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The Role Cox -2 gene expression in uterine disorder

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Abstract
Background: Uterine bleeding refers to the occurrence of lesions resembling endometrial glands and stroma located outside the uterus. The lesions may present as peritoneal lesions, superficial implants, cysts on the ovary, or deep infiltrating disease. Materials and Methods: Study was performed in the Women's and Children's hospital in Al-Muthanna, study specimens were divided into two groups, one for patients called a case and one for control were compared. The patients and control groups were with age ranged between (35-50) years all of them are female. Study was carried out on 60 patients with uterine bleeding and 30 apparently healthy subjects with a control group. All samples were collected in the period (March 2023 till November, 2023). In this study we investigated the correlation between COX-2 genes and uterine bleeding. We screened clinical samples of healthy and patient individuals suspected of uterine bleeding using q RT-PCR. Results: The results revealed a high association between COX-2 gene with the uterine bleeding disease. Conclusions: Our findings suggest that female patients with uterine bleeding in terms of gene regulation, there are genes contribute to the uterine bleeding disease such as COX-2. Several studies evident that those genes are considered as primary factors influence the conditions of uterine bleeding disease.
Keywords *Cyclooxygenase (Cox-2), q RT-PCR*

INTRODUCTION

Uterine bleeding is a chronic gynecological disorder characterized, undergoing cyclic changes with the menstrual cycle. This often leads to severe pain, infertility, and other complications (Zheng H. *et al,*. 2016). Cyclooxygenase-2 (COX-2), or prostaglandin-endoperoxide synthase-2 (HUGO PTGS2), is an enzyme encoded by the PTGS2 gene in humans, and it is one of three cyclooxygenases. This process entails the transformation of arachidonic acid into prostaglandin H2, a crucial precursor of prostacyclin, which plays a significant role in inflammation (Hla et al., 1992). COX-2 consists of three isoforms: Prostacyclin monomers and thromboxanes; Prostaglandins and thromboxanes are primarily generated by COX-1 and COX-2 enzymes (Eren et al., 2010).Numerous isoforms of COX-1 and COX-2 are recognized for their roles in both physiological functions and pathological processes (Simmons et al, 2004). COX-2 is the essential inducible enzyme involved in the inflammatory process. This enzyme serves as a crucial regulatory component in the synthesis of PG, playing a significant role in the mediation of inflammation (Chan et al., 2019).

The level of COX-2 is constantly cyclical and lower in non-secretory phase of the endometrium while in women with normal cycles, the enzyme is found most abundantly in the glandular epithelium. The peak of COX-2 expression goes along with the secretory phase when it is least expressed during initial multiplication and then increases more as the phase progresses (Maia et al,.2005). In female patients with EMS, the COX-2 gene is expressed in both uterineglandular epithelium and endometrial stroma (Goumenou et al,.2004) significantly improved peripheral oxygen saturation and PF, though, is lower than that in the control group (Nandakishore et al,.2014) Breast tissues are dynamic, and its shape and density change throughout menstrual cycle (Ota et. al. , 2001) .

In contrast to the controls, the findings of Cho et al. indicated that during the proliferative phase of the uterus in EMS, COX-2 levels were significantly elevated, while in ovarian uterine lesions during the secretory phase, COX-2 levels were also significantly increased. Additionally, COX-2 exhibits a high sensitivity to pain and may be influenced by the uterine lesions present in patients experiencing chronic stress. Furthermore, the concentration of PTGS2 mRNA in both uterine and ovarian tissue has been demonstrated to have a significant correlation with uterine size and serum CA-125 levels (Cho et al., 2010). In 2018, Mei et al. observed a greater presence of COX-2+CD16- NK cells that had diminished their toxic activity in the abdominal cavity fluid of patients with EMS compared to the control group.

