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# Polymorphism Occurrence in PTGS2gene Sequence in Women with Cervicitis

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**ABSTRACT**

The cervix plays a significant role in confirming the appropriate pregnancy and parturition timing. One hundred blood specimens were taken from 100 women having cervicitis and from 100 apparently healthy women as controls. This work was performed at Al-Khark hospital for delivery and children, in Baghdad city during the period from January 2023 to January 2024. The results showed that the highest infection rate (64.9%) was in the age group (15-24) years, followed by (58.1%) in the age group (35-44) years, (49.3%) in the age group (25-34) years, (47.4%) in the age group (45-54) years, and (25%) in the age group (55-60) years (p=0.03). The results also reveald no significant difference in mean Ca19.9 value among the patients (19.62 ±18.73) compared to the healthy group (19.21 ±18.68), However, a highly significant difference was found in mean Ca125 values between the patients (25.25±0.96) compared to the controls (18.98±17.95) (P<0.001). Also, there was no significant variation between mean Ca 15.3 marker in patient group (22.88±17. 78) compared to the control group (18.86±16.44) (P= 0.72), while there was a highly significant difference between mean CRP level (89.06±0.99) in the patient group compared to the healthy (6.89±0.34) (P<0.001). The plymorfism in PTGS2 gene showed an increse in levels of Ca125 marker 4(20%) with a highly variation (P=0.001), and of CRP levels was 16(80%), with a significant variation (P=0.001), while the levels of polymorphism were found with Ca15.3 marker 3(15%), with significant variation (P=0.07) and Ca19.9 marker 1(5%), with a non-significant difference (P=0.3). No mutation was observed when the rs888160762 SNP of PTGS2 gene was analysed, while a polymorphism happened when the rs20417SNP of PTGS2 gene was analysed by the use of Sanger sequencings. The C homozygous allele was indicated by the single "C" peak, while the G/C heterozygous allele was indicated by the existence of "G" and "C" peaks.

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## 1- INTRODUCTION

The cervix plays a significant role in confirming the appropriate pregnancy and parturition timing. The fetus in the uterus is maintained by the cervix, and the fetus is protected by the cervix from the vaginal canal pathogens. During parturition and pregnancy, the cervix undergoes huge alterations, a process which is related to

collagen degradations and increase in cervical inflammation and immune cell responses [1]. Because of endocrinological, immunological and metabolic changes, the microbiome of the female genital tract can undergo alterations in pregnancy. These symbiotic conditions can result in infection that may ascend upwards to the feto-placental area or can be seeded haematogenously [2]. Uterine cervix cytology is the most commonly employed test and is well known mainly for the cytologic alterations observed in the uterine cervical precancerous lesions and invasive carcinoma [3]. In health care issues, biomarkers are essential because they offer important visions in the prognosis, diagnosis and treatment response as well as personalized medicine. They serve as actual markers that enable early intervention and detection resulting in improved patient's results and lowered cost. Treatment decisions are also guided by biomarkers via disease outcome prediction and individualized treatment plan facilitation [4]. The $7^{th}$ most global cancer is the head and neck cancer which accounts for 3% of the cancers. Either definitive radiation treatment alone or surgery alone leads to a good treatment results in early stage of the disease. Nevertheless, most of newly diagnosed case is presented with local-regionally advanced disease and requires multimodality medication [5]. For ovarian cancer screening, CA125 has become the most promising biomarker; nevertheless, in population-based testing of ovarian cancer, the accuracy of CA125 is still not accepted. In the current review, the role of CA125 in diagnosis and treatment response evaluation were studied [6]. The cancer antigen biomarker 125 (CA-125) is broadly utilized to investigate probable ovarian cancers in symptomatic women who are presented to the primary care. Nonetheless, in this setting, the diagnostic performance of this marker is not clear [7]. The tumor marker role is not well-established in gall bladder cancer (GBC). In this study, the prognostic values of carcinoma embryonic antigen (CEA) and carbohydrate antigen 19–9 (CA19–9) were evaluated in GBC patients [8]. Prostanoids are synthesized by cyclooxygenase (COX), and these Prostanoids play essential roles in living organisms. For Prostanoid synthesis**,** COX enzyme utilizes arachidonic acid, and this Prostanoid plays essential roles in different pathological and physiological conditions [9]. The main goal of this study was evaluation of patient proportion in which metastatic cancer was detected by an elevated CA 15.3 levels. Following ethics committee approval, data was obtained on the metastases detection method as well as CA 15.3 evolution and survival [10]. We can define the pelvic inflammatory disease (PID) as the upper genital tract inflammation because of a woman's infection. The uterus, Fallopian tube, and/or ovaries are affected by this disease. It is typical ascending inflammation that spreads from the lower genital canal [11].

