Acta Scientific CANCER BIOLOGY (ISSN: 2582-4473)

Volume 9 Issue 3 March 2025

Research Article

Significant Association of Methylenetetrahydrofolate Reductase (MTHFR) C677T Polymorphism Increasing Risk Factor in Adenocarcinoma of Ovary in Indian Population of Bihar

Vijay Partap Singh[1], Ajit Kumar Saxena[1]*, Chandan Kumar Singh[2], Sambhawi Sharma[2], Akash Kumar Singh[2] and Rahul Kumar Chaudhary[1]

[1]R.S. Memorial cancer society, Research Center, Kankarbagh, Patna, Bihar, India

[2]Savera Cancer and Multispecialty Hospital, Kankarbagh, Patna, Bihar, India

***Corresponding Author:** Ajit Kumar Saxena, R.S. Memorial Cancer Society, Research Center, Kankarbagh, Patna, Bihar, India.

Received: June 10, 2025

Published: July 03, 2025

Abstract

Introduction: Folate metabolism, play an important role in DNA methylation during proliferation and differentiation of tumor cells through methylenetetrahydrofolate reductase (MTHFR C677T) enzyme has been reported in variety of tumors . Present study has been designed with the aims evaluate the genetic heterogenicity and "risk factor" in heterozygous condition after calculating the frequency of CT genotypes in ovarian cancer patients, and also develop correlation with cellular proliferation (histopathology) in the same

Material and Method: Clinically diagnosed cases (n=125) of ovarian cancer patients with same age matched controls (n=137) were included in the present study. Total (5.0) ml sterile peripheral blood samples were collected and stored at -80°C till further process i.e. isolation of DNA and quantified by Nanodrop spectrophotometer (at wavelengths between 260-280 nm). ARMS-PCR was used for SNP analysis in cancer patients and compare with native controls (lack of familial history of cancer).

Results: Significant findings (p< 0.0002) reveals, 37.5 % frequency of genotype (677CT) were observed in heterogynous condition with Tm value of 83.50 and as compared to the 677TCC genotype (Tm 82.83) between cases controls. condition in ovarian cases. Interestingly, the frequency (33.33%) in heterozygous condition (CT genotype), highest recoded in germ cell tumors and minimal frequency (27.27%) were observed in surface epithelial tumors. Histopathological showing relevant correlation during onset of disease between the frequency (40%) in homozygous (TT rare genotypes) in sex-cord stromal tumors and risk factor (CT genotype) in germ cell tumors.

Conclusion: The present study demonstrates a significant differences in the frequency values between CT and TT genotypes that increase genetic susceptibility with increase "risk factor" in sex- cord stromal tissue in ovarian tumors due to substitution of nucleotide cysteine to thymidine in heterozygous condition (C→T) due to point mutation in folate metabolism associated MTHFR gene polymorphism to reduce 50% intracellular folate contents may increase risk for the development of ovarian cancers in the population of Bihar, hence, MTHFR C677T polymorphism act as potential biomarker for early diagnosis in cancer.

Keywords: Ovarian Cancer; MTHFR C677T Polymorphism; Histopathology; ARMS-PCR.

Introduction

Ovarian cancer, stands as the most common gynecological malignancy after tumors of the cervix and uterus. It is often diagnosed at advanced stages due to the lack of specific clinical symptoms and effective early detection methods, resulting increase of high mortality rates. According to estimates from the GLOBOCAN 2018 database, ovarian cancer continues to pose a major health threat globally ovarian cancer has the highest mortality rate among gynecological malignancies and is associated with poor prognosis, low survival rate, and is the most deadly reproductive cancer among women**.** Present study includes both younger (age group 20-45 year) and older women (age group 45-65 years). The distinction in

age groups might reflect differences in risk factors, as the mechanisms that lead to cancer development can vary depending on a person's age and stage of life. [1-3]. Histopathologically, ovarian cancer , showing great values on the basis of tissue- subtypes i.e. on the basis of germ cell, surface epithelial and sex cord stromal types , and disease burden implying for prevention during screening, and future therapeutic management.

Vitamins B6 and B12 are known to play important roles in various metabolic processes, including DNA synthesis and methylation, which are linked to cancer development [9].

