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# Recent Diagnostic and Biomarker Development for Cervical and Breast Cancer: An overview

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## ABSTRACT

Biomarkers are considered as tremendous gift of modern biomedical sciences to improve the outcome for people with cancer by enhancing detection and treatment approaches. Biomarkers could include a broad range of biochemical entities, such as nucleic acids, proteins, lipids, small metabolites, as well as whole cells or biophysical characteristics of tissues. Detection of biomarkers can be accomplished by a wide variety of methods, ranging from biochemical analysis of blood or tissue samples to biomedical imaging. The primary focus of this review article is to find out the role of biomarkers as invaluable tools for cancer; specially breast and cervical cancer detection, diagnosis, patient prognosis and treatment selection. Besides, the future trend of biomarker development for these two types of cancer is also discussed.

**Keywords:** Biomarker, Cervical cancer, Breast cancer, Diagnostics, HPV.

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# INTRODUCTION

If we look for 'Cancer Biomarker' in Google search engine, we will find almost 38, 60,000 results which clearly indicate that now-a-days, Cancer biomarker has become a buzz word in the field of biological sciences. A biomarker is defined as any characteristic that can be objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological response to a therapeutic intervention.[1] Biomarkers are considered as tremendous gift of modern biomedical sciences to improve the outcome for people with cancer by enhancing detection and treatment approaches. Biomarkers could include a broad range of biochemical entities, such as nucleic acids, proteins, lipids, small metabolites, as well as whole cells or biophysical characteristics of tissues. Detection of biomarkers can be accomplished by a wide variety of methods, ranging from biochemical analysis of blood or tissue samples to biomedical imaging.[2] For many years; research has been carried out in the field of oncology to identify ways of diagnosing cancers at an early, curable stage or to select the optimal therapy for individual patients. The primary focus of this review article is to find out the role of biomarkers as invaluable tools for cancer; specially breast and cervical cancer detection, diagnosis, patient prognosis and treatment selection. Besides, the future trend of biomarker development for these two types of cancer is also discussed.

**Global Burden of Breast and Cervical Cancer**

Cancers of the breast and cervix kill more women than any other forms of cancer in all parts of the developing world. While in the past maternal deaths dominated reproductive mortality in low and middle-income countries, in most countries of Asia and Latin America and some countries of Africa, deaths due to the complications of pregnancy are now outnumbered by deaths from breast or cervical cancer.[3]

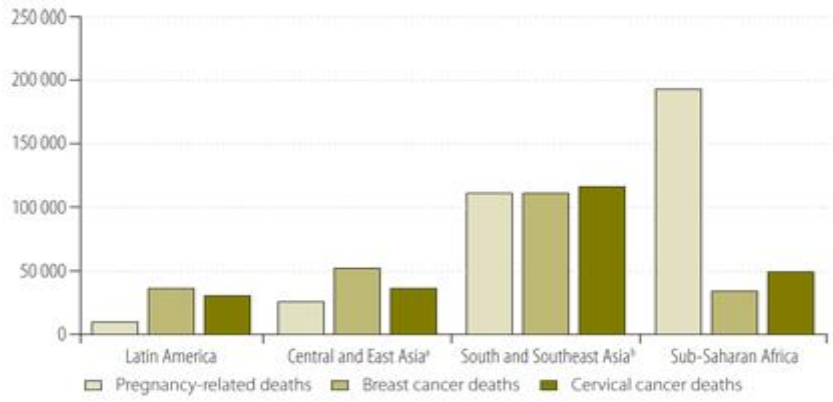


**Figure-1: Deaths from cancers of the breast and cervix compared with pregnancy-related deaths in low- and middle-income countries in four geographical regions, 2008.[3]**

The causes of breast and cervical cancer are related, at least in part, to a woman's sexual and reproductive choices and other exposures in early life – i.e. history of infection with the human papillomavirus (HPV), age at first pregnancy and number of pregnancies, breastfeeding history, diet and physical activity. However, the same reproductive factors that protect against one form of cancer increase the risk of the other form. Women who have early and frequent pregnancies and who breastfeed their children have a lower risk of getting breast cancer but are at increased risk of developing cervical cancer.[3] According to GLOBOCAN, in developing countries 690 000 new cases of breast cancer and 450 000 new cases of cervical cancer occurred in 2008. It is estimated that, by 2030, the incidence rate of breast and cervical cancer would increase to 1.1 million and 730 000 respectively. [30] Developed countries on the other hand, have achieved significant successes in reducing cervical cancer burden over the past six decades, and with annual incidence rates between 4 and 14 per 100,000.Cervical cancer is no longer ranks even among the top ten cancers in these countries. [31] Despite the common misconception that breast cancer is primarily a problem of high-income countries, in 2010 the majority of the world's 425 000 deaths from breast cancer occurred in developing countries (as defined by Forouzanfar et al.)[32]

