 (100.0)	7 (100.0)	2 (100.0)	

Table 9: Significant association between grading of endometrial carcinoma and Cyclooxygenase-2 (COX2) immunohistochemical expression.

Cyclooxygenase-2 (COX2) immunohistochemical expression	Grading of endometrial carcinoma			P value
	Grade 1	Grade 2	Grade 3	
Negative (no expression)	6 (42.9)	2 (16.7)	0 (0.0)	0.161
Weak expression (+)	3 (21.4)	3 (25.0)	0 (0.0)	
Moderate expression (++)	4 (28.6)	5 (41.6)	1 (25.0)	
Strong expression (+++)	1 (7.1)	2 (16.7)	3 (75.0)	
Total	14 (100.0)	12 (100.0)	4 (100.0)	

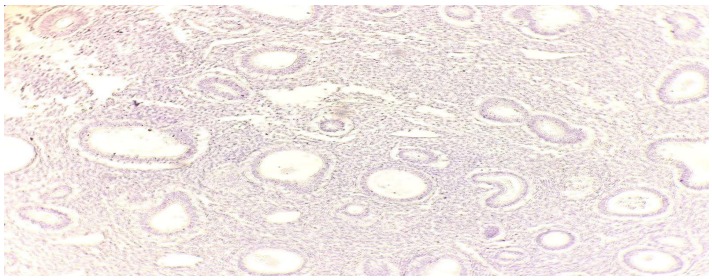

Figure 1: COX2 immunostaining of endometrial hyperplasia without atypia shows negative expression. (10X).

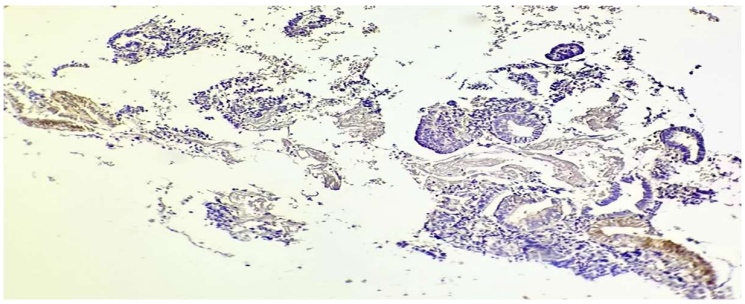

Figure 2: Focal COX2 staining in atypical endometrial hyperplasia. (10x).

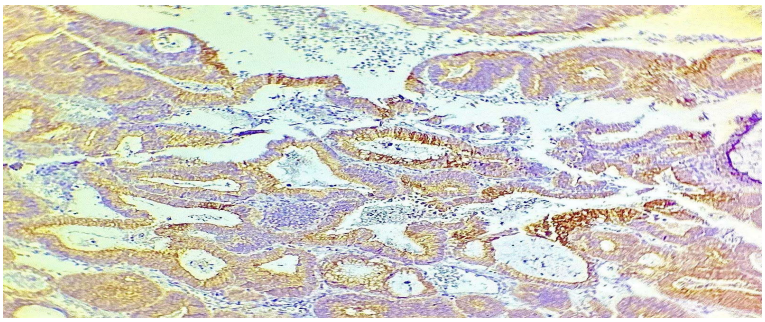

Figure 4: Low grade endometrioid carcinoma show variable weak, moderate, strong COX2 positivity.

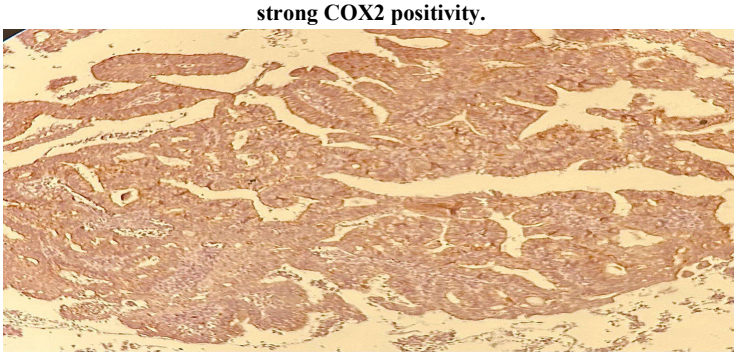

Figure 5: COX2 immunostaining of Grade2 endometrioid carcinoma show diffuse strong cox2 positivity. (40X).

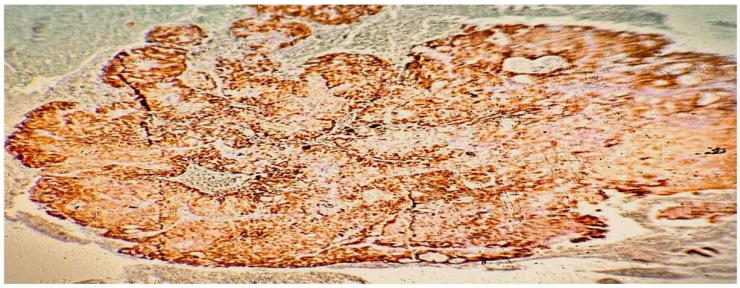

Figure 6: COX2 imunohistochemical staining of high-grade (grade3 with solid growth pattern) endometroid carcinoma, which show diffuse and strong C0X2 positivity. (40X)

DISCUSSION:

This current study include a review of 70 cases, the age range from 31-77 years, with mean age (53.69 ± 11.32) .