According to WHO diagnostic classification guidelines, low folate- metabolism has been significantly associated with an increased risk in the variety of cancers, including breast, colon, endometrial and trophoblastic neoplasia [4-8]. Adenocarcinoma of ovary is difficult to early diagnosis either familial or sporadic in nature. Folate and vitamins B6 and B12 are known to play important roles in various metabolic processes, including DNA synthesis through methylation possibly linked to cancer development [9]. The 5, 10-methylenetetrahydrofolate reductase (MTHFR C677) gene polymorphism is essential for DNA methylation during proliferation of cancer cells and may increase "risk factors" in heterozygous condition after point mutation of nucleotide cysteine substituted to thymidine (C- → T) as shown in figure 2a. The present study has been designed with the aims in the population of Bihar because of poor socioeconomic conditions to evaluates the frequency of MTHFR genotypes (CC, TT and CT) in homozygous or in heterozygous conditions and their correlation in the frequency of different tissue subtyping specificity in ovarian cancer has not been reported earlier.

Martials and Methods

Subjects

Present study included a total of (n = 125) histopathologically confirmed cases of ovarian cancer diagnosed between 2018 and 2022, along with (n = 137) age matched healthy controls. Blood samples and clinical data were collected from Research center, R.S. Memorial Cancer Society, Savera Cancer and Multispecialty Hospital, Patna. The collected data included demographic details, treatment records, and survival outcomes. The present study is approved by Institutional ethical committee (SCMH-IEC/2024/03/01).The patients were categorized into three age-groups: Group 01: <30 years, Group 02: 30-60 years, Group 03: >60 years. A 05 ml peripheral blood sample was collected in sterile EDTA vial their informed written consent from each participant across all age groups. Ovarian cancer patients and healthy women as controls were included in the study for comparative analysis. The study on the analysis of the MTHFR gene polymorphism (C677T) in ovarian cancer patients and control Subjects.

Collection of blood samples from cancer

Blood samples approximate 4.0 ml were collected from each subject under sterile conditions and stored in EDTA vacutainer tube and stored in a deep freezer at - 80ºC till further study.

Isolation of DNA extraction from blood

Genomic DNA was extracted from venous blood samples collected from (n = 125) cases and (n = 137) healthy female controls using a Genomic DNA Kit (Huwel Life Science). The DNA was quantification by Nano drop spectrophotometer using absorbance at wavelengths of 260-280 nm. The quality of the extracted DNA were evaluated on agarose (1.5%) gel electrophoresis after staining with ethidium bromide and bands were visualized on Gel Doc system.

Study of MTHFR C677T gene polymorphism using ARMS PCR

The primers for tetraplex real-time PCR assay were designed for genotyping of MTHFR C677T (http://cedar.genetics. soton.ac.uk/public html/primer1.html) after confirmation by BLAST program at http:// www.ncbi.nlm.nih.gov.to determine the specificity of the primers. To increase the specificity of the reaction a mismatch at the 2 position of the 3' end both the allele-specific primers were selected and further confirm their specificity by software as documented earlier in the literature [10]. To obtain amplicons with distinct melting points, the '*Tm*' values were calculated using analyzer (http://eu.idtdna.com/analyzer/Applications/OligoA). The selection of the primers were based on the amplicons 'Tm' values and following primers were used to determine the genetic heterogeneity-MTHFR-T, 5' - GCACTTGAAG GAGAAGGTGTCTGCGGGCGT-3'; MT MTHFR-C poly G, 5' - GGCGGGCGGCCGGGAAAAGCTG CGTGATGATGAAATAGG-3'; MTHFR-cf, 5' - TGTCATCCCTATTGGCAGGTTACCCCAAA-3'; MTHFR cr, 5' - CCATGTCGGTGCATGCCTTCACAAAG-3'.These group of tetra-primer used for ARMS PCR and SYBR Green used as fluorescent dye for analysis of *Tm* values to evaluate the frequency of mutant allele (C/T) of MTHFR gene in heterozygous condition.

Statistical analysis

Data was analyzed between cases and controls using chi-square test (two- tailed) to assess the significant differences ($p < 001$) between cases and controls. The frequency (%) was also calculates for all the three genotype (CC and TT) in homozygous and CT in heterozygous condition with odds ratios (O.R) and confidence intervals (C.I) at 95% and correlated with tissue specificity.