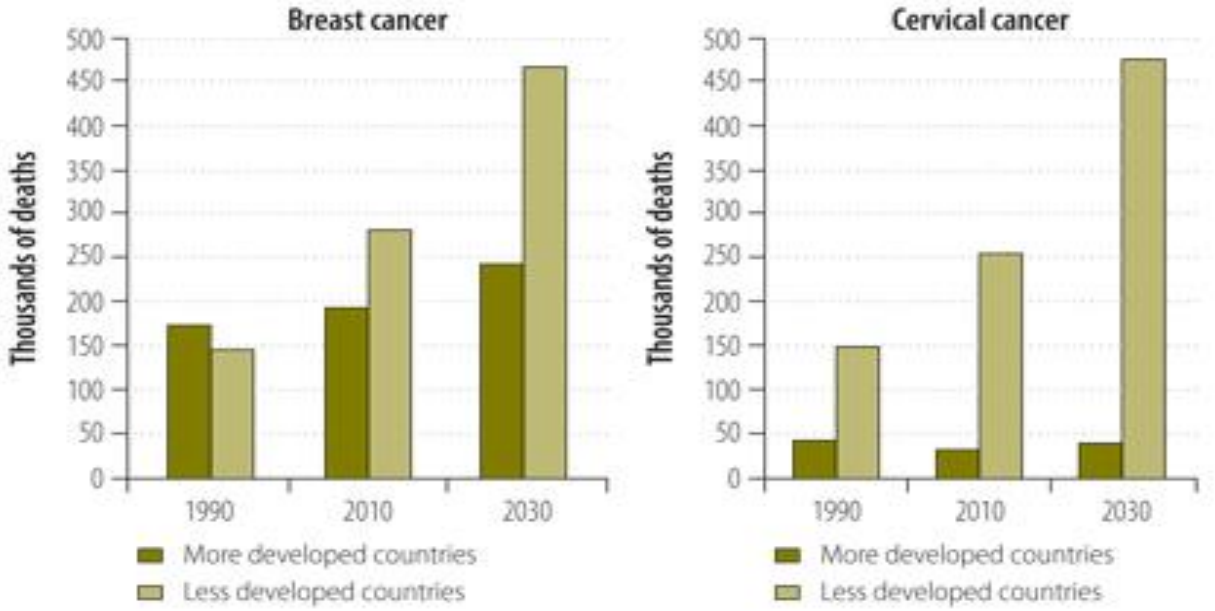


**Figure-2: Estimated global deaths from breast and cervical cancer, by country level of development as defined by GLOBOCAN, 1990, 2010 and 2030. [32]**

In developed settings, the ratio of the number of cervical cancer deaths to the number of new cases was 0.27 in 2008, whereas in less developed regions it was 0.53 and in sub-Saharan Africa it was 0.67. Similarly, the ratio of breast cancer deaths to new cases was just 0.22 in the United States, whereas in less developed regions it was 0.39 and in sub-Saharan Africa it was 0.54.[31]

# MOLECULAR CAUSES OF CERVICAL CANCER

**Human Papillomavirus as the causative agent of Cervical Cancer**

Dr.Haraldzur Hausen and his team in between 1980 to 1982 published their discovery of human papillomavirus (HPV) type 16 and 18 DNAs in cervical cancer cases considered as one of the significant breakthrough in the infectious disease field. Over the last two decades, the HPVs have been well characterized as causative agents for cervical cancer. Viral DNA from a specific group of HPVs can be detected in at least 90% of all cervical cancers and two viral genes, E6 and E7, are invariably expressed in HPV-positive cervical cancer cells.[1] The E6 and E7 gene products target a plethora of cellular functions, with the most important interactions being the inactivation of pRB by E7 and the degradation of p53 by E6. [2] E6 promotes the degradation of tumor suppressor p53 through its interaction with E6AP, an E3 ubiquitin ligase, whereas E7 binds to the retinoblastoma protein (pRB) and disrupts its complex formation with E2F transcription factors. [3] In addition, one function of E6 is to activate telomerase, and E6 and E7 cooperate to effectively immortalize human primary epithelial cells. Though expression of E6 and E7 is itself not sufficient for cancer development, it seems to be either directly or indirectly involved in every stage of multi-step carcinogenesis.[4]

