RESEARCH ARTICLE

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Evaluation of *BRCA1* Gene Promoter Methylation Status in Sporadic Breast Cancer Patients in Southwest of Iran

Hedieh Hassani[1,2], Abdolhassan Talaiezadeh[3,4], Maryam Tahmasebi Birgani[1], Mahdi Bijanzadeh[1,3]*

Abstract

Objective: Similar to other types of cancer, the development of breast cancer is a multi-stage process, consisting of various mutations and epigenetic changes in many genes. Mutations in the *BRCA1* gene, which is a tumor suppressor gene, are considered as the most important types of mutations. The pivotal role of epigenetics is currently considered as the primary key to carcinogenesis. Several studies have previously reported the *BRCA1* epigenetic silencing through promoter methylation in the pathophysiology of breast cancer cells. This study aimed to investigate whether the *BRCA1* gene promoter methylation in peripheral blood cells is correlated with the risk of breast cancer. **Methods:** In the current study, DNA samples were extracted from blood cells belonged to 74 patients with breast cancer as well as 30 healthy individuals, and the *BRCA1* gene promoter methylation status in these two groups was examined using Methylation Specific PCR (MSP). **Result:** out of 74 patients, 2 cases demonstrated methylation in their *BRCA1* gene promoter; however, none of the healthy controls demonstrated methylation status. Among these 74 patients, 13 cases were at the early stages (stage I), and two patients who had *BRCA1* gene methylation (15.4%), were in this group (p=0.02). While 34 and 27 patients were at stages II and III, respectively, showing a negative state of *BRCA1* gene methylation. **Conclusion:** Although 2 out of 74 patients resulted positive for methylation status, the healthy controls demonstrated no methylation. Consequently, there was inadequate evidence to confirm the association between *BRCA1* gene promoter methylation in blood and the risk of developing breast cancer.

Keywords: *BRCA1*- breast cancer- methylation- promoter- peripheral blood

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Introduction

Breast cancer is a multifactorial disease with a multi-stage process, which is associated with intricate variation in gene expression patterns, including the inactivation of tumor suppressor genes and the activation of oncogenes (Pang et al., 2012). Although the majority of breast cancer cases are reported to occur sporadically, about 5-10% of them are considered as inherited (Bosviel et al., 2012; Ferla et al., 2007). The Breast cancer type1 susceptibility protein (*BRCA1*) is a tumor suppressor gene placed on the long arm of chromosome 17 (17q21) and its germline mutations have been observed in 40-50% of hereditary breast cancer cases (Iwamoto et al., 2011; Bal et al., 2012; Zhang et al., 2015, Manchana et al, 2018). The *BRCA1* gene has several functions, including double-strand breaks repair, cell cycle regulation, transcription regulation, and ubiquitination activity (Starita et al., 2003; Yoshida et al., 2004). Although somatic mutations are rare in the *BRCA1* gene, there are ample evidence on the decreased *BRCA1* gene expression among sporadic patients (Bosviel et al., 2012; Daniels et al., 2016; Dobrovic et al., 1997; Zhang et al., 2015). In regard to sporadic patients, gene silencing via epigenetic mechanisms, is known as another mechanism for the inactivation of the *BRCA1* gene in cancer cells (Al-Moghrabi et al., 2014). It has been previously illustrated that methylation occurs in the *BRCA1* gene promoter in approximately 9-44% of patients affected with sporadic breast cancer (Bianco et al., 2000; Birgisdottir et al., 2006; Catteau et al., 1999; Dobrovic et al., 1997; Matros et al., 2005). Epigenetic regulation, especially promoter methylation in human cancer cells, is an important mechanism towards reducing the expression of tumor suppressor genes. As a consequence, abnormal hypermethylation in the genes promoter regions is recognized as an important and primary event during the tumorigenesis process (Bosviel et al., 2012; Wong