Results

In the present study, the MTHFR gene (C677T) polymorphism was analyzed in (n = 125) cases of ovarian carcinoma and (n = 137) age- and sex-matched healthy women as controls, using a highly sensitive and reliable method using four set of primer PCR. This technique amplifies both the wild-type and mutant alleles (C677T) of the MTHFR gene in a single PCR tube. The procedure used for the selection of four primers to amplify the region containing point mutation, with specific amplicons that represent each alleles. The study found that tetra-primer PCR produced amplicons with a melting temperature (*Tm*) values 82.83 in the control group and shifting towards ovarian carcinoma cases ™ values (83.50) showing significant difference of the alleles (C → T) confirming "point mutation" and melting curves (*Tm*) values shown in figure-1a, and details statistical analysis are depicted in table 1.

Table 1: Statistical analysis showing MTHFR C677T gene polymorphism frequency (%) of genotypes in ovarian cancer patients and controls.

S. No	MTHFR Gene polymorphism	Frequency (%)		Odd ratio	Confidence interval	P. value
		ovarian cancer	Control		C I at 95%	
A	Genotype	(n = 32)	(n = 45)	----	---	---
1	CC	15(46.87%)	25(55.55%)	4.6296	2.2419 to 9.5606	p < 0.0001
2	CT	12(37.5%)	20(44.44%)	4.6296	2.0955 to 10.2281	p < 0.0002**
3	TT	05(15.62%)	00(00.00%)	0.1839	0.0103 to 3.2849	p > 0.2496

*Significant differences were observed in heterozygous (CT genotypes) condition between cases and controls.

Data showing the CC genotype frequency in 46.8 % of ovarian cancer cases in homozygous condition and decreasing systematically in CT genotype (37.50%) followed by lowest (15.62%) was fund in TT genotypes. Significant (p < 0.05).difference were observed in heterozygous (CT genotypes) condition. Bar diagram (Figure 1b) showing the lowest frequency of TT homozygous genotype (15.62 %) as compare to CT heterozygous genotype but showing lack of significant difference using two-tailed chi-square test.

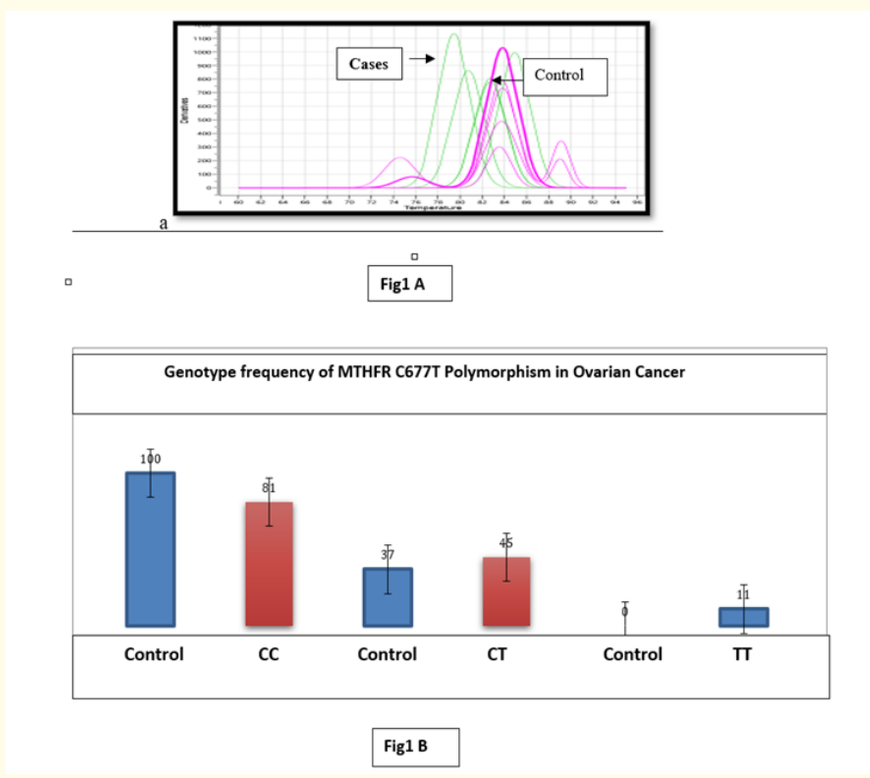

Figure 1: a,b: Diagrams showing ARMS-PCR based analysis of MTHFR C677T polymorphism showing amplification (Tm values) between ovarian cancer and controls .The individual frequency of wild-type (CC), CT and TT genotypes (Figure 1b)showing systematically decreasing trend were observed as shown in two different color codes (brown) ovarian cases with respect to controls (blue).