## 2- MATERIAL AND METHOD

One hundred blood specimens were taken from 100 women having cervicitis and from 100 apparently healthy women as controls. This work was performed at Al-Khark hospital for delivery and children, in Baghdad city during the period from January 2023 to January 2024. The blood samples were put in plane tubes to clot and centrifuged for 15 min. at 3000 rpm for obtaining serum samples, which were kept at -20C until use. The CA 19-9 Elecsys, CA- 125 and CA 15-3 kit and Cobas E411 analyzer system were used to detect CA 15-3, CA 19-9 and CA-125 markers, whereas DNA sequencing and gene detection was performed by using conventional PCR according to the instructions of the manufacturing companies attached in the leaflets and the Primers used were: COX2-F: TGTAAAACGACGGCCAG TCTGAG CACTA CCC ATGATAGA, COX2-R: CAGGAAACAGCTATGACGGGCGAGTAAGGTTAAG. Temp. (°C): 55 and product Size: 760bp.

**Statistical analyses:**
Data of the study were analyzed by using the (SPSS-20 Statistical Package for Social Sciences, Chicago, IL, USA). Relations between qualitative data were studied using Chi-square test.

## 3- RESULTS

The results showed that the highest infection rate (64.9%) was in the age group (15-24) years, followed by (58.1%) in the age group (35-44) years, (49.3%) in the age group (25-34) years, (47.4%) in the age group (45-54) years, and (25%) in the age group (55-60) years (p=0.03), as shown in table (1).

**Table (1): Distribution of cervicitis according to age groups (n=200)**

| Years old | | Cases (n=100) | Controls (n=100) | P-values |
|---|---|---|---|---|
| Age (Years) | (15-24) | 24 (64.9%) | 13 (35.1%) | 0.03 (S) |
| | (25-34) | 34 (49.3%) | 35 (50.7%) | |
| | (35-44) | 18 (58.1%) | 14 (41.9%) | |
| | (45-54) | 18 (47.4%) | 20 (52.6%) | |
| | (55-60) | 6 (25 %) | 18 (75 %) | |

The results also reveald no significant difference in mean Ca19.9 value among the patients (19.62 ±18.73) compared to the healthy group (19.21 ±18.68), However, a highly significant difference was found in mean Ca125 values between the patients (25.25±0.96) compared to the controls (18.98±17.95) (P<0.001). Also, there was no significant variation between mean Ca 15.3 marker in patient group (22.88±17. 78) compared to the control group (18.86±16.44) (P= 0.72), while there was a highly significant difference between mean CRP level (89.06±0.99) in the patient group compared to the healthy (6.89±0.34) (P<0.001), as shown in table (2).

**Table (2): Distribution of tumor markers among the study groups**

| Variables | Groups | Mean±SD | T-test | P-value |
|---|---|---|---|---|
| Ca19.9 | patient | 19.62 ±18.73 | 0.38 | o.72 |
| | Control | 19.21 ±18.68 | | |
| Ca 125 | Patient | 25.25±0.96 | 8.4 | <0.001 |
| | Control | 18.98±17.95 | | |
| Ca 15.3 | Patient | 22.88±17. 78 | 3.3 | 0.72 |
| | Control | 18.86±16.44 | | |
| CRP | patient | 89.06±0.99 | 13.0 | <0.001 |
| | Control | 6.89±0.34 | | |

Table (3) illustrated that the plymorfism in PTGS2 gene showed an increase in levels of Ca125 marker 4(20%) with a highly variation (P=0.001), and of CRP levels was 16(80%), with a significant variation (P=0.001), while the levels of polymorphism were found with Ca15.3 marker 3(15%), with significant variation (P=0.07) and Ca19.9 marker 1(5%), with a non-significant difference (P=0.3).

