Review Article

Molecular Biomarkers for Early Detection and Prevention of Ovarian Cancer—A Gateway for Good Prognosis: A Narrative Review

Abstract

Gynecological cancers are one of the most lethal and deadliest cancers in the world. In India, the prevalence of ovarian cancer accounts for 2.5% to 3%. Despite the availability of improved treatment option along with improved technology, the survival rate of ovarian cancer in the early-stage and the advanced stage is poor. Therefore, due to the heterogeneity of ovarian cancer, to detect it at an early stage and to prevent further mortality turns out to be a big challenge. Researchers are still in the process to identify any single biomarker with good sensitivity and specificity. Various traditional and serum approaches to identify ovarian cancer have been successful in the early stages. The invention of molecular biomarkers such as the use of genomic profiling, DNA methylation, and other approaches have proven to be of higher sensitivity and specificity, which overall affects the prognosis of ovarian cancer. With the use of whole-genome analysis, the detection of possible location of critical tumor suppressor gene (TSGs) in the paired region of chromosomes has been identified, which are associated with *BRCA1* and *BRCA2* which further makes these novel molecular biomarkers as potential biomarkers. Moreover, studies are required to assess the combined use of traditional, molecular biomarkers that might be useful for enhanced sensitivity and specificity for early detection and prevention of ovarian cancer in early stages which will lead to reduced mortality and good prognosis

Keywords: *Molecular biomarkers, ovarian cancer, preventive medicine*

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Access this article online

Website: www.ijpvmjournal.net/www.ijpvm.ir

DOI: 10.4103/ijpvm.IJPVM_75_19

Quick Response Code:

Introduction

Woman reproductive organs constitute five main types of cancer (cervical, ovarian, uterine, vaginal, and vulva) collectively termed as gynecological cancers. Among these, ovarian cancer is the most lethal cancer which if not detected at the earliest stage leads to death. In 2017, more than half of the women died in the U.S due to these diseases and 14,080 out of 22,440 women were diagnosed with ovarian cancer. It is one of the deadliest and fifth most widespread cancer-related death among all gynecological cancer among women in the world. In India, the prevalence of ovarian cancer accounts for 2.5%. The mortality rate of ovarian cancer is up to some extent higher for Caucasoid women than for African-American women.[1]

Ovarian cancer is defined as an abnormal growth of cells that arises from the cells of ovaries. There are different kinds of ovarian cancer but most commonly it arises from the epithelial lining cells of ovaries. Ovarian carcinoma includes cancer of ovaries, fallopian tube, and primary peritoneal (lining tissues of the pelvis and abdomen) cancer, less commonly it includes germ cell tumors and sex cord-stromal tumors.[2]

Histologically, ovarian cancer is further classified as serous, mucinous, endometroid, clear cell, and mixed undifferentiated.[2] Cancer staging is a fundamental principle and one of the first and most important steps used to predict the patient outcome as well as to plan the most appropriate treatment. The most commonly used staging system for the ovarian cancer is FIGO (International Federation of Gynecology and Obstetrics) which provides more accurate prognostic information and better guidance on the management of ovarian cancer. The epithelial ovarian cancer does not present with earlier signs and symptoms and there are no specific efficient biomarkers to detect it which eventually leads it to be diagnosed at advanced FIGO staging. Despite improved treatment option, the survival rates of ovarian cancer with

How to cite this article: Yadav G, Vashisht M, Yadav V, Shyam R. Molecular biomarkers for early detection and prevention of ovarian cancer—A gateway for good prognosis: A narrative review. Int J Prev Med 2020;11:135.

advanced stage FIGO III and IV are only 10–30% when compared with earlier stages (FIGO I and II) (80–95%).[3] In the earlier stage, ovarian cancer presented very few signs and symptoms including premenstrual syndrome, irritable bowel syndrome, and temporary bladder problem.

