



RESEARCH ARTICLE - MEDICAL TECHNIQUES

The Sequence of Prostaglandin-Endoperoxide Synthase 2 Gene in Women with Cervicitis

Maha A. Ahlial^{1*}, Nazar Sh. Mohammed¹, Maysara S. Khalaf¹¹ College of Health & Medical Technology - Baghdad, Middle Technical University, Baghdad, Iraq* Corresponding author E-mail: ahlialmahaaw@gmail.com

Article Info.	Abstract
<p><i>Article history:</i></p> <p>Received 01 July 2022</p> <p>Accepted 17 August 2022</p> <p>Publishing 15 November 2022</p>	<p>Cervicitis is a clinical syndrome characterized by inflammation of primarily the columnar epithelium of the uterine endocervix. It can be acute or chronic. Prostaglandin endo peroxide synthase2 (Cyclooxygenase-2) is a key enzyme in prostaglandin synthesis, which is coded by the PTGS2 gene, and recognized as the master switch that activates the inflammatory response; its induction leads to the biosynthesis of prostaglandins.</p> <p>The current study aims to determine the CA125, CA 19.9, and, CA 15.3 markers level and their relationship with the COX-2 gene.</p> <p>Venous blood samples were obtained from 100 women with cervicitis whose ages ranged from 18 to 60 years, and 100 healthy women as a control group. Detection of CA19-9, CA-125 and CA 15-3 markers was performed by using Cobas E411 analyzer while the CRP test was detected by using Afias CRP instrument. The conventional PCR was used for gene detection and DNA sequencing.</p> <p>The mean± SD of Ca125, CA15.3 and CRP showed a highly significant differences between patient (30.33±1.09), (22.82±1.07), (88.06±5.88) respectively and the healthy control (19.40±0.68), (18.34±0.77), (6.73±0.12) respectively-value >0.001. The mutation in PTGS2 gene with highly CA125 marker 100(100%) and CRP levels 20(80%), while in CA15.3 marker 3(15%) and CA19.9 marker 1(5%).</p> <p>The mutation occurrence in analysis of rs20417 NSP of PTGS2 gene with wild CC GG and the variation C>G G>C.</p>

This is an open access article under the CC BY 4.0 license (<http://creativecommons.org/licenses/by/4.0/>)

Publisher : Middle Technical University

Keywords: Cervicitis; PTGS2 gene; Sequencing.

1. Introduction

The cervix is integral to conception, pregnancy maintenance and timely baby birth. During gestation, the cervix should remain closed although several forces acting upon it [1]. This enables it to act as a barrier to the ascent of vaginal microorganisms and to retain the growing fetus within the uterus [2]. Cervicitis can be defined as the cervical inflammatory response to certain stimuli [3]. It may be chronic or acute; chronic occurs because of insufficient treatment or recurrent inflammation. The non-specific cervicitis is most commonly observed it is unassociated with a Sexual transmitted infection such as gonorrhea or chlamydia [4]. The acute cervicitis often occurs because of an infection such as gonorrhea or chlamydia, while chronic type typically results from non-infectious sources [5]. In several cases, cervicitis remains without symptoms. Symptomatic patients can develop dyspareunia and vaginal discharges, intermenstrual or post-coital bleedings [3]. Sometimes, cervicitis presents in an abnormal form e.g. cervical growth or uterovaginal prolapse, but this occurs rarely and usually because of delayed or untreated conditions [3]. For diagnostic purposes, a culture is taken to search for mucopurulent discharges and specific bacteria such as gonorrhea, chlamydia or Mycoplasma genitalium. Cervicitis may be correlated with preterm childbirth or pelvic inflammatory diseases [6]. In addition, Cytomegalovirus has recently been found to cause cervicitis [7]. Best evidence is currently antibiotic therapies [8]. Tumor markers are used as predictive or prognostic factors. Prognostic factors are used for determining risks of disease outcome in untreated cases or for determining residual risks following treatment, while predictive factors are correlated with the probable resistance or sensitivity to a specific medication. [9]. Estimation of CA-125 blood levels has been suggested as an important tool in the treatment of ovarian cancer. Due to the different conditions that may increase CA-125 serum levels, it is not used for cancer detection, but it is usually used for monitoring chemotherapy response, relapse and disease progression among patients with ovarian cancer [10]. CA-125 test can give significant information for women already known to be affected with ovarian cancer, it has not been found useful as a screening test owing to the unsure association between cancer and levels of CA-125 [10]. Besides ovarian cancer, CA-125 may be increased in patients with conditions like fallopian tube cancer, endometrial cancer, breast cancer, lung cancer and gastrointestinal cancer. It may also be elevated in pregnancy.