[1]*Department of Medical Genetics, Faculty of Medicine, Ahvaz Judishapur University of Medical Sciences, Ahvaz, Iran.* [2]*Student Research Committee, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.* [3]*Cancer Research Center of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.* [4]*Department of General Surgery, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. *For Correspondence: mbijanz@yahoo.com*

et al., 2011). Although the abnormal DNA methylation has been reported as one of the hallmarks of cancerous tissues, several studies have previously suggested that the methylation status of a gene in blood-derived DNA might be associated with a risk of developing breast cancer (Gupta et al., 2014; Hosny et al., 2016; Niwa et al., 2000; Parashar et al., 2018; Severi et al., 2014). According to the high readability and non-invasiveness characteristics of peripheral blood DNA, the assessment of white blood cells DNA, as a biomarker of breast cancers risk, is of particular interest (Brennan et al., 2012; Li et al., 2012). Despite the controversial findings reported in several studies, a considerable number of them have demonstrated that patients and healthy controls have various levels of DNA methylation in peripheral blood (Iwamoto et al., 2011; Kuchiba et al., 2014; Prajzendanc et al., 2020; Wojdacz et al., 2011). Several studies have illustrated that methylation of cancer-related genes, which are derived from white blood cells and breast tumor tissues, are two independent events. Meanwhile, DNA methylation of blood cell genes showed no direct relationship compared to somatic methylation in the case of same genes in tumor DNA. Accordingly, that DNA methylation of some certain cancer related genes might be only specific to peripheral blood DNA (Sturgeon et al., 2014; Wojdacz et al., 2011). The exact functional relationship among white blood cell DNA methylation, cancer tissue methylation status, and their pathophysiological mechanisms is not quite known yet (Tang et al., 2016). In order to assess the association of *BRCA1* gene promoter methylation status in peripheral blood cells of patients with breast cancer risk, we designed a comparison study among the methylation of the *BRCA1* gene promoter in the white blood cells of both the healthy individuals and the patients with breast cancer. If this association was reported as significant, the possibility of using the methylation status of *BRCA1* gene promoter, as a diagnostic biomarker, will be considered in peripheral blood of breast cancer patients.

Materials and Methods

Patients

In this study, 74 patients with breast cancer and 30 healthy individuals with no history of breast cancer in their families, were included. All the blood samples were collected from the Cancer Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran, before performing any radiotherapy or chemotherapy for them. In the current study, the patients' required pathological data, including tumor grade, hormone receptor status, lymph node involvement, metastasis and age at the time of diagnosis, were recorded from their clinical history surveys. Other information regarding menopausal status, menstrual status, and family history of breast cancer, as well as the patients' demographic characteristics, were collected using a questionnaire. The patients were qualified for the analysis if they possessed the following features: 1- Female cases diagnosed with pathology report of breast cancer, and 2- those who never have underwent chemo or radiotherapy, whereas the inclusion criteria regarding the controls were the absence of personal and family history of any type of cancer. This study was approved by the ethical committee of Ahvaz Jundishapur University of Medical Sciences. Moreover, written informed consent was obtained from all the included participants.

DNA extraction and methylation analysis

Genomic DNA was isolated from peripheral whole blood using Blood Genomic DNA Extraction mini Kit (FAVORGEN, Taipei, Taiwan) in terms of the manufacture's protocol and then kept at -20°C. The bisulfite treatment was conducted using EpiTect Bisulfite Kit (QIAGEN, Hilden, Germany) in terms of the manufacturer's protocol. The methylation status of the *BRCA1* gene promoter was examined using Methylation Specific PCR (MSP). The converted DNA was amplified using pairs of specific primers for both methylated and non-methylated sequences that were used in a previous study (Baldwin et al., 2000), as shown in Table1. Thereafter, the MSP was performed in 25 µl of the final reaction containing 100 ng template converted DNA, 2X Blue Master Mix 1.5 mM, and 0.1 µM from each primer, and up to final reaction volume was filled with distilled H2O. Eventually, PCR products were loaded on 2% agarose gel.

Statistical analysis

Methylation frequency was analyzed between the patients and healthy controls using the chi-square (Fisher's exact) test. Similarly, the association between *BRCA1* gene promoter methylation and the patients' clinical and demographic characteristics was assessed using the same test. The odds ratio (OR) values were calculated with 95% confidence intervals (CIs). A two-tailed test with type error level (α) set at 5%, was applied in all the statistical analyses. $P < 0.05$ was considered as statistically significant. All the statistical analyses were performed using SPSS software (version 26; SPSS Statistics, IBM Corporation, Armonk, NY, USA).