Detection of ovarian carcinoma at its earlier stage has become a big challenge for the physicians as well as researchers. Currently, no proven single biomarker with adequate sensitivity and specificity till date has been discovered to detect ovarian carcinoma at an early stage. The researchers are now focusing to find out the suitable and appropriate solution to detect ovarian cancer at an earlier stage through the identification and validation of novel biomarkers using new technologies.

The focus of this review will be on molecular approaches that are currently being employed in the discovery of new ovarian cancers biomarkers. The review will also highlight circulating biomarkers either currently being utilized or in development, that are present in human body fluids such as plasma, serum, and urine.

Molecular Markers in Hereditary Ovarian Cancer

Hereditary breast and ovarian cancer (HBOC) is significantly linked with a high possibility for ovarian and breast cancer when compared to the general population. HBOC is characterized by both ovarian and breast cancer, only breast cancer in males and both ovarian and breast cancer in females. *BRCA1* and *BRCA2* genes are expressed as autosomal dominant with incomplete penetrance and serve as tumor suppressor gene. *BRCA1* and *BRCA2* genes are linked with DNA repair and cellular apoptosis. *BRCA* mutations account for 20%–50% lifetime risk for developing ovarian cancer. *BRCA*-associated ovarian cancers have a good response rate with increased survival of patients based on platinum-based chemotherapy.[4]

The median survival time for the BRCA noncarriers is (37.8 months) lesser when compared to BRCA carriers (53.4) months.[5]

HNPCC (Lynch II syndrome), has a higher tendency for right colon cancer (without polyps) and endometrial-ovarian cancer with autosomal dominant gene expression. Lynch II syndrome has most of the germ-line mutations for *hMLH1, hMSH2, hMSH6* and *PMS2* (DNA mismatch repair). Women have a 12% lifetime risk for developing ovarian cancer with HNPCC syndrome.[6]

Genetic Biomarkers for Ovarian Cancer

BRCA1 and *BRCA2* mutation

Out of all ovarian malignancies, the prevalence of hereditary ovarian cancer accounts for 10 to 15% cases linked with germline mutations in the *BRCA1* and *BRCA2* genes which are mainly involved in genetic testing worldwide. The epithelial ovarian cancer associated with mutations of *BRCA1* and *BRCA2* has a cumulative lifetime risk ranging from 40%–50% and 20%–30%, respectively. The odds of having mutations in *BRCA1* are four times as *BRCA2* mutations.[7]

The hereditary predisposition of ovarian cancer is 10–15% when compared to 5 to 10% of breast cancer cases.[8] With the recent advancements in the genetics of ovarian cancer (*RAD51C* and *RAD51D*)[9,10] *BRCA1* and *BRCA2* are still the most distinguished genes which contribute to the 4.6-fold relative risk of ovarian cancer. The lifetime ovarian cancer risks associated with deleterious mutations in *BRCA1* accounts for 20%–50% and for *BRCA2* it is approximately 10%–20%. Studies have shown that *BRCA1*[11] accounts to significantly younger mean age at the diagnosis of ovarian carcinoma than *BRCA2*[12] mutation carriers which are still significantly less when compared with the general population[13] considering the severe influence of *BRCA1* mutations. Based on histological characteristics, high-grade serous ovarian carcinoma subtype predominantly[14] has both *BRCA1* and *BRCA2* mutation carriers. For the DNA repair, genomic stability maintenance and control of cell cycle checkpoint, genes which have been of utmost importance are *BRCA1* and *BRCA2*.[15] According to Kinzler and Vogelstein's definition,[16] these genes belong to the group of a concierge which is indirectly related to tumor initiation and promotion when weighted against a gatekeeper which is otherwise directly involved. Thus, the caretaker inactivation leads to oncogenic mutation and tumor suppressor gene (TSG) further leading to genomic instability, resulting in the prevention of cell death and function of cell cycle checkpoint, and enabling tumor growth.