**Table (3): Distribution of polymorphism with tumor markers between patients (N=20) and control (N=20) according to cutoff points**

| Type of polymorphism | CRP(ng/dl) | | Ca19.91 | | CA125 | | Ca15.3 | |
|---|---|---|---|---|---|---|---|---|
| | Normal | Increase | Normal | Increase | Normal | Increase | Normal | Increase |
| Yes (n=20) | 4 (20%) | 16 (80%) | 19 (95%) | 1 (5%) | 16 (0%) | 4 (100%) | 17 (85%) | 3 (15%) |
| No(n=20) | 20 (100%) | 0 (100%) | 20 (100%) | 0 (0%) | 20 (100%) | 0 (100%) | 20 (100%) | 0 (100%) |
| P-value | 0.001 (H.S) | | 0.3 (N.S) | | 0.001 (H.S) | | 0.07 (N.S) | |

Upon using the PCR technique, as shown in figures (1) and (2), the Cox2 region amplification in women having cercivitis was factionated on 1.5% agarose gel electrophoresis, stained with Eth. Brom. M:100bp ladders marker Lane 1-10 and Lane 11-20 which resembled 760bp PCR-products.

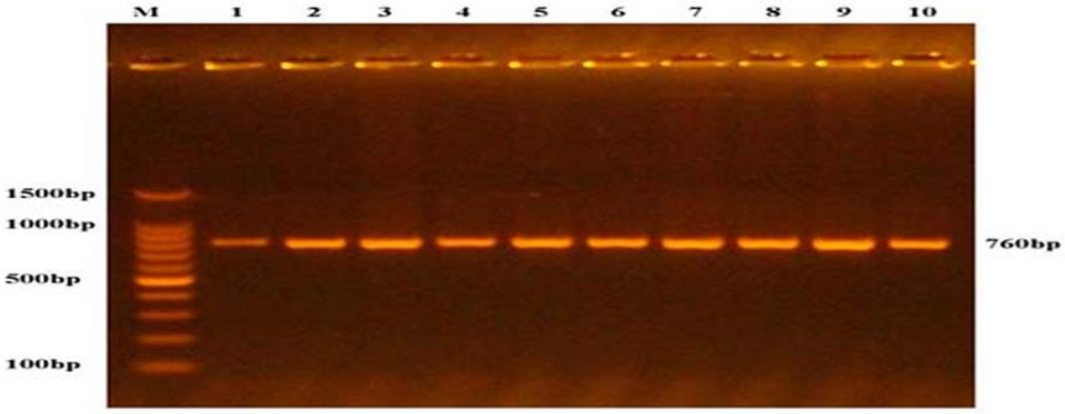


Figure (1): The amplification of Cox2 region in women with cercivitis were factionated on 1.5% agarose gel electrophoresis, stained with Ethedium bromide M:100bp ladder marker Lanes 1-10 resembling 760bp PCR-prodect.