Recent investigations have highlighted the importance of specific inflammatory pathways, notably the COX-2 (Cyclooxygenase-2) pathway, in the pathophysiology of endometriosis. COX-2, an inducible enzyme in the prostaglandin synthesis pathway, plays a crucial role in regulating inflammation, pain, and cell proliferation, which are all significant factors in uterine bleeding. This study investigates the importance of COX-2 gene

expression in uterine bleeding, emphasizing the underlying mechanisms, its contribution to disease progression, and possible therapeutic implications (Benelli R. et al., 2018).

In addition to its involvement in inflammation and pain, COX-2 seems to play a role in the survival and proliferation of endometriotic cells. Elevated COX-2 expression correlates with heightened angiogenesis (the development of new blood vessels), which is a vital component for the proliferation of ectopic uterine lesions. Prostaglandins generated via COX-2 activity enhance the production of vascular endothelial growth factor (VEGF), an essential protein that facilitates angiogenesis, thereby enabling the formation and expansion of uterine lesions within the peritoneal environment (Barbeiro DF. et al. 2016)

Research by Matsuzaki and Darcha (2013) found that COX-2 not only induces VEGF expression but also up regulates anti-apoptotic proteins in endometriotic cells, allowing them to evade cell death and continue proliferating. This ability to avoid apoptosis and promote angiogenesis provides uterine lesions with a unique advantage, making COX-2 a valuable target for therapeutic intervention.

The high expression levels of COX-2 in uterine lesions make it a potential biomarker for diagnosing and monitoring the disease. A biomarker could help in distinguishing uterine tissue from normal uterine tissue, which is particularly useful for non-invasive diagnostic approaches. For instance, elevated COX-2 levels could be measured in serum or peritoneal fluid to aid in early diagnosis or in monitoring treatment efficacy (Li W. *et al.*, 2017). Vigano *et al.* (2009) suggested that evaluating COX-2 expression levels could provide clinicians with a valuable tool for assessing the severity of uterine bleeding. Further research into this area may lead to improved diagnostic methods that are less invasive than current laparoscopic surgery.

MATERIAL AND METHODS

Control and Patient Groups :

The study was executed on 30 control subjects, 60 patient ,whose ages ranged from 35 to 50 years (all of them were female). The selection of women's patients depends on a number of criteria that will be discussed in the following subsections. All patients in this study were diagnosed with uterine bleeding. The medical history of each patient that was taken regarding age, duration of bleeding, history of hormonal therapy, and weight. All control in this study were diagnosed without uterine bleeding. All women who expect to be healthy without any uterine disorders.

Blood Sample collection:

Blood samples were collected in EDTA tubes, and immediately after collection, 1 ml of the blood sample was transferred to a 15 ml plane tube than 14 ml of RBC lysis solution was added. After centrifuging the tube at 13,000 rpm for 1 minute, discard the supernatant for removing the red blood cell, and in a final step, 2 ml of RNA Later solution was added and keep the samples until use.

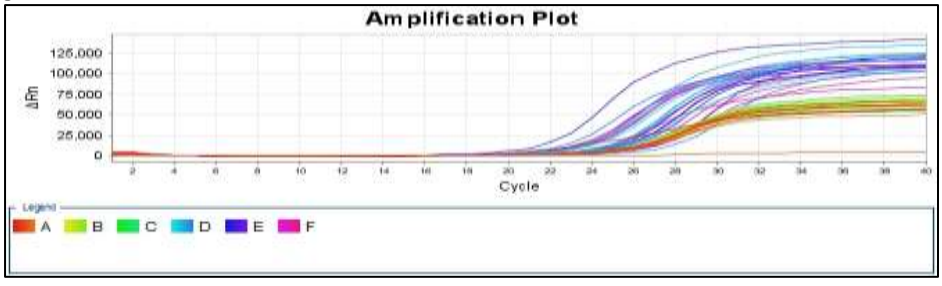
Determination of Cyclooxygenase-2 gene and Interleukin-23 gene:

The identification of the Cyclooxygenase-2 gene and the Interleukin-23 gene through PCR (Polymerase Chain Reaction) employs the One-step RT-PCR (real-time PCR) methodology. This technique integrates the reverse transcriptase step within the same tube as the PCR reaction, thus facilitating a one-step process. The reverse transcription (RT) and qPCR processes are performed within the same reaction well.