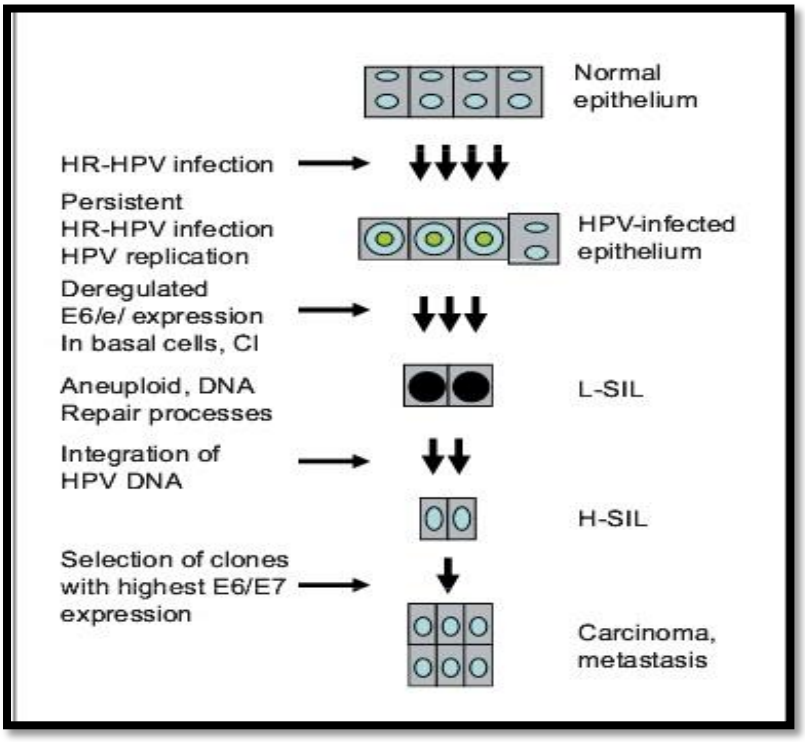


**Figure-3: The process of HPV Carcinogenesis [4]**

Infection by HPV requires that virus particles gain access to the epithelial basal layer and enter the dividing basal cells. Although the exact mechanism by which the viruses gain entry is not

completely known but it can be assume that Heparansulphate proteoglycans may play a role. Other secondary receptors such as α6-integrin have been proposed also.[5] Some literatures suggest that HPV-16 penitrate the cell through endocytosis.[6, 7] In case of productive infection, the viral DNA remain stable and not integrated into the host's genome. Here, E2 protein play important role in initiating viral replication and genome segregation. [8] In addition, E2 protein also act as a transcription factor and regulate the viral early promoters (p97 in HPV16; p99 in HPV31) and control expression of the viral oncogenes (E6 and E7).[4] Meanwhile, E7 protein contribute in disrupting the association between pRB and E2F (transcription factors), irrespective to the presence or absence of external growth factors. E2F subsequently trans activates cellular proteins required for viral DNA replication such as cyclins A and E. E7 also associates with other proteins involved in cell proliferation, including histone deacetylases,[9] API transcription complex10 and the cyclin dependent kinase inhibitors p21 and p27. It appears that the ability of E7 to drive cells through mitosis in differentiating epithelium may be limited to those cells that express p21 and p27 at low levels or express sufficient E7 to overcome the block to cell-cycle progression. This is important given that deregulated expression of the viral oncogenes is a predisposing factor in the development of HPV-associated cancers.[11]The continuous activity of E6 and E7 proteins leads to genomic instability, accumulation of oncogene mutations, further loss of cell-growth control and ultimately cancer.[4]

**Molecular Causes of Breast Cancer**

Breast cancer can be a perfect example of diseases caused by genetic mutation. About 10% of the breast cancer cases are due to the mutation in two genes named: BRCA1 and BRCA 2. [12] Most breast cancers that occur in women with germ line BRCA1 mutations are estrogen receptor-negative (ER-) and typically lack expression of progesterone receptor (PR) and human epidermal growth factor receptor (HER) 2 over expression (so-called 'triple-negative 'breast cancers).[13] Human epidermal growth factor receptor 2 (HER2) is a gene that produce HER2 protein that regulates the breast cell repairing and growing. If the HER2 gene mutates, it causes an uncontrolled increase in HER2 protein. This causes cells to grow and divide out of control, which may lead to cancer. In about one of every five (20 percent) of breast cancer cases, HER2 genes don't function correctly.[14] The mortality of breast cancer Patients may vary depending on the presence or absence of hormone receptors. Breast cancer patients with tumors that are estrogen receptor (ER)-positive and/or progesterone receptor (PR)-positive have lower risks of mortality after their diagnosis compared to women with ER- and/or PR-negative disease. [15] In a study carried by Arteaga and colleagues addressed the role of mutations in the phosphatidylinositol 3-

