International Research Journal of Pharmacy and Medical Sciences
ISSN (Online): 2581-3277

Exploring Blood-Based Methylation of BRCA Genes in Breast Cancer: Advances, Applications, and Challenges

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Abstract*—Breast cancer is a leading cause of cancer-related deaths in women worldwide. While BRCA1 and BRCA2 gene mutations are well-established risk factors, epigenetic alterations—particularly promoter hypermethylation—have emerged as critical mechanisms in breast cancer development, especially in sporadic cases. This review highlights the role of DNA methylation in silencing BRCA1 and BRCA2 genes and focuses on its detection in peripheral blood samples such as plasma, serum, and blood cells. Multiple studies have reported a significant association between BRCA1 promoter hypermethylation in blood and breast cancer presence, including aggressive subtypes like triple-negative breast cancer (TNBC). Blood-based methylation analysis offers a non-invasive, accessible approach for early detection, risk prediction, and therapy monitoring. Techniques like methylation-specific PCR (MS-PCR) have demonstrated promising sensitivity but face limitations, including methodological variability and limited quantitative accuracy. Research also reveals conflicting results regarding the consistency and predictive value of BRCA methylation across populations. Therefore, while blood-based methylation of BRCA genes shows strong clinical potential, further standardization and large-scale validation across diverse populations are essential for its integration into routine breast cancer care.*

***Keywords*—** *Breast cancer, BRCA1, BRCA2, DNA methylation, promoter hypermethylation, blood-based biomarkers, epigenetics, early detection, non-invasive screening, TNBC.*

I. INTRODUCTION

Breast cancer (BC) is among the most common cancers worldwide and a leading cause of cancer-related death among women. It is a complex disease involving many factors such as genetics, environment, and lifestyle [1]. Globally, breast cancer represents one in eight cancer diagnoses, with over 2.3 million new cases and approximately 685,000 deaths in 2020 [2]. According to Globocan 2022, breast cancer accounted for 11.6% of all new cancer cases, with significant variation across regions due to differences in healthcare access and screening availability [2].

Breast cancer is a significant public health problem in India, where it accounts for 13.5% of new cancer cases and 10.6% of cancer deaths, particularly in southern states like Tamil Nadu and Telangana [3]. Risk factors for breast cancer can be categorized as unchangeable (genetic mutations, family history, early menstruation) and changeable (obesity, alcohol use, sedentary lifestyle, hormone therapy) [4]. Among genetic risk factors, mutations in BRCA1 and BRCA2 genes are crucial, as they significantly increase lifetime breast cancer risk [5][6].

II. BIOLOGICAL BACKGROUND

The BRCA1 and BRCA2 genes are tumor suppressor genes vital for repairing DNA damage, maintaining genomic stability, and preventing tumor formation [48]. The location of BRCA2 is chromosome 13q12-13 [7], while the location of BRCA1 is chromosome 17q21. Mutations in these genes lead to defective DNA repair, resulting in uncontrolled cell division and cancer. Women with BRCA1 mutations have up to an 80% lifetime risk of breast cancer, whereas BRCA2 mutations confer up to a 55% risk [8].

In addition to genetic mutations, BRCA genes can also be silenced through epigenetic mechanisms, notably DNA methylation. DNA methylation involves adding methyl groups to cytosines within CpG islands, especially in gene promoters. Promoter hypermethylation results in the loss of gene expression. This process commonly occurs in sporadic breast cancers and represents an alternative papthway to gene inactivation besides genetic mutations [9][10].

III. RESEARCH UPDATES

Recent studies have increasingly focused on detecting BRCA1 and BRCA2 methylation in peripheral blood samples (plasma, serum, and blood cells). This method provides a non-invasive way to detect breast cancer early and screen for it.

Promoter hypermethylation of BRCA1 is frequently reported in blood samples from breast cancer patients. Al-Moghrabi et al. (2011) detected BRCA1 methylation in 28% of breast cancer patients compared to 10.9% in cancer-free women [11]. Similarly, Iwamoto et al. (2011) found higher methylation frequencies in blood from breast cancer patients (21.5%) compared to controls (13.5%), highlighting a statistically significant association between blood methylation and breast cancer risk [12].

Ullagaddi Sahana P. and Kulkarni Bhushan B., "Blood-based Methylation of BRCA1 and BRCA2 Genes in Breast Cancer" *International Research Journal of Pharmacy and Medical Sciences (IRJPMS)*, Volume 8, Issue 4, pp. 53-55, 2025.