Nevertheless, *BRCA1* and *BRCA2* are responsible for the maintenance of genomic stability as they control cell growth and hence are considered TSGs.[17]

The *BRCA1* and *BRCA2* proteins are implicated in the refurbishment of DSBs (double-stranded breaks) through the HR pathway. Use of substitute pathway is actually useful for refurbishing the DSBs which is mainly due to deficiency of *BRCA1* or *BRCA2* leading to accretion of mutation events which results in a greater chance of chromosome instability.[10,18]

Various studies associated with *BRCA1* and *BRCA2* mutations have been carried out in different parts of the world. One such study was done in Greece, where a cohort of 592 patients were screened for commonest *BRCA1* mutations for sporadic OC, out of which 27 mutations of *BRCA1* were carriers (4.6%)[11]

In Belgium, a study among 193 sporadic cases of breast and ovarian cancer by de Leeneer *et al*. for *BRCA1/2* stated that there were 3 carriers out of 7 with both breast and ovarian cancer women (42.9%) but no carrier were found among

6°C patients.[19] In Poland, 21 out of 151 consecutive OC patients accounted for *BRCA1/2* mutations (13.9%)[20] while for 74 Russian patients, the prevalence of the *BRCA1/2* mutation was 19%.[21] In Korea, *BRCA1/2* mutations patients with a positive family history of ovarian cancer patients were 13 of 40 (33%) while 23 had no positive family history out of 283 patients (8%).[22]

RAD51C

RAD51C was first recognized by Meindl *et al*. as a rare hereditary breast and ovarian cancer (HBOC) gene.[23] Meindl and his coassociates screened 1100 hereditary breast (HBC) and HBOC families for *RAD51C* mutations and hypothesized that *RAD51C* biallelic mutations causing Fanconi anemia would be similar to *BRIP1* and *BRCA2* and monoallelic mutations would cause HBOC. *RAD51C* [*RAD51* homolog C], is a member of the *RAD51* gene family, located on chromosome 17q23, which is expressed with the highest level in testis, followed by the heart muscle, spleen, and prostate, and other various human tissue and organs that encodes strand-transfer proteins in various human tissues.[24,25]

BRCA1, BRCA2, PALB2, BRIP1, and *RAD51C* are involved in DNA damage repair by homologous recombination pathway.[26]

For women at the age of 80, there is a 10% risk of developing ovarian cancer carrying a *RAD51D* mutation. In a consanguineous family with high penetrance, a homozygous biallelic mutation in the *RAD51C* gene showed Fanconi anemia-like disorder that was associated with heterozygous mutations with high penetrance[27] while an increased risk of breast and ovarian cancer were associated with rare heterozygous mutations with high penetrance.[26]

A study conducted by Meindl *et al*. examined 1100 affected individuals from pedigrees with gynecological cancers that were negative for mutations in *BRCA1* and *BRCA2* from German families and found that there was no mutation in families with only breast cancer as well as healthy control while both breast and ovarian cancer had six pathogenic *RAD51C* mutations.

ATM mutations

The two genes which have been recognized as high penetrance allele are *BRCA1* and *BRCA2* for ovarian cancer; while the third gene is *ATM* with high penetrance alleles. Ataxia Telangiectasia Mutated (*ATM*) gene is situated at the short arm chromosome of locus 11q22-23, encoding a large protein belonging to a family of PI3K related kinases.[28,29] The function of this protein is to regulate various cellular responses to genotoxic stress.

The main function of *ATM* is that it acts as an activator of the DNA damage response cascade after DNA double-strand breaks.[30] *ATM* exists as a homodimer, which upon activation dissociates into active monomers via autophosphorylation at Ser1981 after DNA damage. At the DNA damage site, *ATM* is recruited and in its active form by the property of direct and indirect phosphorylation events of a large number of proteins, activates a signaling cascade which eventually activates cell-cycle checkpoints and the initiation of DNA repair.[30]

p53 mutation

p53 gene is so-called as a tumor-suppressor gene. During the past several decades, a lot of promising research at the molecular level is being carried out for understanding these gene mutations which still remains a challenge for the researchers to serve for the community.[31]