Nomenclature			
CA	Cancer antigen	PCV	Packed cell volume
CBC	Complete blood count	PG	Prostaglandin
CRP	C- reactive protein	PTGS2	Prostaglandin endo peroxide synthase2
COX	Cyclooxygenase	STI	Sexual transmitted infection
DNA	Deoxyribonucleic acid	SD	Standard deviation
EDTA	Ethylene diamine tetra acetic acid	SPSS	Statistical Package for the Social Sciences
ELISA	Enzyme Linked Immunosorbent Assay	SNP	Single nucleotide polymorphisms
HB	Hemoglobin	RPM	Round per minute
PCR	Polymerase chain reaction		

2. Material and Method

In this study, venous blood samples were obtained from 100 women with cervicitis whose ages ranged from 18 to 60 years, and 100 healthy women as a control group 25 to 52 years. The study was conducted at Al-Elwiya maternity teaching hospital in Baghdad city during the period from November 2021 to June 2022. The blood samples were divided into two parts, the first part was put in EDTA tubes for hematological investigations (CBC and molecular technique), for molecular technique the blood samples stored at -20C until use, and the second part was placed in plane tubes, then centrifuged after clotting at 3000 rpm for 15 minutes to obtain serum, which is stored at -20C until use.

2.1. Estimation of tumor markers

Detection of CA 19-9, CA- 125 II and CA 15-3 II markers was performed using CA 19-9 CalSet, Elecsys CA- 125 II and Elecsys CA 15-3 II kits (Roche, Germany) and Cobas E411 analyzer (Roche, Germany).

2.2. Estimation of CRP

The CRP test was detected by using the CRP kit (Boditech, Korea) and AFIAS CRP instrument (Boditech, Korea).

2.3. Hematological investigation

The complete blood count including, Hb, PCV, lymphocyte and neutrophil counts carried out using the Mindray Auto hematology analyzer.

2.4. Molecular detection

2.4.1. DNA Extraction

Genomic DNA was isolated from Blood Sample according to the protocol of Geneaid Extraction (Presto™ Mini g DNA Kit Geneaid, Taiwan) [11].

The conventional PCR (Geneaid, Taiwan) was used for PTGS2 gene amplification and DNA sequencing PCR product. agarose gel electrophoresis was adopted to confirm the presence of amplification. PCR was completely dependable on the extracted DNA criteria (Agarose, Ethidium Bromide Solution (10mg/ml), GoTag Green Master Mix, Nuclease Free Water, TAE 40X, Quantifluor dsDNA System Promega, USA). Cervical swabs were taken from all studied groups (patients and controls), and Pap smears were performed for all of them.

2.4.2 Primer preparation

Macrogen Company supplied these primers in a lyophilized form. Lyophilized primers were dissolved in nuclease-free water to give a final concentration of 100pmol/μl as a stock solution. A working solution of these primers was prepared by adding 10μl of primer stock solution (stored at freezer -20 C) to 90μl of nuclease-free water to obtain a working primer solution 10pmol/μl. sequence of forward (f) and reversed (R) primer (Macrogen, Korea) for detection of COX2 gene, see Table 1.

Table 1 COX2 gene

Primer Name	Sequence 5' - 3'	Annealing Temp. (°C)	Product Size (bp)
COX2-F	TGTAAAACGACGGCCAGTCTGAGCACTACCCATGATAGA	55	760
COX2-R	CAGGAAACAGCTATGACGGGCGAGTAAGGTTAAGAAAG		

2.5. Statistical analyses

Statistical analyses were performed using SPSS (To analyze current data, SPSS v.23 programs were utilized.). Data were introduced as mean ± SD or numbers and percentages. The student t-test was used to compare between women have cervicitis and healthy group.