Histopathological findings as shown in figure 2a with high grade adenocarcinoma of ovarian carcinoma with hyper chromatic nuclei with atypical mitotic activity. Similarly in another case showing the *plasmanea locules* was one of the characteristic features of mucinous carcinoma (Figure 2b) for confirmation of pathological findings. There is significant correlation between histopathological observation of three different layers i.e germ- cell, surface epithelial and sex cord tumors. MTHFR C677T gene polymorphism. The highest frequency of CT genotypes (27.77 %) was observed in surface epithelial in heterozygous condition and again highest frequency (40.00%) was observed in homozygous condition with TT genotype in sex-cord tumors as details are documented in table-2 and bar diagram (Figure 3) showing systematically down regulation of genotype frequency i.e. CC and TT in homozygous and in CT heterozygous conditions.

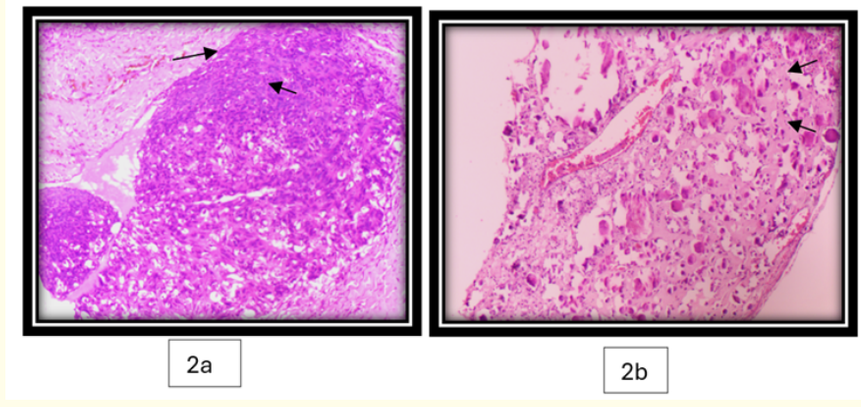

Figure 2: Histopathological finding showing high grade adenocarcinoma of ovary with hyper chromatic nuclei and atypical mitotic activity (2a), and in another case *plasmanea locules* - characteristic features of mucinous carcinoma (Figure 2b) was observed (arrow head), after H and E staining of transverse sections.

Table 2: Differential frequency (%) observed between genotypes and different cell types in ovarian cancer patients.

Type of Histology	No. of Case	CC	CT	TT
Germ Cell Tumor	12	06 (50.0%)	04(33.33)	02((16.66%)
Surfaces Epithelial Tumor	18	08((44.44%)	05(27.77)	03(16.66%)
Sex-cord stromal tumor	05	02(40%)	01(28%)	02(40%)

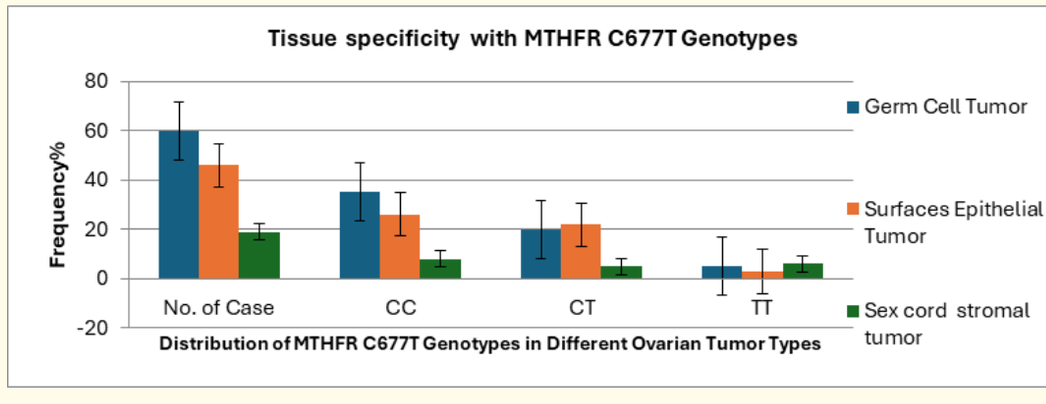

Figure 3: Bar diagram showing the significant correlating between the frequency (%) of MTHFR C677T genotypes CC,TT in homozygous and CT genotype heterozygous condition with tissue subtypes in ovarian tumors: Germ cell tumors in CT genotype in: 33 % cases while TT rare genotype showing in 16.66 % cases only, suggesting genetic heterogenicity is due tissue specificity during progression of disease.