kinase (PI3K) pathway in breast cancer progression. PI3K serves as a major signaling hub downstream of HER2 and other receptor tyrosine kinases and mutations in the genes constituting this pathway occur in >70% of breast cancers, making it the most frequently mutated pathway in this disease.[16] Among the epigenetic alterations descried in breast cancer, DNA promoter methylation has been extensively studied and observed in genes involved in several critical signaling pathways that initiate and promote breast tumorigenesis. In addition to DNA hypermethylation, global methylation levels have been observed to decrease with breast cancer progression. In close connection with DNA methylation changes, histone modifications, especially the acetylation and methylation of histone lysines, play an essential role in the nucleosomes remodeling and gene expression regulation in breast cancer.[17]

**Molecular Diagnostics and Biomarker for Detecting Cancer**

Molecular diagnostics is a technique used to analyze biological markers in the genome and proteome—the individual's genetic code and how their cells express their genes as proteins—by applying molecular biology to medical testing. [18] As an intrinsic part of DNA technology, molecular diagnostics are rooted in the April 1953 discovery of the DNA double helix. In 1976, for the first time of history, Kanand his colleagues carried out, prenatal diagnosis of a Thalassemia, using hybridization on DNA isolated from fetal fibroblasts. Also, Kan and Dozy, in 1978, applied Restriction Fragment Length Polymerase (RFLP) analysis to pinpoint sickle cell alleles of African descent. These diagnostic techniques provided the means of establishing similar approaches for the identification of other genetic diseases, like phenylketonurea, cystic fibrosis and so on.[19] Cancer is a collection of more than 100 different diseases, and, for many cancers, the molecular characteristics have not been fully classified. The diagnosis of cancers is still based largely on morphological examination of tumor biopsy specimens, as it has been for decades, but this approach has significant limitations for predicting a given tumor's potential for progression and response to treatment. The first example of cancer biomarker is the presence of Bence Jones Protein in Urine of the malignant myeloma patients and tumor specific antigen carcinoembryonic antigen (CEA) in colon carcinomas.[20] For the classification of cancer, Lander's group use genomic signatures as a biomarker.[21] A long-standing goal of cancer research has been to identify the molecular mechanisms of cancer development and designing molecular markers for the early detection and to target those mechanisms with drugs specifically designed to attack them. For example, a current advancement in the field of breast cancer is, measuring the expression of the estrogen receptor for prognosis and identifies women who are likely to benefit from anti-estrogen therapy.[22]Furthermore, considering the over expression of HER2 (a growth factor receptor) in

breast cancer as a biomarker for prognosis and for treatment with different chemotherapeutic drugs is another significant improvement in molecular diagnosis and biomarker fields.

| DATE | DISCOVERY |
|---|---|
| 1949 | Characterization of sickle cell anemia as a molecular disease |
| 1953 | Discovery of the DNA double helix |
| 1958 | Isolation of DNA polymerases |
| 1960 | First hybridization techniques |
| 1969 | In situ hybridization |
| 1970 | Discovery of restriction enzymes and reverse transcriptase |
| 1975 | Southern blotting |
| 1977 | DNA sequencing |
| 1983 | First synthesis of oligonucleotides |
| 1985 | Restriction fragment length polymorphism analysis |
| 1985 | Invention of PCR |
| 1986 | Development of fluorescent in situ hybridization (FISH) |
| 1988 | Discovery of the thermostable DNA polymerase – Optimization of PCR |
| 1992 | Conception of real time PCR |
| 1993 | Discovery of structure-specific endonucleases for cleavage assays |
| 1996 | First application of DNA microarrays |
| 2001 | First draft versions of the human genome sequence |
| 2001 | Application of protein profiling in human diseases |

**Figure-4: The key breakthroughs in the field of molecular biology, which influenced the development of Molecular Diagnostic system later. [19]**