3. Results

3.1. Demographic picture of studied groups

The distribution of groups according to age groups is shown in Table 2 which documented that the means age of women with cervicitis were 34.43±11.89 years versus 39.05±13.53 for control group with (*p*-value= 0.01), this table also documented the most age group attacked with

cervicitis was 25-34 years with 34 (49.3%) from total study cases (N=100), while the less cases of cervicitis was within age group 65-75 years, these differences between the frequency and percentage was statistically significant (p -value=0.03). The results of this study also showed the most cases of cervicitis were recorded among the women with residency in the rural areas with 51 (51.0%), versus 49 (49.0%) cases was recorded in the urban regions.

Table 2 Demographical characters of studied group (N=200)

Parameters	Case (N=100)	Control (N=100)	P-value
Age groups (M±SD)	34.43±11.89	39.05±13.53	0.01 (S)
Age (Years)	(15-24)	13 (35.1%)	0.03 (S)
	(25-34)	24 (64.9%)	
	(35-44)	34 (49.3%)	
	(45-54)	18 (58.1%)	
	(55-64)	13 (41.9%)	
	(65-75)	18 (52.6%)	
Residency	Rural	5 (21.7%)	0.7 (N.S)
	Urban	1 (50.0%)	
	Rural	49 (49.0%)	
	Urban	51 (51.0%)	

3.2. Estimation of tumor markers

The mean Mean± Std., of tumor marker Ca125 there were a highly significant differences among patient (30.33±1.09) front the healthy control (19.40±0.68), Ca15.3 patients (22.82±1.07) and the healthy control (18.34±0.77), and CRP the patients (88.06±5.88) and the healthy control (6.73±0.12), P-value <0.001 (H.S). While it was no significant differences in Ca19.9 markers between the patients (19.62±0.79) and the healthy control (19.21±0.71), P-value (0.7), as shows in Table 3.

Table 3 Comparison of the levels of studied parameters among cases (N=100) and control (N=100)

Tumor marker	Study Groups	N	Mean+SE	T-test	P-value
CA19.9	Case	100	19.62±0.79	0.38	0.7 (N.S)
	Control	100	19.21±0.71		
CA125	Case	100	30.33±1.09	8.4	<0.001 (H.S)
	Control	100	19.40±0.68		
CA15.3	Case	100	22.82±1.07	3.3	0.001 (H.S)
	Control	100	18.34±0.77		

3.3. Estimation of CRP

The mean differences between the CA markers and the control group, the mean of Ca19.9 (19.62) compared to control (19.21), the mean of Ca125 (30.33) compared to control (19.4) and the mean of Ca15.3 (22.82) but the healthy control (18.34). While the mean of CRP was (88.06) and the control group 6.73, as show in Fig.1.