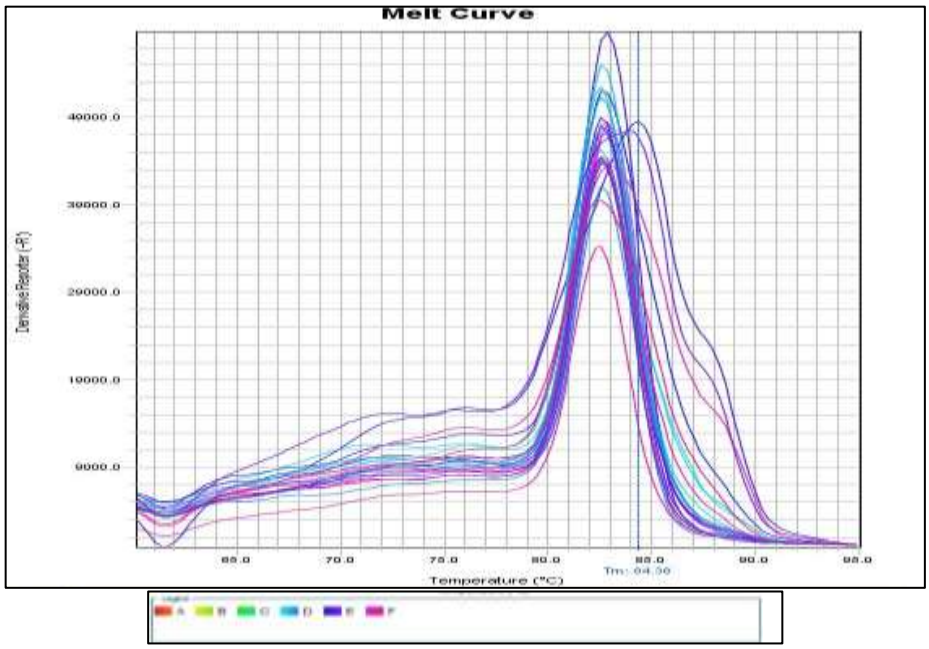
Results and Discussion :

Gene Expression

By using Quantitative RT- PCR, the assessment of COX2 was done in a group of infected with uterine bleeding patients the result show that the CT value indicated of COX2, in comparison with housekeeping gene as a control.



Figure(1): The Quantitative RT- PCR of COX-2.



Figure(2): The melt curve of COX-2.

The majority of the tested clinical samples showed positive CT value genes COX-2. The negative control showed no CT values confirming a negative result thus the sensitivity of the used q RT-PCR. as shown in table (1).
We have set a standard CT-values as 18-24 is considered as a positive signal while more than 24 CT value is a negative signal. The latter was applied over all the tested clinical samples and genes.
House Keeping gene (H. K. gene) or control gene , which are often referred to as H.K. genes. COX-2 level was measured and compared to the H.K gene, as this gene might be changed under variable conditions .
The result showed that the expression of COX-2 was increased in patients prognosis to endometrial compared to H.K gene; suggested that the correlation between COX-2 and uterine bleeding is significant.

Table (1): The RT-PCR result of IL-23 and COX-2 genes.

Gene types	**RT –PCR**	NO.	**Percentage**
COX-2	Positive	17	57 %
	Negative	13	43 %
H.K	Positive	2	7 %
	Negative	28	93 %

The table (2) illustrates the mean CT value of COX-2 compared to H.K. gene.
The estimated mean CT values of COX-2 (31.81) after the comparison between the patients set samples and the control gene.

Table (2): Quantitative CT value measurement of COX-2 in patient samples.

Gene types	**NO.**	Mean ±SD	P. Value
COX-2	30	31.81 ± 30.89	0.05
H.K	30	32.63± 30.32	0.05

In figure (2) expression rates of the targeted genes compared between the control group and patient samples.