**Classification of Cancer Biomarkers**

Efforts have been made in the recent past to define and classify Cancer Biomarkers in different approaches however scientists could not able to come in a general agreement yet. Broadly, any biologically derived entity or processes which lead to a cancer diagnosis (in prognosis, screening and risk assessment), at the stage of diagnosis or post diagnosis (in therapy and treatment module) are potential candidates as cancer biomarkers.[23] Various markers are currently being investigated for their ability to guide treatment decision-making and management. There are known as Predictive and Prognostic biomarkers.[24] Predictive biomarkers are sometimes referred as response markers when they are used to assess the administering drug. For instance, Herceptin® is useful in breast cancer lesions showing only Her2/Neu over expression, whereas tamoxifen is the preferred treatment for other breast cancer lesions. Thus Her-2/Neu is a predictive cancer biomarkerfor a

subset of breast cancer therapies.[25] A diagnostic cancer marker can be stage, tissue, relapse, follow-up and age specific.[23] HPV is considered to be a diagnostic cancer biomarker for uterine and cervical cancers as it is present in >90% cancer lesions.[26] Many of the biomolecules like; DNA, RNA, Protein are currently being used as important biomarkers. Cell-free circulating DNA carries not only tumor-specific changes in its sequence but also distinctive epigenetic marks, namely DNA methylation, in certain GC-rich fragments. These fragments are usually located within the promoters and first exons of many genes, comprising CpG islands. Analysis of DNA methylation using cell-free circulating DNA can facilitate development of very accurate biomarkers for detection, diagnosis, prediction of response to therapy and prognosis of outcomes. Recent data suggest that benign and inflammatory diseases have very specific methylation patterns within cell-free circulating DNA, which are different from the pattern of a malignant tumor of the same organ. In addition, specific methylation patterns have been detected for cancers of different organs, so a differential diagnosis of site-specific cancer appears feasible.[27] with the advent of transcriptomic technologies such as microarrays and next-gen RNA sequencing, RNA biomarkers have grown exponentially and hold great promise for a wide range of research and clinical applications. Overtime, many micro RNAs (miRNA) are found to have links with several types of cancer. That is why they are sometimes referred to as 'Oncomirs'. Cell-free miRNA are highly stable in blood, are over expressed in cancer, and are quantifiable within the diagnostic laboratory. An important feature of miRNA is that, their expression profiles can be used to classify human cancers and establishing a correlation between disease prognosis and therapeutic outcome.[28]

**Table 1: Classification of Cancer Biomarker**

| Based on Disease State | Based on Bio-molecules | Based on Other Criteria |
|---|---|---|
| Prediction Biomarkers | DNA Biomarkers | Imaging Biomarkers |
| Detection Biomarkers | RNA Biomarkers | Pathological Biomarkers |
| Diagnosis Biomarkers | Protein Biomarkers | In Silico Biomarkers |
| Prognosis Biomarkers | Glyco Biomarkers | |

**Biomarkers for Cervical Cancer**

The most promising cytological biomarkers for cervical cancer screening are p16(INK4a)/Ki-67 dual immunostaining, methylation of CADM1 and MAL and viral integration. [33] Hypermethylation of three genes, MYOD1, CDH1, and CDH13, was observed in sera of cervical cancer patients as determined by MethyLight technology of methylation analysis.[34] Presence of Papilomavirus genome itself is a risk factor for Cervical Cancer.In several studies, Methylation of HPV16 and HPV18 specific genes has also been reported. Preliminary reports indicate hypermethylation of

SPARC, TFPI2, RRAD, SFRP1, MT1G, and NMES1 genes in samples isolated from cervical cancer patients. [35, 36, 37] Some predominant biomarkers that are useful in various stage of cervical cancer development are discussed below

**p16(INK4a):** This biomarker is a cyclin-dependent kinase inhibitor which significantly over expressed in cancerous and precancerous cervical tissues. It has markedly correlation with viral oncoprotein E7 which disrupts pRb, a key regulatory factor. The disturbance of the pRb pathway leads to a compensatory over expression of p16ink4a through a negative feedback loop. [38] In a consequence, over expression and cellular accumulation of p16ink4a happen which is a specific marker of cervical precancerous lesions and can be measured through immune cytochemical staining of histology and cytology slides and using ELISA (Enzyme-linked Immunosorbent Assay). There is substantial quantitative and morphological heterogeneity in methods for defining p16ink4a positivity.[39]

**E6/E7 Oncoprotein:** When HPV infection moves from transient to transforming stage the E6/E7 mRNA and protein expression goes high.[40] Hence, multiple studies suggests the detection of these mRNA as the identifying tool of cervical pre-cancer stage.[39] DNA-based assays show high sensitivity but poor specificity in detecting high-grade cervical lesions. On contrary, mRNA based assays of the oncoproteins E6 and E7 show higher specificity but lack either detection of all high-risk HPV genotypes or the capacity to specify the detected genotypes.[41] A study was carried out where the mRNA of eight most prone genotypes in cervical cancer (HPV16, -18, -31, -33, -35, -45, -52, and -58) were analyzed. The result suggested that, mRNA testing with real-time PCR may be a useful tool in investigation of as well as in primary screening for cervical neoplasias.[42]

