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Immunohistochemical Expression of Cyclooxygenase-2 in endometrioid carcinoma

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Abstract:

Background and objectives: Cyclooxygenase-2 functions as an enzyme which synthesize prostaglandins from Arachidonic acid Cyclooxygenase -2 is one of the important factors in maintaining the endometrium during menstrual cycle. The Aim of this study was to assess expression of Cyclooxygenase-2 in endometrioid carcinoma by immunohistochemistry to explore association between Cyclooxygenase-2 expression and a certain number of clinicopathologic variables.

Methods: A retrospective study was carried out for seventy-eight blocks that were preseved in formalin and embedded in paraffin of endometrioid carcinoma were obtained and randomly selected from a private laboratory in Erbil city during October 2020 to October 2022. COX-2 rabbit monoclonal antibody was used to satin slides obtained from endometrioid carcinoma blocks and the expression was scored by multiplying the number of stained cells by the intensity of staining.

Results: Cyclooxygenase-2 expressed in 15.4% of cases of study cases. It is down-expressed in high grade endometrioid carcinoma while over-expressed in cases with myometrial invasion ≥50%. Regarding other clinicopathological parameters like, lymphovascular invasion, tumor stage and lymphnode status, Cyclooxygenase-2 showing down-expression, there is no existence of notable link between the expression of Cyclooxygenase-2 and clinicopathological parameters, including: Age(p=0.197), Tumor grade(p=0.069), Myometrial invasion(p=0.261), Lympho vascular invasion(p=0.516), Lymph node status(p=1000) and Tumor stage(p=0.872).

Conclusion: Cyclooxygenase-2 is expressed in a limited number of endometrioid carcinoma and there is lack of significant association between Cyclooxygenase-2 expression and clinicopathological parameters.

Keywords: COX-2, Endometrioid carcinoma, Immunohistochemistry.

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Introduction:

Uterine corpus carcinoma holds the position of the sixth most prevalent cancer among women worldwide. The incidence is continuously rising,[1,2] According to International Agency for Research on Cancer the incidence rate of endometrial cancer may reach more than 50% worldwide by 2040.[3] In Kurdistan region the incidence rate is also increasing, according to a survey in Erbil, uterine cancer holds the seventh position among the most frequently diagnosed cancers universally, and in the specific context of Duhok, it stands as the tenth most common form of cancer.[4] The risk factors of endometrial carcinoma are estrogen excess, tamoxifen therapy, hereditary factors, infertility, increasing age, thyroid disease, Polycystic ovarian syndrome, obesity, diabetes and hypertension.[5] Bokhman before thirty years identified two different pathogenetic classifications of endometrial carcinoma according to estrogen dependence, type 1 endometrioid carcinoma which is (estrogen-dependent) and type 2 non-endometrioid which is (non-estrogen-dependent).[6] Endometrioid carcinoma is categorized based on (The Cancer Genome Atlas) into four molecular subtypes: Ultra mutated (POLE mutated), Hypermutated/Microsatellite instability (MSI), copy number high/serous like subtype, copy number low.[7] Endometrioid carcinoma classified based on (International Federation of Gynecology and Obstetrics/ FIGO) grading system into Grade 1 tumors have ≤5%, grade 2 (6-50%), grade 3 >50% solid component, (grade1/2) regarded as (low grade) and grade 3 regarded as (high grade).[8]Cyclooxegenase-2 (COX-2), alternatively called prostaglandin-endoperoxide synthase, it is mostly present in endoplasmic reticulum.[9] It is an enzyme which synthesize prostaglandins from arachidonic acid, playing an important role in inflammatory response and it is highly affected by tumor promoters, growth factors and prostaglandins.[10] Cyclooxygenase-2 also has role in carcinogenesis which can be confirmed by overexpression in a variety of malignant tumors while it is expressed only in a limited number of cells.[11] It has been determined that COX-2 enhances activities that are conducive to tumor growth through a number of mechanisms, like angiogenesis, resistance to programmed cell death, modification of host immune surveillance, increased DNA mutation frequency, increased function of peroxidase enzymes, all of which contribute to the cancer's invasiveness.[12] It has been discovered that COX-2 gene promoter region was hypomethylated in three diseased participants that is endometriosis, endometrioid carcinoma of ovary, endometrioid endometrial cancer.[13] Cyclooxygenase-2 is one of the important factors in maintaining the endometrium during menstrual cycle.[14] Several investigations have verified the overexpression of COX-2 in tissue samples from endometrioid carcinoma.[9] It has been proposed that the enzyme level might be used for the treatment response and degree of myometrial invasion.[15] Considering the availability of both (selective and non-selective COX2 blockers), blocking of COX2 activity seems to be a potential technique for treating malignancies.[16] A study reported tumor inhibiting effect of celecoxib through both (COX-2-dependent and -independent) pathways in endometrioid carcinoma.[17]This study aimed to assess frequency of immunohistochemical COX-2 expression in endometrioid carcinoma and to investigate its association with some clinicopathologic parameters, such as: age, tumor grade, myometrial invasion, lymphovascular invasion, lymph-node status and tumor stage.