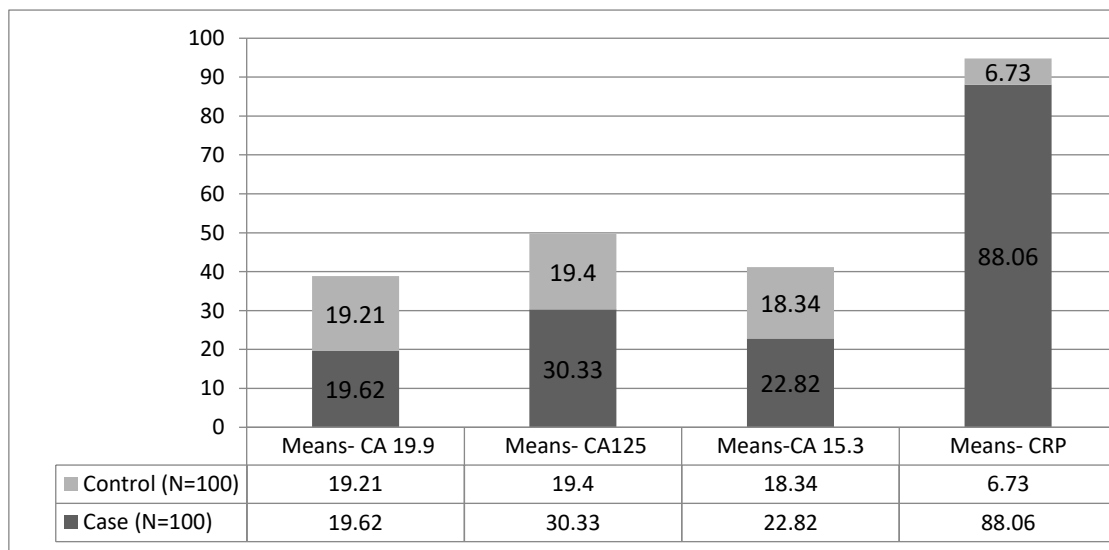


Fig 1. The levels among studied groups and control groups

3.4. Hematological investigation of studied groups

A complete blood picture of the distribution of mutation was show in Fig. 2, the levels of lymphocytes with 12(60%), the decreases of Hb levels 17(85%), PCV 14(70%0 and Neutrophils 2(10%).

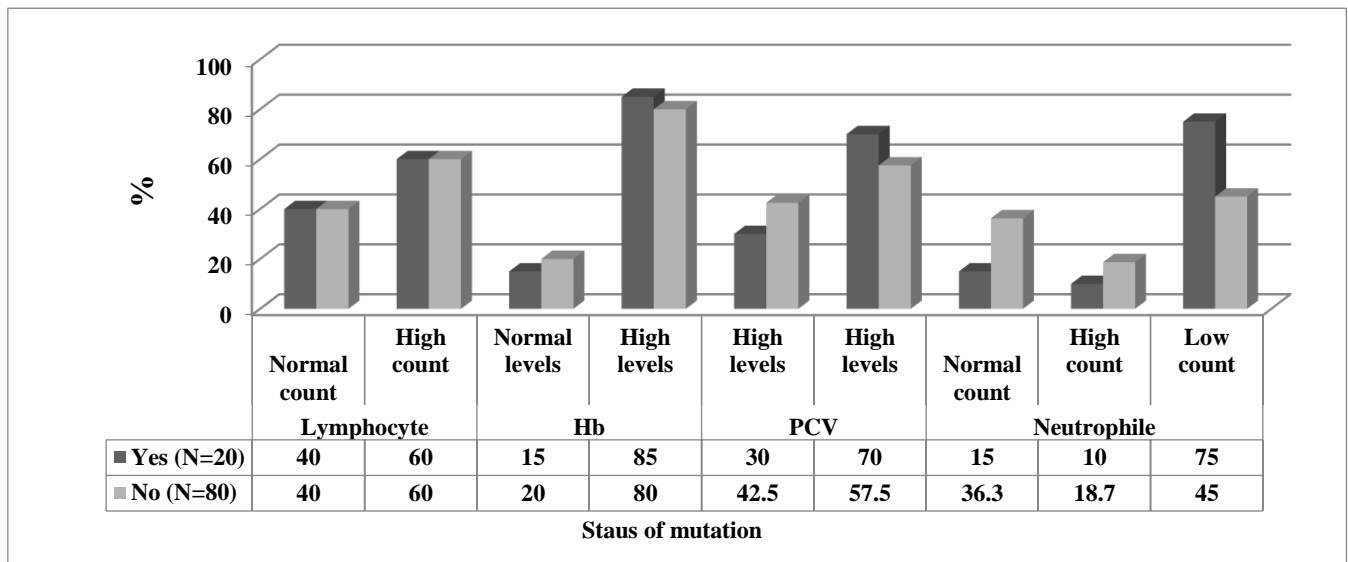


Fig 2. Complete blood picture profile and type of mutations among patients

3.5. Detection of PTGS2 mutation

Amplification of Cox2 regen in women with cervicitis were factionated on 1.5% agarose gel electrophoresis stained with Ethidium bromide M:100bp ladder marker 1-10 resemble PCR with 760 pb as shows in Fig. 3.