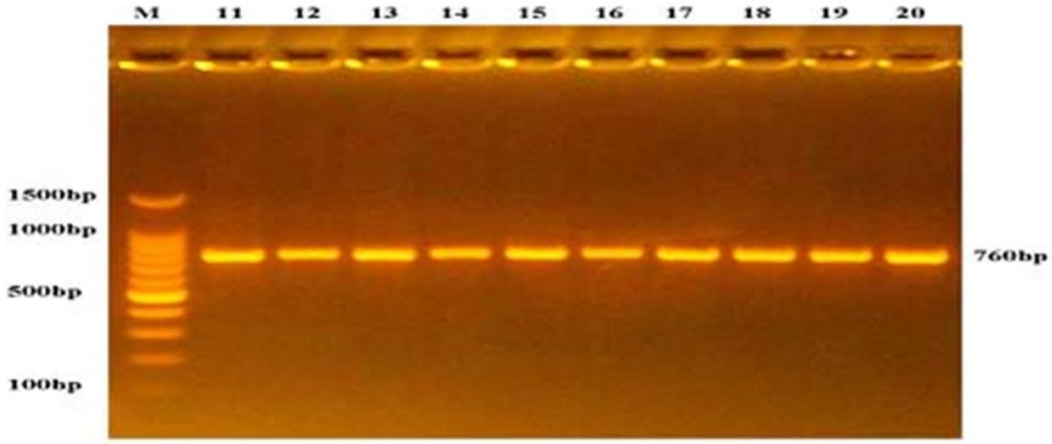


Figure (2): The amplification of Cox2 regen in women with cercivitis were factionated on 1.5% agarose gel electrophoresis stained with Ethedium bromide M:100bp ladder marker Lanes 11-20 resembling 760bp PCR-prodect

Table (4) and figure (3,4) demonstrated that no mutation was observed when the rs888160762 SNP of PTGS2 gene was analysed, while a polymorphism happened when the rs20417SNP of PTGS2 gene was analysed by the use of Sanger sequencings. The C homozygous allele was indicated by the single "C" peak, while the G/C heterozygous allele was indicated by the existence of "G" and "C" peaks.

| PTGS2 GENE ID 5844 | | |
|---|---|---|
| **SNPs** | **patients rs888170768** | **patients rs204172** |
| **Wild** | **CC** | **GG** |
| **Variation** | **C>G** | **G>C** |
| **Samples** | | |
| **a** | **CC** | **GC** |

| | | |
|---|---|---|
| **b** | **CC** | **GC** |
| **c** | **CC** | **GC** |
| **d** | **CG** | **GC** |
| **e** | **CC** | **GC** |
| **C1** | **CC** | **GC** |
| **C2** | **CC** | **GC** |
| **C3** | **CC** | **GC** |
| **C4** | **CC** | **GC** |
| **C5** | **CC** | **GC** |
| **C1** | **CC** | **GG** |
| **C2** | **CC** | **GG** |
| **C3** | **CC** | **GG** |
| **C4** | **CC** | **GG** |
| **C5** | **CC** | **GG** |

**Table (5): Polymorphism occurred in PTG-S2 GENE ID-5743 SNPs rs20417, Wild CC GG & the variations C>G G>C in comparison with the controls**

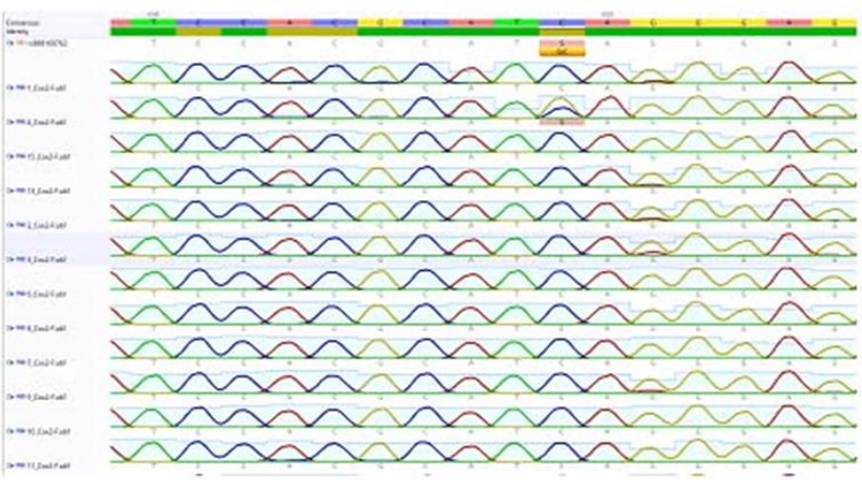

Figure (3): Analysis of rs888160762 SNP of PTGS2 gene using Sanger sequencing. The single "C" peak indicates C homozygous alleles, while the presence of "G" and "C" peaks indicates G/C heterozygous alleles