Discussion

In eukaryotic system folate metabolism is the relevant factor for proliferation of tumor cells during events of oncogenesis. Etiopathology of tumor biology is still not clear but definitely, genetics and epigenetics factors play an important role in transition of epithelial to mesenchymal cells involving significant role of transition transcription factor (SOX4), cytokeratin, epithelial cell adhesive molecule (EpCAM) and metastatic vimentin markers. Meta-analysis comprehensively examined the associations between methylenetetrahydrofolate reductase (MTHFR) polymorphisms C677T and A1298C gene increase "risk factor" in variety of diseases including recurrent pregnancy loss and congenital heart defects [10,11]. Dietary supplement including MTHFR gene polymorphisms increase risk for the development of cervical and colorectal cancer [12,13]. MTHFR C677T, is a key enzyme that involved for DNA metabolism and maintaining genomic instability. The loss of heterozygosity (LOH) in ovarian tumors supports the hypothesis that substitution of nucleotide cysteine to thymidine (C→T), the point mutation increase risk factor during progression of disease [14-16]. Different frequencies of MTHFR genotypes might have varying due cellular subtype proliferation of age-specific histopathologically based findings.

Other cellular sub-types in low-grade serous carcinomas, differ in etiology due to genetic mutations and fragmentation of chromosomes to make the aggressive behavior of the disease [15]. The age specificity correlation between young and older-age -group in the present study is difficult to predict due to small sample size or unknown environmental factors. Although, ovarian carcinogenesis is based on variety of factors such as endocrine dysregulation, variations in karyotypes (complex chromosomal rearrangement) or gene mutations together dysregulation of cell signaling through p53 mutation involved in the etiopathology of adenocarcinoma of ovary more complex for the early management by the clinicians [17]. Although, histopathological variation based on cytoarchetecture after tissue biopsy have own limitation due to variation in tissue specificity (sub-typing) [18,19]. Hence, genetic markers such as BRCA1 or BRCA2 gene becomes essential tool for confirmation of hereditary and spontaneous mutation in high-grade serous carcinoma due to TT genotype in homozygous condition. Serous carcinoma shows positive immunohistochemically positive staining for cytokeratin, (CA-125), estrogen receptor (ER), and tumor suppressor (p53) gene expression [20-22]. Clinicians are trying to protect after maintaining folate equilibrium followed by protect the tumor growth in colorectal cancer patients and improve DNA repair mechanisms individually for CT or TT genotype in homozygous or in heterozygous condition.

Conclusion

Present study concludes that frequency of genotypic variation of MTHFR C677T gene polymorphism are varying significantly in germ cell subtyping due to genetics heterogenicity (CT genotypes) and increase "risk factor" followed by increasing frequency of TT genotype in homozygous condition to confirm in sex-cord stromal tumor during progression of disease in ovarian tumor. Hence, MTHFR C677T gene polymorphism increase frequency of T allele due to "point mutation" confirm the onset of disease could be use as genetic biomarker for tissue specificity and genetic susceptibility followed by confirmation of diagnosis and proper management by the clinicians to reduce mortality. Although, the efforts are continuing by the scientist to find epigenetic causative factors and reverse or reduce fragmentation of chromosomes or DNA damage in such patients otherwise the study will be remain un clear.

Acknowledgments

Authors are thankfully acknowledge to the Indian Council of Medical Research (New Delhi) and Department of Surgical Oncology, Savera Multispecialty Hospital Patna (Bihar). Authors (AKS) also extended acknowledgement to the patients/ family who participate and cooperate in the study.

Bibliography

1. Bray F., *et al*. "Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries". *Ca-A Cancer Journal for Clinicians* 68.6 (2018): 394-424.

2. Momenimovahed Z., *et al*. "Ovarian cancer in the world: epidemiology and risk factors". *International Journal of Women's Health* 11 (2019): 287-299.