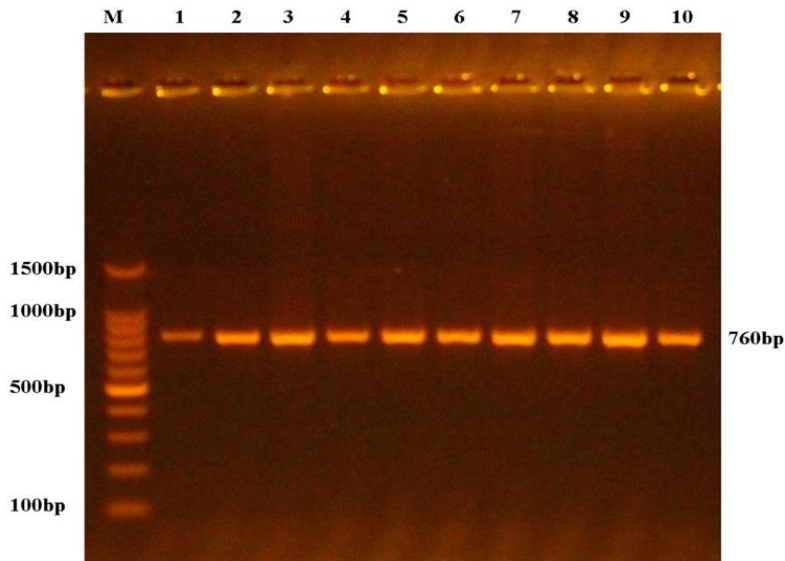


Fig 3. The amplification of Cox2 region in women with cervicitis

3.6. Gene sequencing

There are no mutation occurrence for analysis of rs888160762 Single nucleotide polymorphisms of PTGS2 gene using Sanger sequencing. Single “C” peak indicative of C homozygous alleles. Presence of the “G” and “C” peak indicative of G/C heterozygous allele.as show in Fig. 4.