**Retinoblastoma protein Cytokeratin -CK14 and CK13:** This special type of protein help in the assessment of an individual Cervical intraepithelial neoplasia (CIN's) lesion's potential for progression and regression.[43] This cytokeratins are also known as the risk signature for Primary lung squamous cell carcinomas (SCC).[44]

**Markers of aberrant S-phase induction**

The cell cycle activation caused by HPV oncogenes in changing infections is characterized by aberrant S-phase induction.[45] An assay detecting two proteins indicating aberrant S-phase induction, topoisomerase IIA (TOP2A) and mini chromosome maintenance protein 2 (MCM2) is commercially available (ProEx™ C by Becton Dickinson)[46]

**Potential Biomarkers for Cervical Cancer**

**PAX1 methylation:** PAX1 genes isolated from the DNA of the cervical scrapings have great potential as a biomarker for cervical cancer screening. When incorporating PAX1 detection into

current screening protocol, the efficacy of screening could be greatly improved. A recent study in Yuan's General Hospital (2014) reported that, PAX1 genes from the Cervical scrapings of the patients had a sensitivity and specificity of 86% and 85%, respectively, whereas when used as a co-test with the Papanicolaou (Pap) test, the sensitivity and specificity were 89% and 83%, respectively. It also reduce the unnecessary referral for colposcopy and biopsy up to 60%.[47]

**Serum microRNA-205:** miR-205 is significantly over expressed in human cervical cancer tissues and promotes proliferation and migration of cervical cancer cells.[48] Besides, it also act as an oncogene by modulating the expression of multiple cancer-related target genes. [49] A study reported that, patients with high serum miR-205 levels had a significantly lower survival rate than those with low expression levels, and serum miR-205 was an independent risk factor for poor prognosis.[50] These results suggested that serum miR-205 could be used as a potential predictor of prognosis in cervical cancer.

**Other Biomarkers that need clinical validation:** Other cellular makers such as MCM5 and CDC6, Survivin and CEA have also been evaluated in various stages of cancer cell development. Most of them are marked by non-uniformity in determination of end points and limited sample sizes. [39] Although some of the biomarkers are very promising for this purpose, no studies have evaluated how accurately these biomarkers classify or predict the outcome. Additional clinical trials are needed to determine the true clinical value of these promising cytological biomarkers.

## FUTURE TREND OF CERVICAL CANCER BIOMARKER

Cervical cancer prevention is at a transition from cytology-based screening programs to HPV-based prevention. With primary prevention using vaccines and secondary prevention using a highly sensitive HPV DNA test with long-term negative predictive value at hand, extending screening intervals will be crucial for these programs to work. New biomarkers will be important to decide who among the HPV-positive women needs to be referred for further evaluation or treatment. Large studies are currently underway for various triage biomarker candidates. It can be expected that the first screening programs based on primary HPV testing and new biomarkers as secondary tests will be implemented in a few years. It will be important to reserve treatment for those women who are at risk of developing cancer, rather than treating any high-grade lesion. Prospective biomarkers may play an important role in these therapy decisions. The implementation of new prevention strategies will be very different in each healthcare setting; in a few years, we can expect to see a wide variety of cervical cancer prevention programs existing in parallel.

**Biomarkers for Breast Cancer**

For many years, in addition to the conventional clinical prognostic factors of breast cancer, established molecular biomarkers such as estrogen receptor and progesterone receptor have played a significant role in endocrine therapy. Estrogen receptors' expression is well established as biomarker because of its ability to provide the index for sensitivity to endocrine treatment. While the absence or presence of the ER is used to obtain treatment decisions, little attention has been paid on the value of the quantitative expression levels as a predictive indicator. [51] Absence of Progesterone receptor expression is also a powerful, independent prognostic variable in primary breast cancer even in ER-positive cases.[52] The expression of the PgR is strongly dependent on the presence of ER. Tumors expressing PgR but not the ER are uncommon and represent <1% of all breast cancer cases in some large series. For this reason, tumors with PgR expression lacking ER expression should undergo a retesting of their ER status to eliminate false ER negativity.[53]