Results

BRCA1 gene promoter methylation in patients and controls

The DNA samples extracted from peripheral blood cells of 74 breast cancer patients and 30 healthy controls were qualitatively evaluated using the MSP method. The participants' age was in the range of 30 to 83 years old. The mean age of the patients and controls were 48.03±11.13 and 48.17±10.29 years old, respectively. Besides, no significant difference was found between the controls and patients (p=0.953). The demographic characteristics of the patients and healthy individuals are shown in Table 2. Out of 74 breast cancer patients, 2 cases (2.7%) showed promoter methylation of the *BRCA1* gene, whereas the healthy controls showed no the *BRCA1* promoter methylation in peripheral blood cells (Figure 1). Consequently, no evidence was found regarding any association between *BRCA1* promoter methylation and breast cancer susceptibility (P=0.9, OR=0). All the subjects' demographic characteristics such

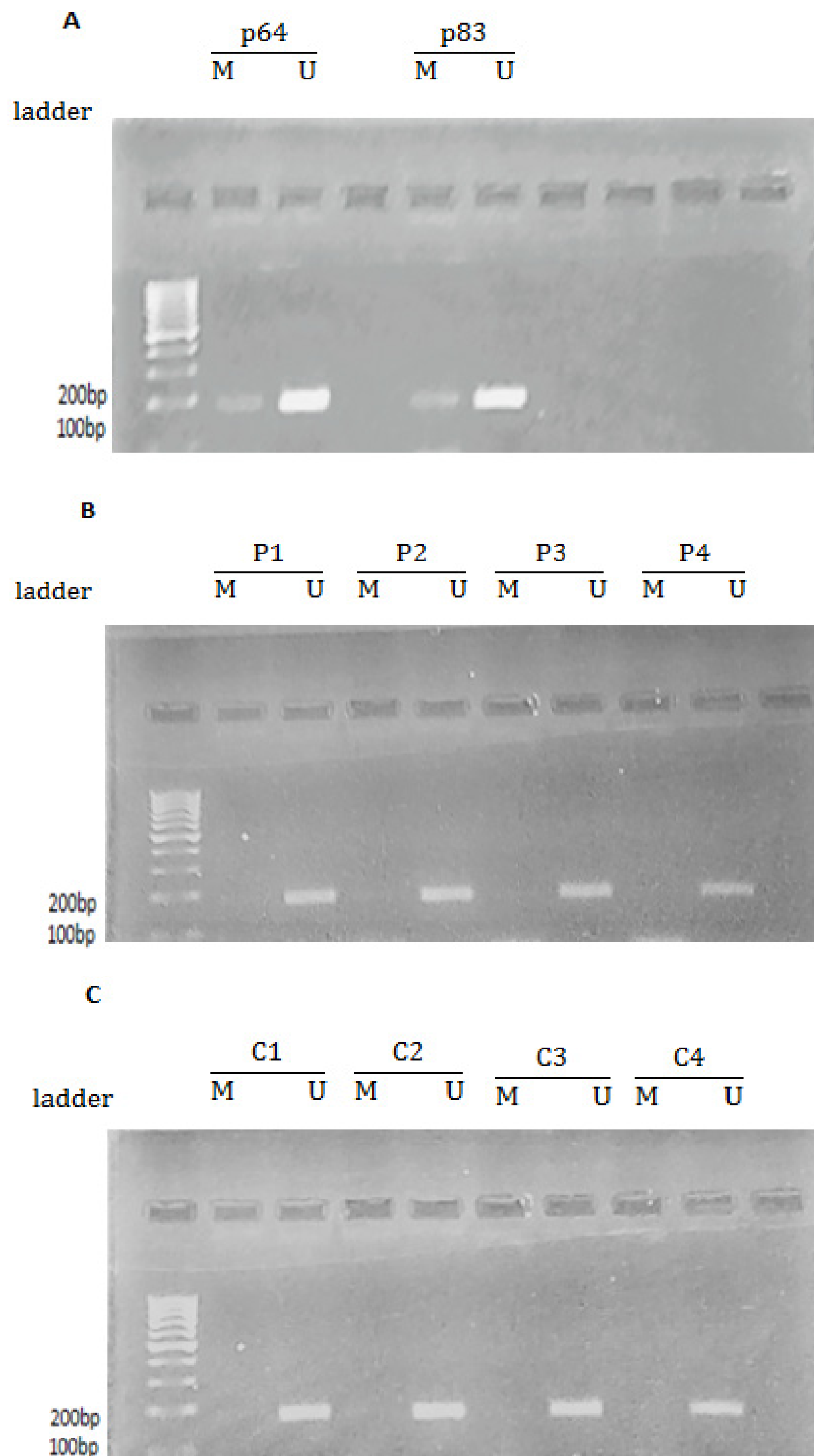

Figure1. Representative Results of MS-PCR Analysis for *BRCA1* Gene in the Breast Cancer Patients and Controls. A. Two patients were positive for *BRCA1* promoter Methylation. B and C Unmethylated status in *BRCA1* gene promoter in the patients and controls, respectively. C, Controls; P, Patients; M and U correspond to Methylated and Unmethylated samples respectively.

as age, menopausal status, menstrual age, marital status, age at the time of the first pregnancy, number of children, and lactation were assessed, in order to determine whether an association exists between promoter methylation of *BRCA1* and their demographic characteristics. Two patients showing promoter methylation were under premenopausal condition, and their menstrual age was 13 years old, who were also single and aged under 50 years old. Overall, none of the demographic characteristics showed any significant relationship with the methylation status of the *BRCA1* gene (Table 3).

The correlation of BRCA1 gene promoter methylation with clinical features of the patients with breast cancer

The association between *BRCA1* gene promoter methylation in peripheral blood cells and the patients'

Table 1. Primer Sequences for Methylation-Specific PCR Chain Reaction used for *BRCA1* Gene Promoter

Gene	Primer Name	Sense Primer	Antisense Primer	Annealing Temp (°C)	Size (bp)
BRCA1	Unmethylated	GGTTAATTTAGAGTTTTGAGAGATG	TCAACAAACTCACACCACACAATCA	61	182 bp
	Methylated	GGTTAATTTAGAGTTTCGAGAGACG	TCAACGAACTCACGCCGCGCAATCG	65	182 bp