Wong et al. (2011) showed that women with early-onset breast cancer, lacking germline BRCA mutations, exhibited increased constitutional BRCA1 promoter methylation in blood, suggesting inherited epigenetic susceptibility [13]. Gupta et al. (2014) also demonstrated significant BRCA1 methylation in peripheral blood DNA of patients with triple-negative breast cancer (TNBC), a subtype particularly aggressive and hard to treat [14].

Contrasting findings exist, with Hassani et al. noting very low BRCA1 methylation rates (2.7%) in blood samples from breast cancer patients, indicating variability and the need for further investigation [15].

Research on BRCA2 methylation in blood samples remains limited. However, preliminary studies, such as Gacem et al. (2012), have indicated potential clinical relevance, noting associations between BRCA1/BRCA2 methylation and improved patient survival [16].

IV. Clinical Potential

Detecting methylation of BRCA1 and BRCA2 genes in blood samples has substantial clinical potential, especially for breast cancer screening, early detection, and prognosis.

As a less intrusive technique, blood-based methylation assays provide an advantage. Current breast cancer screening relies heavily on mammography, which can miss early-stage cancers in dense breast tissue [17]. Methylation markers could complement mammography by identifying high-risk individuals earlier.

Several studies highlight the potential of BRCA1 methylation as an early biomarker. Promoter hypermethylation occurs even in normal breast tissues adjacent to tumors, suggesting methylation changes occur early during cancer formation [18]. Thus, detecting these methylation patterns in blood could enable early intervention.

Moreover, BRCA1 methylation is associated with more aggressive breast cancer subtypes, such as TNBC, indicating potential use for prognosis and risk stratification [19]. Gupta et al. (2014) noted a significant association between peripheral blood methylation and specific breast cancer subtypes, reinforcing its prognostic value [14].

Circulating tumor DNA (ctDNA) methylation analysis further expands clinical possibilities. CtDNA, released from tumor cells into the bloodstream, allows real-time monitoring of cancer progression and treatment effectiveness [20][21]. Thus, BRCA methylation tests could guide personalized treatment strategies, tracking response to therapies and disease recurrence.

V. LIMITATIONS

Despite promising findings, several limitations affect the reliability and practical use of blood-based BRCA methylation tests.

Firstly, methodological issues persist. Methylation-specific PCR (MS-PCR), though highly sensitive, yields qualitative rather than quantitative results and may miss methylation heterogeneity across different CpG sites [22][1]. False positives due to improper primer design remain problematic, requiring rigorous validation protocols to ensure accuracy [23].

Secondly, study findings vary significantly, highlighting the need for large-scale validation. Differences in population genetics, environment, and lifestyle factors likely contribute to conflicting results. Most research has focused on Western populations, with limited data from regions like South Asia [19]. Thus, broader studies across diverse populations are necessary.

Thirdly, the clinical translation of these tests faces practical hurdles, including cost and accessibility of advanced techniques (like next-generation sequencing or pyrosequencing) [24][25]. The complexity and expense of such methods limit widespread implementation, particularly in resource-constrained settings.

Finally, while methylation testing holds promise, it remains uncertain whether these markers alone provide sufficient predictive accuracy to replace existing screening methods. Comprehensive evaluation alongside existing diagnostic tools is necessary to confirm their clinical value.

VI. Conclusion

Blood-based methylation detection of BRCA1 and BRCA2 genes represents a promising advance in breast cancer diagnostics, offering a non-invasive approach for early detection, risk assessment, prognosis, and treatment monitoring. BRCA1 promoter hypermethylation, in particular, has consistently shown potential as a valuable biomarker.

Although there is promise, there are still notable gaps in research and constraints. Methodological inconsistencies, population variability, and resource constraints hinder the widespread adoption of these tests. Moreover, further large-scale studies across diverse populations, including underrepresented regions such as India, are necessary to validate the utility of blood-based methylation assays.

Future efforts should prioritize refining detection methods, standardizing procedures, and assessing combined diagnostic approaches to maximize clinical accuracy and benefit. With these advancements, blood-based BRCA methylation testing could become an integral component of personalized breast cancer care, significantly improving outcomes through early detection and

Acknowledgment

The authors thank all participants, clinical staff, and the Dr. Prabhakar Kore Basic Science Research Centre (BSRC), KAHER for their support in conducting this study.

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