**HER2**

Around the year 1982-84, new oncogene is discovered by a group of scientists at Massachusetts Institute of Technology (M.I.T) (R.A. Weinberg Group), Rockefeller and Harvard University. This gene is also known as Her2, ErbB2 or p185(for encoding a phosphoproteinof 185,000 dalton). [54] HER2 stands for Human Epidermal growth factor Receptor. HER2 receptors are found on the surface of some normal cells but in HER2 breast cancer there is a much higher than normal amount on each cell. This produces too much HER2 protein on the cell which is thought to cause the cancer cells to grow and divide rapidly.[55]The HER2 gene encodes a transmembrane tyrosine kinase receptor. Activation of this receptor occur in a ligand-independent way particularly when the receptor is mutated or over expressed.[55]The HER2 status can be determined by histological tests or ELISA method that measures the HER2 protein level in the blood serum. Among the tissue test, the immune histo chemistry (IHC) and Fluorescence In Situ Hybridization (FISH) are the two tests approved by FDA (Federal Drug Administration).[56] Various reports suggest that there is a strong correlation between changes in serum HER2/neu levels and the clinical course of patient's metastatic breast cancer, regardless of their treatment regimen. [57] The overall correlation between clinical course of disease and serial changes in the circulating serum HER2/neu is approximately 86 %.[58] Inspired by the results, within the last few years, many more promising agents targeting HER2 have been designed and developed including monoclonal antibodies and tyrosine kinase inhibitors.

**BRCA 1/BRCA 2**

*BRCA1*germline mutations have been correlated to the increasing risk of developing breast cancer.[59] Hence,*BRCA1* located in chromosome 17q21, has been identified as a breast cancer

susceptibility gene.[60] This gene encodes a nuclear phosphoprotein that plays a role in maintaining genomic stability, and it also acts as a tumor suppressor. The encoded protein combines with other tumor suppressors, DNA damage sensors, and signal transducers to form a large multi-subunit protein complex known as the BRCA1-associated genome surveillance complex (BASC). This gene product associates with RNA polymerase II, and through the C-terminal domain, also interacts with histone deacetylase complexes. This protein thus plays a role in transcription, DNA repair of double-stranded breaks, and recombination. Mutations in this gene are responsible for approximately 40% of inherited breast cancers and more than 80% of inherited breast and ovarian cancers.[61]

**Ki67**

Ki67 is a non-histone protein that expressed in proliferating cells and absent in quiescent cells. This unique property makes Ki67 as an emerging marker of cancer cell proliferation.[62] Although the aggressiveness of cancer cells mostly correlated with tumor grade and Ki67 indexing, Ki67 is still not recommended regularly for determining prognosis in newly diagnosed breast cancers due to its lack of reproducibility.[63] Difference studies have been carried out to evaluate the correlation of Ki67 with other biomarkers. Ki67 has been found to have strong relation with tumor grading.[64]The relationship with Estrogen Receptor has been predominantly described as an inverse correlation with lower proliferative activity in ER-positive tumors.[65] However, A common debate about **Ki67** is the standardization and categorization of this protein for prognosis tests.

**Estrogen receptor**

Estrogens are reported to involved in the promotion of human breast cancer. Estrogen receptors are one of the most important biological factors in the field of oncology. ER biomarkers work effectively as predictive biomarker as there is strong correlation between the level of expression of ER and the response of endocrine therapy. ‘Estradiol’- an estrogen receptor protein that act as mitogen for the cancer cells, is subjected to endocrine therapies hence act as effective biomarker. The absence of estrogen receptors are considered as a positive indication of chemotherapy.[69] The estrogen receptor (ER) is the target for the drugs tamoxifen and fulvestrant. In spite of this receptors’ importance as a therapeutic target, ER-negative tumors have a more aggressive character and a different metastatic pathway than ER-positive tumors.[71]Thus, the proteins that are negatively regulated by ER may constitute biomarkers and therapeutic targets for breast cancer. Higher levels of ER are associated with a lower risk of recurrence when receiving adjuvant tamoxifen. ER-negative breast cancer cases are more likely to gain a pathological complete response with neoadjuvant chemotherapy than the ER-positive patients, with response rates of 7–8

vs 21–33% respectively.[70] PGRMC1 (progesterone receptor membrane component-1), a hormone receptor component is found to be alleviated in the ER-negative tumor and can function as a predictive biomarker in breast cancer. Although, PGRMC1 is not a progesterone receptor but binds to P-450 proteins, an unknown steroid-binding protein, and PAIR-BP1 (plasminogen activator inhibitor mRNA-binding protein), and PGRMC1 is linked to pro-survival signaling in cancer.[71]