clinicopathological characteristics was assessed. Out of 74 patients, 13 cases were at the early stages (stage I), and two patients who had *BRCA1* gene methylation (15.4%) were included in this group (p=0.02). As well, 34 and 27 patients were at stages II and III, respectively, showing a negative state of *BRCA1* gene methylation. Additionally, two positive cases of *BRCA1* gene methylation were observed to be similar in other clinicopathological features, including tumor grade, hormone receptor status, and both metastasis and lymph node involvement. They were negative for progesterone, estrogen and human epidermal growth factor receptors, in tumor grade II and had no lymph node or organ metastasis. It is noteworthy that no significant association was found between other clinicopathological features and the *BRCA1* gene methylation status (Table 4).

Discussion

In the current research, we aimed to assess whether the promoter methylation of the *BRCA1* gene in peripheral blood cells has an association with the risk of developing breast cancer. For this purpose, *BRCA1* gene promoter methylation was evaluated using the qualitative MSP method and 2 cases out of 74 breast cancer patients, demonstrated *BRCA1* gene methylation in their peripheral blood cells. Furthermore, these two subjects were at stage 1, tumor grade 2, and had triple-negative status. In addition, none of the healthy controls showed the methylation status. Nonetheless, no significant association was found between *BRCA1* gene promoter methylation in peripheral blood cells and the risk of developing breast cancer. In the present study, due to the lack of tumor tissue samples obtained from the included patients, it was not possible to compare the methylation status of the *BRCA1* gene promoter in the patients' peripheral blood cells and the related tumor tissue samples. While Wojdacz et al, examined the methylation status in *BRCA1*, *BRCA2*, *APC*, and *RASS1A* genes in the tumor tissues of 75 patients with breast cancer and their blood samples using the quantitative MS-HRM method. Correspondingly, 4% of these patients showed methylation in *BRCA1* and APC genes in both tumor and blood samples. As well, 4.3% and 2.7% of patients showed *BRCA1* and *APC* genes' methylations only in blood samples, respectively. The results of the above-mentioned study suggested that the methylation status of some cancer-related genes in peripheral blood DNA is not directly related to the methylation of the same genes in tumor tissue DNA and might be specific only to the peripheral blood DNA (Wojdacz,Thestrup,Overgaard, et al., 2011). In another study, Wojdacz et al. examined the promoter methylation of the *BRCA1* gene along with that of the *BRCA2*, *APC*,