The protein of *p53* (also known as TP53) binds to a specific site at DNA and regulates cell multiplication. Multiplication of cells that have continual DNA damage is blocked by *p53* protein but if the damage is beyond repair, cell death occurs via apoptosis through *p53* gene. Due to mutation, *p53* gene gets deactivated and the damaged cells continuously proliferate which eventually leads to carcinogenesis.[32] it has been found that 50% of invasive epithelial ovarian cancer contain an abnormal *p53* gene, although in almost no borderline epithelial cancers this gene has been detected.[33,34] Mutations in *p53* are mainly from endogenous origin but exogenous exposure like tobacco smoke may also account to a certain extent[35] which are most common but transient.[36]

Thus, *p53* transitions might occur during normal cell proliferation due to random errors in DNA synthesis. Consequently, Fathalla- Pike hypothesis stated that ovulation may also induce *p53* transitions which increase ovarian epithelial cell proliferation. During ovulation-induced proliferation these random changes in *p53* gene arise from errors which disable the protein, thereby providing the gateway to other cellular damage further leading to cancer. *p53* mutations are usually seen and expressed in its early and localized stage but one would see them equally in both localized and advanced cancers.

Recent Molecular Biomarkers of Ovarian Cancer

Due to significant heterogeneity among the various ovarian cancer subtypes, it becomes quite difficult to search for new biomarkers. The recent advancement of genomics, transcription, and proteomic profiling with the help of tumours in serum, plasma, and urine acts as a newer source for identifying potential cancer screening markers.

Whole genome analysis

The whole genomic analysis includes comparative genomic hybridization (CGH), LOH (loss of hybridization), spectrokaryotyping (SKYP), and serial analysis of gene expression (SAGE). Due to the advancements in technology these molecular markers are now being used

in rapid diagnosis and prognosis of the risk of the ovarian cancer patients. These molecular markers have higher sensitivity and specificity and are now considered as potential biomarkers.

Loss of heterozygosity analysis (LOH)

LOH denotes the lack of tumor suppressor gene in both the region of paired chromosomes which is a usual phenomenon in a cancer gene. The chance of determining possible locations of critical tumor suppressor genes and the identification of possible cancer biomarkers is provided through the loss of heterogeneity analysis.

Modification in polymorphic markers to homozygous state in the tumour DNA from a heterozygous state in the germline DNA is the most common genetic events in numerous cancer types which results in loss of heterozygosity.[37]

Polymorphic markers (microsatellites or single-nucleotide polymorphisms) are the best way to predict loss of heterozygosity which are easily identified in a human germline DNA and cancer cells by the presence of heterozygosity at a genetic locus and absence of heterozygosity in the cancer cells at a particular locus.[38]

Patients who have germline mutations in tumor cells show loss of heterozygosity in genes *BRCA1* and *BRCA2* which results in loss of wild type allele. These genes regulate the DNA repair pathway by binding to *RAD51* which produces proteins.[38]

Comparative genomic hybridization (CGH)

Comparative genomic hybridization is also called an *in-situ* hybridization technique which is a whole-genome assay that detects gains or losses of gene copy number at the chromosomal level. With the use of this assay, a number of chromosome regions with abnormal gene copy number in ovarian cancer have been identified and have been further evaluated as potential prognostic markers.[39]

In primary ovarian cancer, the most common gains were revealed in chromosome 8 and 8q (i.e. 36–75% of tumors), and the most common losses has been found to be at 8p (>30% of tumors).[40]

At the chromosomal level, CGH is also being used to differentiate histological subtypes of ovarian cancer (serous and nonserous), defining whether there is chromosomal gain or loss in each group. Among the histological variants of ovarian cancer, the serous group had more frequent chromosomal imbalances than nonserous cancers and discrete copy number anomaly were identified at 11 and 12. Moreover, in a serous group of cancers, the most common anomaly was the addition of 1q and 8q and deletion of 8p and 17p.