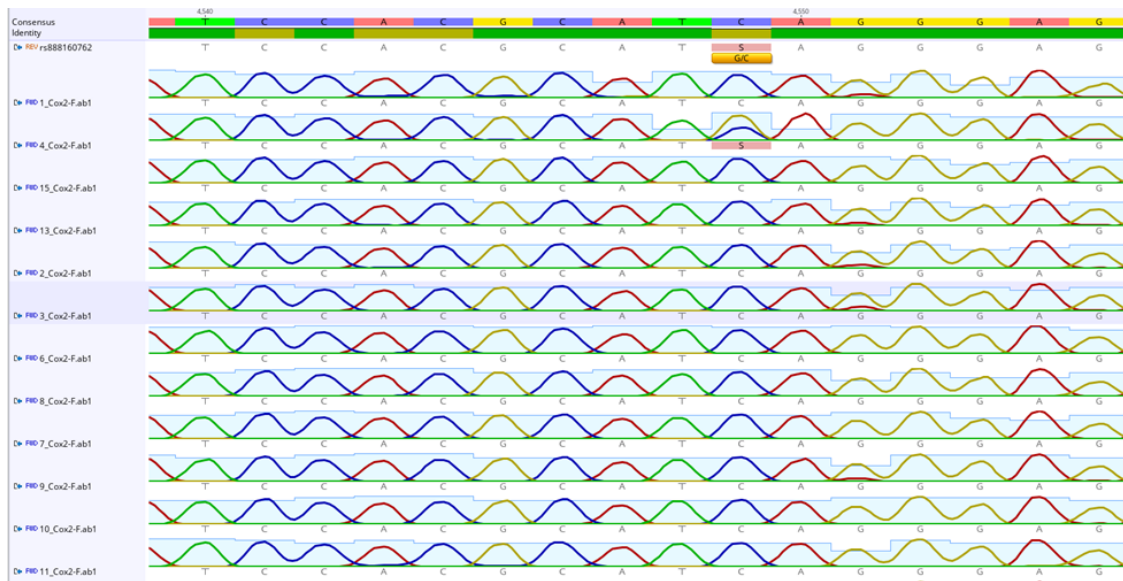


Fig 4. Analysis of rs888160762 Single nucleotide polymorphisms of PTGS2 gene

4. Discussion

The cervix is integral to conception, the maintenance of pregnancy, and timely delivery of the baby. The distribution of studies groups according to the age groups 34.43 ± 11.89 in comparison with the control group, (39.05 ± 13.53) . The ages (25-34), 34 (49.3%), where more prevalence in women has cervicitis than other ages. These findings disagree with (Zhang, et al., 2018) who reported that among women with cervical intraepithelial lesions aged 65–69 years (38.8%) was significantly higher than that of the other age groups [11]. The mean \pm SD, of tumor marker Ca125 were a highly significant differences between patient (30.33 ± 1.09) and the healthy control (19.40 ± 0.68) , these results were matched with (Charkhchi, et al., 2020) who reported that the tumors biomarker CA125 has been used as the primary ovarian cancer marker for the past four decades and gave a highly significant levels [12]. The mean differences among the CA markers compared to control group. The mean \pm SD of CA19.9 (19.62) front control (19.21), the mean \pm SD of CA125 (30.33) and the control (19.4) and the mean \pm SD of CA15.3 (22.82) but the healthy control (18.34). While the mean \pm SD of CRP was (88.06) and the control group 6.73. Bian, et al., (2017) reported that the CA125 levels were a high in patients with endometrial Cancer or cervicitis that develops into cancer [13]. The mutation in PTGS2 gene with highly CA125 marker 20(100%) and CRP levels 16(80%), while in CA15.3 markers 3(15%) and CA19.9 marker 1(5%). Charkhchi, et al, (2020) explained that the more mutation occurrence in different organs of women bodies with highly levels of CA125 marker and other tumor markers [14]. There is no mutation occurrence for analysis of rs888160762 NSP of PTGS2 gene using Sanger sequencing. Single “C” peak indicative of C homozygous alleles. Presence of the “G” and “C” peak indicative of G/C heterozygous allele [15]. But the mutation occurrence for analysis of rs20417 NSP of PTGS2 gene with wild CC GG and the variation C>G G>C. Genetic variation of SNPs in the *PTGS2* gene is reported to be capable of creating tissue milieu favoring tumor digenesis. Some studies have implicated that the common SNP rs20417 has association with PCa risk. Zhang, et al., (2015) found results between SNP rs20417 and risk of PCa either at genotypical level or at allele level (CC > GG, 1.00, 95% CI 0.87 to 1.15; CC + GC > GG, 95% CI 0.96 to 1.06; CC > GC + GG, 95% C>G, 95% GC> GG, 95% [16].

5. Conclusion

According to the results, most patients' ages were between 25 to 34 years, which was more prevalent in women with cecivitis than other ages. The mean differences among the Ca markers compared to the control group showed that the mean Ca19.9 was (19.62), Ca125 was (30.33) and Ca15.3 was (22.82) with non-significant differences. A noticeable rise in levels of CRP in cases of cervical infections, which corresponds to an increase in levels of CA125 marker. No mutation occurrence for analysis of rs888160762 NSP of PTGS2 gene using Sanger sequencing. Single “C” peak indicative of C homozygous alleles. Presence of the “G” and “C” peak indicative of G/C heterozygous allele.

Acknowledgement

Great thanks to the staff in Al-Elwiya maternity teaching hospital in Baghdad.

Ethical Approval

Ethical approval for this study was granted from the ethical committee of the Iraqi Ministry of Health (no. 2969).

Reference

- [1] Myers, K.M. Feltovich, H. and Mazza, E., et al. The mechanical role of the cervix in pregnancy, J. Biomech. 48 (2015) 1511e1523.
- [2] Gravett, M.G. Rubens, C.E. and Nunes. T.M., Global report on preterm birth and stillbirth (2 of 7): discovery science, BMC Pregnancy

Childbirth (10 suppl. 1) (2010) S2.