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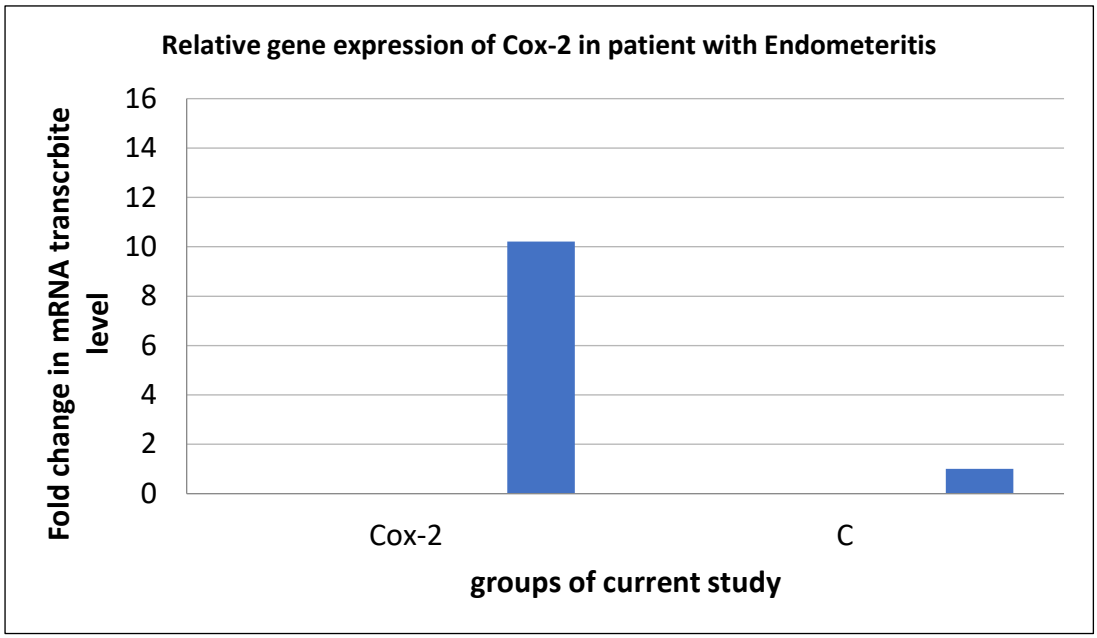

Figure(2): The represents the expression rate of COX-2.

Analysis of tables (4) revealed sample 6 exhibited optimal regulation for expression levels of COX-2, whereas samples 4, 13, and 16 showed highest regulation, and the remaining samples exhibited down regulation. These differential expression patterns may be attributed to a variety of factors, including the influence of other genes and patient-specific characteristics.

Table (4): The highest expressed samples of COX-2

Patient no.	Ct value	Fold change	History association		
			Duration of heavy bleeding	Period regular	Hormonal therapy
1	26.72	3.62	1year	regular	Yes
2	24.11	22.13	8days	regular	Yes
3	24.52	1.38	14day	irregular	yes
4	25.82	32.85	3days	regular	No
5	21.95	3.09	15day	irregular	No
6	25.41	1.06	30day	irregular	No
7	28.21	1.58	30day	irregular	No
8	24.81	3.44	15day	irregular	No
10	25.13	11.69	10days	regular	No
11	20.01	15.97	10days	regular	No
13	22.90	38.75	14day	regular	No
14	25.57	6.56	30day	irregular	No
15	24.43	11.75	10days	irregular	No
16	22.55	57.00	30day	irregular	No
17	23.54	6.74	10days	irregular	No
18	21.25	7.77	20day	irregular	No
19	21.75	8.21	6days	irregular	No
20	25.07	5.33	13day	irregular	No
21	21.35	7.22	20day	regular	No
22	25.82	1.48	15day	regular	No
23	26.61	1.96	60day	irregular	No
24	23.53	3.19	14day	regular	No
25	26.61	1.95	30day	regular	No
26	23.53	15.61	90day	regular	No
28	26.32	4.56	20day	regular	No
30	23.92	28.63	25day	irregular	No