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Material and methods:

Seventy-eight formalin-fixed paraffin-embedded blocks of hysterectomy specimens diagnosed as endometrioid carcinoma were randomly selected from a privately-owned laboratory situated within the Erbil city limits during October 2020-October 2022. Two sections were prepared from each block, one stained with H&E for the purpose of histological analysis, while the second section was utilized for immunohistochemical assessment of COX-2 expression. The microscopical classification & grading of endometrioid carcinoma cases were carried out according to FIGO grading system [18] and the pathological staging was done depending on eighth edition of the American Joint Committee on Cancer (AJCC).[19] Ethical approval was obtained from the Ethics Committee of Kurdistan Higher Council of Medical Specialties.Four µm thick sections were cut and mounted on charged slides. After drying at 60 °C for one hour, slides were subjected to deparaffinization and rehydration at the temperature of the surrounding environment (20-25 °C), Then positioned in a xylene bath and after five minutes incubation they were put in ethanol for the next three minutes. Lastly, the immersion of the specimens in distilled water was performed for thirty seconds. Epitope retrieval was carried out by using a specific method in 10 mmol/L citrate buffer 1:10 ratio with distilled water. Immunohistochemical staining was performed using COX-2 rabbit monoclonal antibody, catalog no: BSB 5362, a concentrated dilution of 0.5 ml at a ratio of 1:100 was administered to the tissue sections. The reactivity for COX-2 was considered as positive when staining of the cytoplasm was observed, and scoring of COX-2 immunoreactivity was done and reviewed by two expert pathologists. With every staining procedure, control slides were created specifically for positive and negative controls. A specific example diagnosed as colorectal adenocarcinoma was used as a positive control for COX-2 expression, while negative controls were created by removing the (primary antibody) and by using (N-Universal negative control).For scoring of the expression of COX-2 expression, the immunoreactive score (IRS) was used which multiplies both the proportion of cells showing immunoreactivity and the intensity of staining as follows:The proportion of immunoreactive cells: 0 (0%), 1 (1-25%), 2 (26-50%), 3 (51-75%) and 4(76-100%) assessing the ratio of carcinoma cells with positive staining compared to the total population of carcinoma cells. The intensity of the staining was scored as: 0 (Negative), 1 (Weak), 2 (Medium), and 3 (Strong). Cyclooxygenase Immunohistochemical scoring results ranged from (0-12): 0 point: (-) Negative, (1-4) points: (+) Weakly positive, (5-8) points: (++) Moderately positive, (9-12) points: (+++) Strongly positive.A staining score equal or more than 5 is regarded as positive[11,16]The data analysis was performed using the Statistical Package for the Social Sciences (SPSS, version 25). The data sets were subjected to a comparative analysis by using (Chi Square Test of Association). Fisher′s exact test was utilized when the expected frequency (Value) of over 20% of the cells in the tables was below 5. A (p value of ≤ 0.05) was deemed to have statistical significance.

Results:

The study involved the participation of Seventy-Eight women who were diagnosed with endometrioid carcinoma. The average age of the participants was 60.1 years with a standard deviation 11.6, the middle value of the participants′ ages was 60 years and the age of the participants ranged from 34 to 85 years. Approximately half of the female participants (47.4%) were above the age of 60.It is evident in Table 1 that the majority (93.6%) of the tumors were categorized as

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low grade and tumors classified as stage T1 accounted for 83.3% of the cases; ≥ 50% of the myometrium was invaded by 69.2% of the tumors. There was lympho-vascular invasion in 14.1% of patients. Lymph-node metastasis was detected in 5.1% of the patients. A positive score for COX-2 expression was found in 15.4% of the patients Table (1).

Table (1): Endometrioid carcinoma characteristics.