3. Chan JK., *et al*. "Stages III and IV invasive epithelial ovarian carcinoma in younger versus older women: what prognostic factors are important?" *Obstetrics and Gynaecology* 102.1 (2003): 156-161.

4. Kurman Robert J., *et al*. "World Health Organization Classification of Tumors of the Female Reproductive Organs. 4th edition., *International Agency for Research on Cancer* (2014).

5. Wentzensen N., *et al*. "Ovarian Cancer Risk Factors by Histologic Subtype: An Analysis From the Ovarian Cancer Cohort Consortium". *Journal of Clinical Oncology* 34.24 (2016): 2888-2898.

6. Giovannucci E., *et al*. "Folate, methionine, and alcohol intake and risk of colorectal adenoma". *Journal of the National Cancer Institute* 85.11 (1993): 875-884.

7. Tworoger SS., *et al*. "Intake of folate and related nutrients in relation to risk of epithelial ovarian cancer". *American Journal of Epidemiology* 163.12 (2006): 1101-1111.

8. Saxena Ajit K., *et al*. "ARMS-PCR based SNP analysis of MTHFR C677T allele using Syber green in pancreatic tumor". *British Journal Of Medical and Health Research* 11.12 (2016): 1-6.

9. Ye S., *et al*. "An efficient procedure for genotyping single nucleotide polymorphisms". *Nucleic Acids Research* 29.17 (2001): E88-98.

10. Saxena Ajit K. *et al*. "Evaluation of Methylenetetrahydrofolate reductase C677T gene polymorphism associated risk factor in the patients of recurrent pregnancy loss". *International Journal of Medical Genetics and Genomics* 4.2 (2012): 25-28.

11. Saxena Ajit K., *et al*. "Penetrance of Methylene Tetrahydrofolate Reductase C677T Gene Polymorphism and Karyotypic Variations Associated Increase Genetic Susceptibility in the cases of Congenital Heart Defects". *Biomedical Journal of Scientific and Technical Research* 38.1 (2021): 30051-30057.

12. Ma J., *et al*. "Methylenetetrahydrofolate reductase polymorphism, dietary interactions, and risk of colorectal cancer". *Cancer Research* 57.6 (1997): 1098-1102.

13. GK Singh., *et al*. "p53 gene inactivation modulates methylenetetrahydrofolate C677T gene polymorphism associate risk factor for the development of cervical carcinoma-A tissue specific genetic heterogeneity". *The Journal of Clinical and Experimental Oncology* 23 (2009): 167-178.

14. Ali AT., *et al*. "Epidemiology and risk factors for ovarian cancer". *Przeglad Menopauzalny* 22.2 (2023): 93-104.

15. Lheureux S., *et al*. "Epithelial ovarian cancer: Evolution of management in the era of precision medicine". *CA Ca-A Cancer Journal for Clinicians* 69.4 (2019): 280-304.

16. Anupma Singh., *et al*. "In Human allele specific variation of MTHFR C677T and A1298C associate risk factor for the Development of Ovarian Cancer". *Journal of Experimental Therapeutics and Oncology* 11.1 (2015): 67-70.

17. Lheureux S., *et al*. "Epithelial ovarian cancer: Evolution of management in the era of precision medicine". *Ca-A Cancer Journal for Clinicians* 69.4 (2019): 280-304.

18. Saxena Ajit K., *et al*. "Differential expression of Vimentin gene modulated by MTHFR C677T gene polymorphism in circulating tumor cells isolated from breast cancer patient". *Genetics and Molecular Research* 23.1 (2024): 1-10.

19. Viel A., *et al*. "Loss of heterozygosity at the 5,10-methylenetetrahydrofolate reductase locus in human ovarian carcinomas". *British Journal of Cancer* 75.8 (1997): 1105-1110.

20. Timmermans M., *et al*. "No improvement in long-term survival for epithelial ovarian cancer patients: A population-based study between 1989 and 2014 in the Netherlands". *The European Journal of Cancer* 88 (2018): 31-37.

21. Zhou L., *et al*. "Ovarian endometrioid carcinoma and clear cell carcinoma: A 21-year retrospective study". *Journal of Ovarian Research* 14 (2021): 63.

22. Clarke-Pearson DL. "Clinical practice. Screening for ovarian cancer". *The New England Journal of Medicine* 361 (2009): 170-177.