The CGH is used to detect copy number changes by replacing metaphase chromosomes with a high resolution (10 Mb for detecting deletion and not less than or equal to 3 Mb for high-level amplifications) through hybridization target mapped with sequenced clones. The high resolution of array CGH allows us to detect copy number changes plotted onto glass slides, governed by the size and density of the nucleotide sequences which are determined by fine-mapping with the specific determination of the boundaries and amplitude.[40]

DNA methylation

DNA methylation is linked with gene expression through its epigenetic mechanism. A cytosine residue of CG (CpG) dinucleotides is the area where the DNA methylation occurs. DNA modification occurs predominantly on guanosine followed by cytosines in the DNA sequence. These CpG dinucleotides are linked with promoter regions and are usually clustered in small segments of DNA termed CpG islands. In the promoter region of a gene, location of CpG island for a given stretch of cytosines which is methylated, such a region would be termed as ‘hypermethylated (silenced by methylation). On the other hand, in a CpG island when a given stretch of cytosines in the promoter region of a gene is not methylated, in this case, it would be ‘hypomethylated (not silenced by methylation).[41]

In ovarian cancer, gene promoters where anomalous methylation of CpG islands occur is associated with loss of gene expression, DNA methylation provides a substitute pathway which is linked with the functional loss of TSG resulting in gene deletion.[42]

One of the most studied TSG associated with ovarian cancer in *BRCA1* and *BRCA2*. In the case of ovarian cancer, numerous other conventional TSGs endure hypermethylation. Ovarian tumors with discrete carcinogenic mechanism have TSGs that are involved in DNA mismatch repair (MMR). Germ-line mutations in the hMLH1, hMSH2, MGMT genes results in defective MMR.[43]

Oncogenes, DNA satellites, and DNA reparative elements mainly result in DNA hypomethylation. In addition to the hypermethylation (promoter-associated CpG islands), overexpression of protein expressed genes resulting from global hypomethylation and specific hypomethylation plays a significant role in ovarian cancer. Hypomethylation in the centromere disrupts the similar elements through gene transcription at chromosomal translocations further leading to genomic instability.[44] Hypomethylation is increased from normal tissue to ovarian cancer as well as it has increased in an advanced stage. Hypomethylation also increases from normal tissue to low grade to high-grade ovarian cancer.[27]

Specktrokaryotyping

Specktrokaryptyping showed both simple numerical, structural, and complex aberrant changes involving the use

of cytogenetic analyses of ovarian carcinomas. Analysis of ovarian carcinomas by conventional cytogenetic methods cannot determine the highly abnormal karyotypes with conformity.

Cytogenetic study analysis showed an independent deleterious effect related to ovarian carcinomas with chromosomal aberrations on 1p1 and 3p1. The major drawback of this study was difficulty in identifying specific recurrent structural aberrations in a very large chromosomal region containing numerous genes. Subsequently, with the advancement in the molecular cytogenetic study (i.e. spectral karyotyping) over molecular studies in identifying recurrent chromosomal alterations, there has been a landmark achievement in identifying the disruption or breakpoints in the chromosomal regions containing various genes.[42]

Conclusions

Various tumor biomarkers have become important in the management of ovarian cancer. These markers have been useful in the early diagnosis and treatment, early prognosis, and detecting recurring diseases. The single tumor markers always have limited sensitivity and specificity for differentiating benign and malignant lesions. Hence, to overcome the limited sensitivity and specificity, molecular markers play an important role in assessing risk at an earlier stage and differentiating benign and malignant tumor among high-risk patients. Various combinations have proven to be useful in improving the sensitivity and specificity of serum or urine markers for the early detection of invasive ovarian cancer as ovarian cancers have differential expression of various biomarkers. The review highlights the newer molecular approaches for ovarian cancer that will improve patient compliance, early screening, and detection that would decrease morbidity and mortality. Further research needs to be done to identify and explore newer markers with increased sensitivity, specificity, cost-effective and painless procedure with early detection of the malignant lesion.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Received: 28 Feb 19 **Accepted:** 12 Sep 19
Published: 03 Sep 20

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