	No.	**(%)**
Tumor grade		
Low	73	(93.6)
High	5	(6.4)
Myometrial invasion		
<50%	24	(30.8)
≥50%	54	(69.2)
Lymphovascular invasion		
Negative	67	(85.9)
Positive	11	(14.1)
Lymphnode status		
Negative	74	(94.9)
Positive	4	(5.1)
Tumor stage	65	(83.3)
T1	5	(6.4)
T2	**7**	(9.0)
T3	1	(1.3)
T4		
COX 2 expression score		
Negative	66	(84.6)
Positive	12	(15.4)
Total	78	(100.0)

There was no significant correlation observed between the expression of COX-2 and any of the factors, including: Age (p = 0.663), Tumor grade (p = 0.577), Myometrial invasion (p = 0.745), Lympho vascular invasion (p = 0.675), Lymph node status (p = 0.110) and Tumor stage (p=1.000) as presented in Table (2).

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Table (2): COX 2 expression by the studied factors:

	COX 2 expression			
	Negative	Positive	Total	p value
Age (years)				
≤ 60	34 (82.9)	7 (17.1)	41 (100.0)	
> 60	32 (86.5)	5 (13.5)	37 (100.0)	0.663*
Tumor grade				
Low	62 (84.9)	11 (15.1)	73 (100.0)	
High	4 (80.0)	1 (20.0)	5 (100.0)	0.577**
Myometrial invasion				
< 50%	21 (87.5)	3 (12.5)	24 (100.0)	
≥ 50%	45 (83.3)	9 (16.7)	54 (100.0)	0.745**
Lymphovascular invasion				
Negative	57 (85.1)	10 (14.9)	67 (100.0)	
Positive	9 (81.8)	2 (18.2)	11 (100.0)	0.675**
Lymphnode status				
Negative	64 (86.5)	10 (13.5)	74 (100.0)	
Positive	2 (50.0)	2 (50.0)	4 (100.0)	0.110**
Tumor stage				
T1	55 (84.6)	10 (15.4)	65 (100.0)	
T2	4 (80.0)	1 (20.0)	5 (100.0)	
T3	6 (85.7)	1 (14.3)	7 (100.0)	
T4	1 (100.0)	0 (0.0)	1 (100.0)	1.000**
Total	66 (84.6)	12 (15.4)	78 (100.0)	

*By Chi Square Test. **By Fisher's exact test.

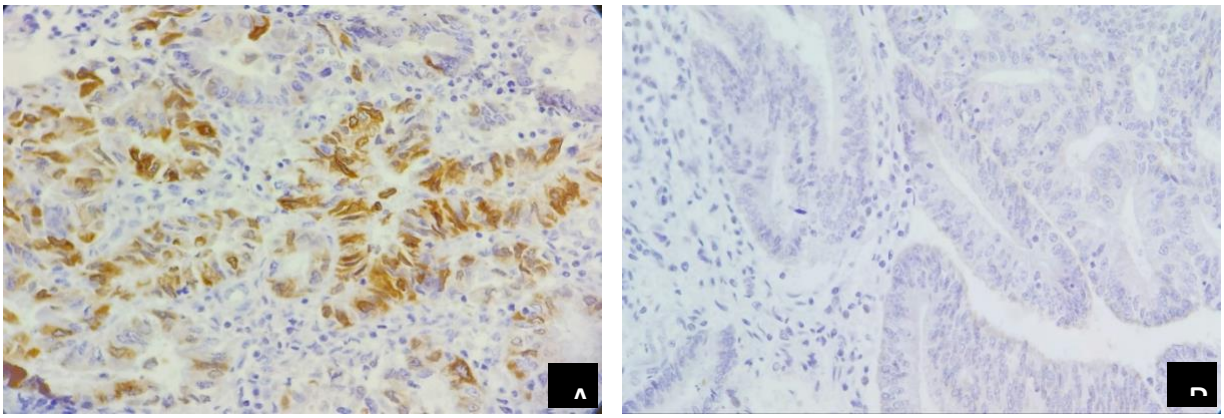

Figure (1): Expression of CoX-2. **A**. Positive COX-2 expression (x400). **B**. Negative COX-2 expression (x400**)**

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Discussion:

Cyclooxygenase-2 is a catalytic enzyme that promotes carcinogenesis, progression and decreased apoptosis.[20] It has been found that COX-2 expression was significantly associated with risk and development of endometrioid carcinoma[5] Before many years a number of studies were done about COX-2 immunohistochemical expression and they identified that primary endometrial carcinoma exhibits a significant proportion with high expression of COX-2.[10, 11, 14, 16, 21]Based on many studies COX-2 over expression predict poor prognosis in endometrioid carcinoma.[5] In different studies variable COX-2 expression was reported in endometrioid carcinoma, which is arranged from 16% up to 90.2%.[10, 11, 14, 16, 21]In this particular study, COX-2 was detected in 15.4% of the cases, comparing to the results of other studies, as Lyndin et al. revealed that COX-2 expressed in 60% of cases,[16] Ferrandina et al. reported COX-2 expression in 39.1% of their studied cases,[10] While Ferrandina′s study at 2005 revealed that 50% of the patients exhibited COX-2 expression,[22] while COX-2 expressed in 53% of cases in the study was done by OHNO,[14] 77% by NASIR[21] and 90.2% by Deng.[11] The finding of this study is close to Lyndin et al. results who discovered that 16% of endometrioid carcinoma showed positive COX-2 expression.[16] In contrary our finding was not in line with Deng et al. who revealed a much higher percentage of positive expression which was 90.2%,[11] The discrepancy in results can be explained by the difference in sample size, variable antibody sources, and different properties of antibodies, duration, type and method of antigen retrieval, fixation of the tissues and method of scoring. Also a study identified that type 2 endometrial carcinoma (non-endometrioid) have higher levels of these proteins and higher expression of COX-2.[16] Other research revealed that only grade I (low grade) endometrioid carcinoma showing apical cytoplasmic staining while in all other endometrial carcinoma types staining pattern is at periphery of cytoplasm and membrane which is regarded as moderate to strong positivity.[16]Consistent with Ferrandina′s findings, this study also observed that the average age of participants (with standard deviation) was 60.1 (11.6) years. Additionally, almost half of the female participants (47.4%) were aged 60 or above,[10] there was no significant association between COX-2 expression and age of patients, this finding is in line with the results reported by Ferrandin et al,[10] Although most women are diagnosed in postmenopausal age and its rare in young adults.[23]In the present study, most of them 93.6% of tumors are of low grade and 6.4% are of high grade, in concordance with other studies in which most of the cases are of low grade,[11,24] among low grade cases 15.1% were positive while 84.9% were negative and among high grade cases 20% of them were positive, other studies have reported an elevated prevalence of COX-2 positivity from low-grade to high-grade cases,[25] consistent with the findings of Lyndin et al., no significant correlation was observed between COX-2 expression and tumor grade in this study.[16]In current study ≥ 50% of the myometrium was invaded by 69.2% of the tumors, in which 16.7% of them showing COX-2 expression, that is in alignment with other studies in which COX-2 expression was higher in cases with deep myometrial invasion,[10] In spite of the absence of a statistically significant association between COX-2 expression and myometrial invasion, the same result identified by other studies,[10,11,22] in contrary down expression of COX-2 was recognized in those tumors that invading ≥50% of myometrium by Deng et al. and Ferrandina et al.[11, 22]Our study clarified that there is lymphovascular invasion in 14.1% of patients, in which 18.2% of them showing COX-2 expression

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while 85.1% of them showed no lymphovascular invasion and among them 14.9% expressed COX-2. This result was not found to be statistically significant similar to the research which is done by Deng et al.[11] According to a study, patients with lymphovascular invasion experienced a higher occurrence of relapses compared to those without lymphovascular invasion.[26]
The findings of this study revealed that lymph node invasion was present in 5.1% of the cases and about half of them expressed COX-2 while in not invaded lymph nodes 86% of them showing COX-2 expression. other studies also recognized decreased expression of CoX-2 with lymph node metastasis,[10, 22] however, there is no significant relationship observed between Cox-2 expression and the status of lymphnodes, this outcome also identified by other studies.[10, 22].It has been found that 83.3% of patients were of stage T1 in which 15.4% of them showing COX-2 expression, while those of stage T2 expressed COX-2 in 20% of cases but when extends beyond the uterus T3 showing down expression and when the tumor invades the bladder and bowel mucosa T4 there is no COX-2 expression although among 78 cases only one case was at stage T4, according to other studies with increasing stage COX-2 showing down expression [10, 22, 24] but this result was not found to be statically significant in the same line with these studies.[14, 16]

Conclusion:

Cyclooxygenase-2 is expressed in a limited number of endometrioid carcinoma while there is no significant association between Cox-2 expression and clinic-pathological parameters further studies required to evaluate expression of COX-2 in association with endometrioid carcinoma progression and metastasis.

Conflict of interest: None

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