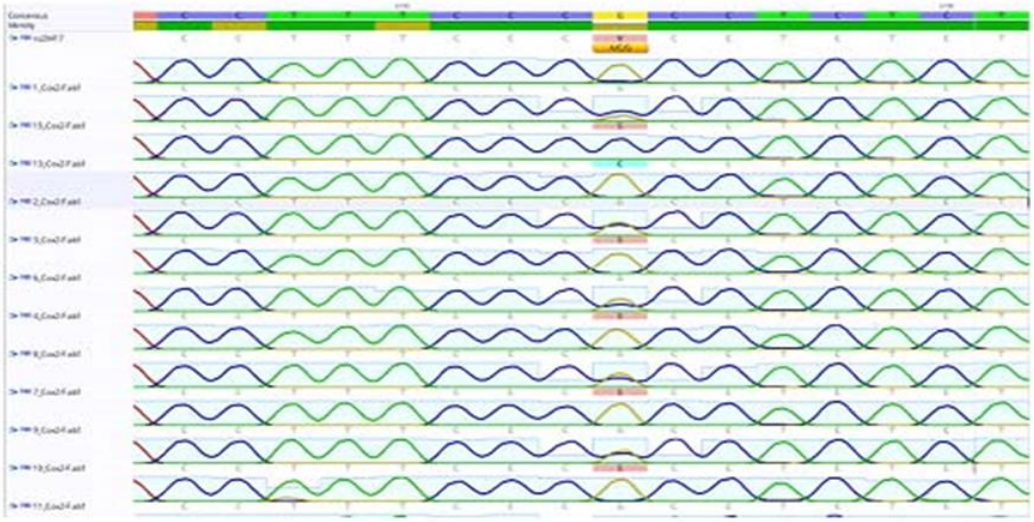

**Figure (4): Analysis of rs20417 SNP of PTGS2 gene using Sanger sequencing. The single "C" peak indicates C homozygous alleles, while the presence of "G" and "C" peaks indicates G/C heterozygous alleles.**

## 4- DISCUSSION

The results showed that the highest infection rate (64.9%) was in the age group (15-24) years, followed by (58.1%) in the age group (35-44) years, (49.3%) in the age group (25-34) years, (47.4%) in the age group (45-54) years, and (25%) in the age group (55-60) years (p=0.03). These findings agreed (Jennings, *et al.*, 2023) who stated that the cervical intraepithelial lesion rate was significantly higher (38.8%) in the age group (65–69) years than other age groups [12, 13]. No significant variation was found between mean Ca19.9 in patients (19.62 ±18.73) compared to healthy group (19.40±0.68) and between mean Ca15.3 levels (20.80±0.74) and the controls (19.21 ±18.68). But, the mean Ca125 was important variation (25.25±0.96) versus the controls (18.98±17.95) P<0.001. These findings were in agreement with (Charkhchi, et al., 2020) who demonstrated that Ca125 marker was used as a main marker in ovarian carcinoma for the last few decades showing highly significant values [14]. In addition, there was no significant variation between mean of Ca 15.3 marker (22.88±17. 78) compared to control group (18.86±16.44) (P= 0.72,) but there was signifivant variation between CRP (89.06±0.99) compared to healthy (6.89±0.34) P<0.001. Such findings coincided with (Orsolini, et al., 2022) who revealed a highly significant increase in the levels of CRP in ovarian carcinoma and cervicitis [15]. Polymorphism in Prostaglandin-endoperoxide synthase2 gene showed an increase in levels of Ca125 marker 4(20%) with a highly variation (P=0.001), and of CRP levels was 16(80%), with no significant difference (P=0.4). Suliman, et al, (2023) revealed that the highest polymorphism happened in females with high Ca tumor markers levels [16]. Also, these results were in a harmony with (Yoon, et al., 2024), who gave showed that there is a direct relationship with the increase in the levels of C-reactive protein and polymorphism in the prostaglandin- end peroxide synthase PTGS2 gene [17]. There was no polymorphism happened in the analysis of rs888170768 NSP of Prostaglandin-endoperoxide synthase2 gene (Cario, et al., 2020) [18]. On the other hand, Kosumi, et al., (2019) stated that the pathways of Prostaglandin-end peroxide synthase-2 (PTGS-2, cyclooxygenase-2, COX-2-prostaglandin E2 (PGE-2) promoted tumor progressions [19]. In view of the evidence which suggests that in cancer cells, increased PGE-2 synthesis by BRAF mutations, it is hypothyzed that the relationship between tumor Prostaglandin-endoperoxide synthase2 gene (COX-2) expressions and colorectal carcinoma mortalities may be stronger in BRAF mutated tumor than in BRAF wild-type tumor (Kosumi, et al., 2019 and Signorile, at al., 2023) [20].