In the presented study, there is a significant correlation between histopathological diagnosis (endometrial carcinoma and endometrial hyperplasia with or without atypia) with the age group of patients which shows that the incidence of endometrial carcinoma higher in older age group than endometrial hyperplasia mean age for carcinoma cases (57.97 ± 12.72), for atypical endometrial hyperplasia (53.90 ± 9.43) and for endometrial hyperplasia without atypia (47.05 ± 7.39) this agree with a study from Kingdom of Bahrain done by **Ganesh** [9] who studied the relation between age and endometrial carcinoma incidence and found that 56% of all cases (16 cases) of endometrial carcinoma with age group 60y and above.

The clinical presentation of patients in this presented study; most women with endometrial carcinoma complain from postmenopausal bleeding in comparison to women with endometrial hyperplasia with and without atypia who mostly suffer from heavy and irregular menstrual bleeding(as 18 cases out of 30 EC cases presented with PMB while only 2 cases out of 20 cases of endometrial hyperplasia without atypia suffering from PMB and remaining 18 ones suffering from HMB) , this significant correlation between the clinical presentation and endometrial carcinoma risk agrees with a study from the UK done by **Pennant et al.**[10] which found that the risk of endometrial carcinoma in premenopausal women with abnormal uterine bleeding is low and higher in PMB.

About COX2 IHC expression, we found a statistically significant association with the patient's age and clinical presentation as there is an increase in COX2 expression in older women and in women with PMB, this nearly agree with **M. Lyndin** [11] (30 cases of endometrial carcinoma used with mean age 56.2 ± 8.4 years old) and found that COX2 in EC tissue was detected in older women (p value = 0.0220), no another study found for comparison.

This current study found a significant increase in COX2 expression with progression from endometrial hyperplasia without atypia to atypical endometrial hyperplasia to endometrial carcinoma (p value < 0.001).

Majority of endometrial carcinoma cases show positive expression for COX2 (22 out of 30 cases) mostly with moderate to severe expression, less expression in atypical endometrial hyperplasia (13 out of 20 cases) with moderate to weak expression and mostly negative to weak and focal in endometrial hyperplasia without atypia (only 4 out of 20 cases show weak positive expression), this result nearly agree with study from Turkey done by **Hande Ece Kalkan et al.** [12] in which total of 120 cases were examined (30 cases as control group , 30 cases for carcinoma and 30 cases for each endometrial hyperplasia with and without atypia) the study show increase in COX-2 expression in endometrial carcinoma in comparison to the remaining groups and strong expression with progression to carcinoma.

Regarding endometrial carcinoma staging, in this current study, there is a significant correlation between the FIGO tumour stage of EC and cox2 expression as all patients with stage 3 show strong cox2 expression (+++), 6 of 7 stage 2 cases between moderate to strong expression while from 9 cases of stage 1b (>50% of myometrium invasion) 2 negative cases and 1 case with weak expression and from 12 cases of stage 1A (<50% of myometrial invasion), 10 cases between negative to weak expression and no case with strong expression, this agrees with a study from China done by **Lin Deng** [13] (which included 61 patients with EC in different stage and found that overexpression of cox2 showed relationships with factors related to EC progression, such as FIGO stage ($P < 0.05$).

Sunitaś study, 2018 from India in which a total of fifty cases of endometrial carcinomas were reviewed in different stages from stage 1 to stage

4 and show an increase in COX-2 positivity from 27% in Stage I to 100% in Stage IV that is also agree with this current study.

However, this current study is not compatible with study from Ukraine done by **M. Lyndin** [11] (in which 30 cases of endometrial carcinoma were reviewed, rabbit polyclonal cox2 were used, and the study did not find a relationship between the FIGO tumour stage and COX2 levels (p value= 0.14) .

Regarding the FIGO grade of endometrial carcinoma, in this current study (3 out of 4 grade 3 cases) show strong expression with cytoplasmic and membranous staining of solid growth and high grade degree nuclear atypia and remaining one with moderate expression that differ significantly from low grade (6 out of 14 grade 1 cases) were negative to cox2 and only one case showstrong positivity, while grade 2 show variable cox2 expression from negative to strong positivity. However this study shows no statistical significance between the tumour grade and cox2 expression ,this disagrees with **M. Lyndin,** [11] (among 30 cases of EEC , 7 correspond to grade 1 , 16 to grade 2 and 7 to grade 3) found significant association and increase in cox2 expression with advanced grade (p=0.0054) but agrees with **Sunita study, 2018**[14] (fifty cases of endometrial carcinoma were examined and the result that COX-2 immunopositivity was 50%, 28%, and 41% in Grade 1, Grade 2, and Grade 3 respectively),this variation may be due to the low number of patients in our study, especially in G3 or differences in the type of antibody used .

Therefore, our study results are consistent with variable cox2 expression according to histopathological diagnosis, which increases with progression from endometrial hyperplasia to endometrial carcinoma; it is not possible to depend on the presence of COX2 as a tumour cell indicator because of its potential presence in the endometrial hyperplasia and in normal endometrium (as seen in some previous study). [14]

CONCLUSION:

- COX2 immunohistochemical expression increased in endometrial carcinoma, associated closely with parameters of tumor aggressiveness such as advanced tumour stage and grade, this makes the possibility of the use of COX2 inhibitors as adjunct endometrial carcinoma treatment and to decrease recurrence after treatment, also the possibility for it use in prevention of endometrial carcinoma in high risk groups.
- Endometrial carcinoma with strong positivity for COX-2 expression have a poor prognostic significance.

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