**Table 2: Putative estrogen receptor related breast cancer biomarkers [72]**

| S.I | Putative Biomarkers | ERα (+) | ERα (-) | Function |
|---|---|---|---|---|
| 1 | 611- CTF | - | + | Resistance to treatment |
| 2 | FKBPL | + | - | Regulation of ER expression |
| 3 | BP1 | - | + | Cell Proliferation |
| 4 | Superoxide Dismutase | + | - | Anti-oxidant |
| 5 | Ral A Binding Protein | + | - | Tumorigenesis-Metastasis |
| 6 | Galectin-1 | + | - | Apoptosis |
| 7 | UridinePhosphorylase 2 | + | - | Contributes to drug efficacy |
| 8 | Cellular retinoic acid binding protein 1 | + | - | Cell growth and differentiation |
| 9 | Protein S100-A11 | + | - | Tumorigenesis |
| 10 | Nucleoside Diphosphate Kinase A | + | - | Metastasis Suppressor |
| 11 | Rho GDP-Dissociation inhibitor 1 | - | + | Resistance to drugs |

**Progesterone receptor**

Expression of the progesterone receptor (PR) is heavily dependent on estrogen and rarely seen in estrogen receptor–negative tumors. Metastatic disease that expresses both estrogen receptor and progesterone receptor responds better to anti-estrogen therapy than that which is estrogen receptor positive but progesterone receptor negative.[71] The progesterone receptor can be a very good therapeutic guide for early stage breast cancer. One study suggested that, PR expression is independent prognostic variable. The study reached for the association of PR expression with different issues and found that, PR expression is significantly associated with overall survival, breast cancer specific survival and disease free survival ($p<0.0001$).[73] Genome-wide analysis by a study identifies hundreds of genes that are significantly more prognostic than PR in ER+ breast cancer, suggesting that other candidate prognostic biomarkers are likely to outperform PR for predicting patient survival in ER+ breast cancer. Moreover, there are still not sufficient evidence to establish that PR is a competitive predictive marker in ER+ breast cancer comparing other predictive biomarkers.[74]

## OTHER EMERGING BIOMARKERS FOR BREAST CANCER

A new type of combination biomarker with different novel factors (e.g, ER, PgR, HER2, and Ki67) whose can distinguish between different subtypes of breast cancer is under evaluation phase.[61]Now-a-days,candidate-marker approaches and genome-wide epigenetic and transcriptomic

screening of different breast cancer tissues and body fluids provided new promising biomarker panels, allowing breast cancer prognosis and monitoring of therapy efficacy. These biomarkers are now under clinical trials.[66] About 15% of the women around the world diagnosed as "Triple-Negative Breast Cancer" (TNBC). This TNBC cases are rare and in this type of cancer, the patient is not responsive to therapies targeting HER2, estrogen and progesterone receptors. BCL-2, an isofom protein can categorized the TNBC cases in to two groups: lower risk with expression and higher risk without expression. This protein biomarker can enable the clinicians to analyze someone's tumor tissue before treatment and see how they will respond.[67] Apart from that, there are some other emerging biomarkers like Cyclin D1, Cylcin E etc. Cyclin D is a member of the cyclin protein family involved in regulating cell cycle progression. The synthesis of cyclin D is initiated during G1 and drives the G1/S phase transition.[75]This are over expressed at the mRNA and protein level in over 50% of breast cancer cases including 15% in which a gene amplification occurs.The cyclin E protein amplified in the breast cancer cell line. They are proved to have an important role in tumorigenesis.[76] Besides, DNA methylation has shown promise as a potential biomarker for early detection, therapy monitoring, and assessment of prognosis or prediction of therapy response. In particular, paired-like homeodomain transcription factor 2 (PITX2) DNA methylation has been validated using a robust assay for paraffin-embedded tissue for clinically relevant outcome prediction in early breast cancer patients treated by adjuvant tamoxifen therapy.[77]

## CONCLUSION

The great technological advancement in the past decade provides the researchers the opportunity to characterize the molecular basis of cancer. Researchers are now able to design drugs targeted at specific molecules. New diagnostics, or biomarkers, for cancer are now urgently needed to determine which of these new drugs a patient is most likely to benefit from. Similarly, more cost effective and efficient biomarkers are now needed for better prediction and diagnosis.[78]For example, the circulating micro RNA (miRNA) are now getting attention of the researchers for detecting breast cancer as the process is convenient and the results are more specific. Future DNA-based screening tests might detect methylation or other epigenetic modifications of DNA that occur specifically in cancer. There is a possibility that in near future the concentration of research might shift from cancer infected organs to the signaling pathway.[79]Since biomarker research advances towards personalized medicine, the potentiality relies on identifying the population that would respond to the drug. Emphasis should be givento develop biomarker assay and methods of using these markers need to be validated to eliminate the odds. In future,

biomarker research may focused on the transition of biomarkers from the development phase to clinical applications for drug trials.[80]

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