- [3] Singh N, Arora A. An Extreme Case of Chronic Cervicitis Mimicking Cervical Cancer and Causing Third-Degree Prolapse. *Journal Of Gynecologic Surgery* [serial online]. December 2014;30(6):380-382.
- [4] Pollett S, Calderon M, Heitzinger K, Solari V, Heitzinger K, Solari V, Montano S & Zunt J. Prevalence and predictors of cervicitis in female sex workers in Peru: an observational study. *BMC Infectious Diseases* [serial online]. April 30, 2013;13:195.
- [5] Efosa O, and Uwadiogwu A. Cytopathological Examination and Epidemiological Study of Cervicitis in Commercial Sex Workers (Csws) in Coal City (Enugu), Nigeria. *Ethiopian Journal of Health Sciences* [serial online]. July 2015;25(3):225-230.
- [6] Lusk M, Garden F, and Rawlinson WD, W NaingZ, G CummingR, KonecnyP. Cervicitis aetiology and case definition: a study in Australian women attending sexually transmitted infection clinics. *Sexually Transmitted Infections* [serial online]. May 2016;92(3):175-181.
- [7] Abou M, and Dällenbach P. Acute cervicitis and vulvovaginitis may be associated with Cytomegalovirus. *BMJ Case Reports* [serial online]. April 19, 2013;2013 Available from: MEDLINE, Ipswich, MA. Accessed March 22, 2017.
- [8] Taylor, S. Stephanie N., LensingS, SchwebkeJ, LillisR, Leandro A. Mena, Anita L. Nelson, Rinaldi A, SaylorL, McNeil L, and Jeannette Y. Lee. Prevalence and treatment outcome of cervicitis of unknown etiology. *Sexually Transmitted Diseases* [serial online]. May 2013;40(5):379-385.
- [9] Speers, CW. and Hayes, DF. Tumor Biomarkers. In: De Vita, Hellman, and Rosenberg's *Cancer: Principles and Practice of Oncology*. 11th ed. LWW Wolters Kluwer; (2018):974-975.
- [10] Hu, M. Lan, Y. and Lu, A. Ma, X. and Zhang, L. "Glycan-based biomarkers for diagnosis of cancers and other diseases: Past, present, and future". *Progress in Molecular Biology and Translational Science*. (2019), 162: 241–252.
- [11] Juwita, E. Puspandari, N. N. and IndahParamita, R. et al., Whole genome sequencing data of *Escherichia coli* isolated from bloodstream infection patients in Cipto Mangunkusumo National Hospital, Jakarta, Indonesia, C.-H. Lai, C.-W. Chang and F.-T. Lee et al. / *Data in Brief* 30 (2020) 105550.
- [12] Zhang, Ch. Huang, Ch. Xiang Zheng Xi and Pan D. Prevalence of human papillomavirus among Wenzhou women diagnosed with cervical intraepithelial neoplasia and cervical cancer, *Infectious Agents and Cancer* (2018) 13:37.
- [13] Charkhchi, P. Cybulski, C. and Gronwald, J, Oliver Wong F, A. Narod S and R. AkbariM . CA125 and Ovarian Cancer: A Comprehensive Review, *Cancers (Basel)*. 2020 Dec; 12(12): 3730. doi: 10.3390/cancers12123730.
- [14] Bian, J. Sun, Xi. Li, Bo. and Ming, L., Clinical Significance of Serum HE4, CA125, CA724, and CA19-9 in Patients With Endometrial Cancer, *Technol Cancer Res Treat*. 2017 Aug; 16(4): 435–439. doi: 10.1177/1533034616666644.
- [15] Charkhchi, P. Cezary Cybulski,2 C. and Jacek Gronwald, et al., CA125 and Ovarian Cancer: A Comprehensive Review, *J. of Cancers (Basel)*. 2020; 12(12): 3730
- [16] Pietruszewska, W, *, Wojciech Fendler, W. and Marta Podwysocka M. et al., Expression of Transcript Variants of PTGS1 and PTGS2 Genes among Patients with Chronic Rhinosinusitis with Nasal Polyps, *Diagnostics* 2021, 11, 135. <https://doi.org/10.3390/diagnostics11010135>.
- [17] Zhang, L. Yan Zhang, Zhang, Xu., and Hong, B. Prostaglandin-endoperoxide synthase 2 (PTGS2) rs20417 polymorphism and prostate cancer risk: a meta-analysis. *Int J Clin Exp Med*. (2015). 8(11): 20454–20462.