This research highlights the value of COX-2 as both a biomarker for endometriosis and a potential focal point for developing targeted treatments aimed at reducing inflammation and controlling uterine-tissue growth ,the result supported by others report significantly with McDuffie *et al.* (2005) also showed that COX-2 expression levels could be influenced by hormonal fluctuations in uterine tissue, particularly progesterone. Still, this hormone-mediated expression did not distinguish healthy uterine tissue from benign conditions.

Additionally, research by Guan *et al.* (2007) indicates that inflammation-mediated pathways could elevate COX-2 expression in uterine tissue, though this is more evident in pathological samples than in normal uterine tissues. This aligns with the hypothesis that COX-2 might contribute to pathological processes rather than being an inherent feature of healthy uterine tissue.

Research shows that uterine tissue from patients with endometriosis has significantly higher COX-2 expression than that of non- uterine tissue, indicating that COX-2 plays a role in sustaining the inflammatory environment necessary for ectopic tissue survival (Banu et al., 2008).

These genetic and epigenetic changes suggest that COX-2 expression in uterine bleeding is not merely a response to inflammatory stimuli but may also be a result of heritable and modifiable changes that contribute to the persistent inflammation and other pathogenic processes in uterine bleeding, this inflammatory environment supports the establishment and growth of uterine lesions through mechanisms such as cell proliferation, adhesion, and resistance to apoptosis (Jung *et al.,* 2013).

The results obtained in our study are consistent with earlier reports on the inter-relationship between the uterine bleeding was associated with higher COX-2 expression for patients with defect uterine tissue than that of non- uterine tissue, indicating that COX-2 plays a role in sustaining the inflammatory environment necessary for ectopic tissue survival (Banu *et al*., 2008). In turn, this inflammatory environment supports the establishment and growth of uterine lesions through mechanisms such as cell proliferation, adhesion, and resistance to apoptosis (Jung *et al*., 2013).

Vigano *et al*., (2009) suggested that evaluating COX-2 expression levels could provide clinicians with a valuable tool for assessing the severity of uterine bleeding. Further research into this area may lead to improved diagnostic methods that are less invasive than current laparoscopic surgery.

In each of these studies, COX-2 expression levels in patients with uterine disorders closely mirrored those found in healthy uterine tissue, particularly during the secretory phase of the menstrual cycle when COX-2 expression naturally peaks (Matsuda *et al.,* 2018). This lack of difference suggests that COX-2 may not serve as a primary factor in uterine disease pathology but rather as a secondary response to other molecular changes, other enzymes, performing similar functions in inflammatory response (Takano *et al.,* 2019). This hormonal regulation might mask COX-2 differences between pathological and non-pathological tissues. Recent studies suggest that COX-2 expression in uterine disorders might be influenced by local factors in the tissue microenvironment, which can vary significantly even within different areas of the same tissue sample (Lee *et al.,* 2019). This variation could lead to inconsistencies in COX-2 expression patterns.

The latter finding is in line with our results as indicated uterine tissue is heavily influenced by hormones like estrogen and progesterone, which could affect COX-2 expression differently based on menstrual cycle phases or exogenous hormone levels (Yamamoto *et al.,* 2020). This redundancy could mean that COX-2 inhibition alone may not significantly impact uterine inflammation. Some studies suggest that COX-2 expression may vary across different disease stages or may only be up regulated in advanced cases of uterine pathology (Xu *et al.,* 2021). Thus, measuring COX-2 in early-stage or mild conditions could yield results similar to healthy controls.

CONCLUSION:

Our findings suggest that female patients with uterine bleeding in terms of gene regulation, there are genes contribute to the uterine disease such as COX-2. Several studies evident that this gene is considered as primary factors influence the conditions of uterine disease, thus we investigated the gene expression of these gene.

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