## 5- CONCLUSION

İt can be concluded that no mutations took place in the rs888170768 SNP analysis in Prostaglandin-endoperoxide synthase2 genes, while a polymorphism was shown to occur in the rs204172 SNP analysis of Prostaglandin-endoperoxide synthase2 genes.

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# حدوث تعدد الأشكال في تسلسل جين PTGS2 لدى النساء المصابات بالتهاب عنق الرحم

## الخلاصة:

يلعب عنق الرحم دوراً هاماً في تأكيد التوقيت المناسب للحمل والولادة. تم أخذ مائة عينة دم من 100 امرأة مصابة بالتهاب عنق الرحم ومن 100 امرأة سليمة ظاهرياً كمجموعة سيطرة. تم إجراء هذا العمل في مستشفى الكرخ للولادة والأطفال بمدينة بغداد خلال الفترة من يناير 2023 إلى يناير 2024. أظهرت النتائج أن أعلى معدل إصابة (64.9٪) كان في الفئة العمرية (15-24) سنة، تليها (58.1٪) في الفئة العمرية (35-44) سنة، (49.3٪) في الفئة العمرية (25-34) سنة، (47.4٪) في الفئة العمرية (45-54) سنة، و (25٪) في الفئة العمرية (55-60) سنة (ص = 0.03). كما أظهرت النتائج عدم وجود فرق معنوي في متوسط قيمة Ca19.9 بين المرضى (19.62 ± 18.73) مقارنة بمجموعة السيطرة (19.21 ± 18.68)، ومع ذلك، وجد فرق معنوي للغاية في متوسط قيم Ca125 بين المرضى (25.25 ± 0.96) مقارنة بمجموعة السيطرة (18.98 ± 17.95) (P <0.001). كما لم يكن هناك تباين معنوي بين متوسط علامة Ca15.3 في مجموعة المرضى (22.88 ± 17.78) مقارنة بمجموعة السيطرة (18.86 ± 16.44) (P = 0.72)، بينما كان هناك فرق معنوي للغاية بين متوسط مستوى CRP (89.06 ± 0.99) في مجموعة المرضى مقارنة بالأصحاء (6.89 ± 0.34) (P <0.001). أظهرت التغيرات الشكلية في جين PTGS2 زيادة في مستويات Ca125 العلامة 4 (20٪) مع تباين كبير (P = 0.001)، ومستويات CRP كانت 16 (80٪)، مع تباين كبير ( P = 0.001)، في حين تم العثور على مستويات تعدد الأشكال مع Ca15.3 العلامة 3 (15٪)، مع تباين كبير ( P = 0.07) و Ca19.9 العلامة 1 (5٪)، مع فرق غير مهم (P = 0.3). لم يتم ملاحظة أي طفرة عند تحليل SNP rs888160762 لجين PTGS2، بينما حدث تعدد الأشكال عند تحليل SNP rs20417 لجين PTGS2 باستخدام تسلسلات سانجر. تم تحديد الأليل المتماثل C من خلال وجود قمة "C" واحدة، في حين تم تحديد الأليل المتغاير G/C من خلال وجود قمتي "G" و"C".