Table 2. Demographic Features of Breast Cancer Patients and Healthy Controls

	Parameters	Patient n:74 (%)	Control n:30 (%)
Age	Under 50	38 (51.4%)	17 (56.7%)
	Above 50	36 (48.6%)	13 (43.3%)
Menopausal status	Pre	44 (59.5%)	18 (60%)
	Post	30 (40.5%)	12 (40%)
Age at menopause (year)	Under 50	2 (6.7%)	1 (8.3%)
	Above 50	28 (93.3%)	11 (91.7%)
Age at menarche (year)	Under 12	49 (66.2%)	15 (50%)
	13-14	24 (32.4%)	11 (36.7%)
	Above 15	1 (1.4%)	4 (13.3%)
Marriage	Yes	57 (23%)	24 (80%)
	No	17 (77%)	6 (20%)
Child number	Under 2	24 (32.4%)	15 (50%)
	Above 2	50 (67.6%)	15 (50%)
Breastfeeding	Yes	43 (58.1%)	14 (46.7%)
	No	31 (41.9%)	16 (53.3%)
Age at first pregnancy	Under 30	55 (74.33%)	19 (63.3%)
	Above 30	2 (2.7%)	2 (6.7%)
	Nulliparity	17 (22.97%)	9 (30%)

Table 3. Demographic Features of Breast Cancer Patients and Their Association with *BRCA1* Promoter Methylation in Peripheral Blood Cells

Parameters		Total 74 (%)	Me(+)[a] 2 (%)	Me(-)[b] 72 (%)	p-value
Age	<50	38 (51.4)	2 (5.3)	36 (94.7)	
	50≤	36 (48.6)	0 (0)	36 (100)	0.494
Menopausal status	Pre	44 (59.5)	2 (4.5)	42 (95.5)	
	Post	30 (40.5)	0 (0)	30 (100)	0.511
Age at menopause	<50	2 (6.7)	0 (0)	2 (100)	
	50≤	28 (93.3)	0 (0)	28 (100)	-
Age at menarche	≤12	49 (66.2)	0 (0)	49 (100)	
	13-14	24 (32.4)	2 (8.3)	22 (91.7)	0.129
	15≤	1 (1.4)	0 (0)	1 (100)	
Marriage	Yes	57 (77)	0 (0)	57	
	No	17 (23)	2 (11.8)	15 (88.2)	0.05
Child number	<2	24 (32.4)	2 (8.3)	22 (91.7)	
	2≤	50 (67.6)	0 (0)	50 (100)	0.102
Breastfeeding	Yes	43 (58.1)	0 (0)	43 (100)	
	No	31 (41.9)	2 (6.5)	29 (93.5)	0.172
Age at first pregnancy	< 30	55 (74.3)	0 (0)	55	
	30≤	2 (2.7)	0 (0)	2	0.104
	Nulliparity	17 (23)	2 (11.8)	15 (88.2)	

[a], Me(+): BRCA1 promoter methylation positive in peripheral cells; [b], Me(-): BRCA1 promoter methylation negative in peripheral cells

Table 4. Association between *BRCA1* Promoter Methylation and Clinico-Pathological Features

Parameters		Total n (%)	Me(+)[a] n (%)	Me(-)[b] n (%)	p-value
Cases		74	2 (2.7)	72 (97.3)	
Controls		30	0 (0)	30 (100)	
Grade	I	8 (10.8)	0 (0)	8 (100)	
	II	48 (64.9)	2 (4.2)	46 (95.8)	0.999
	III	18 (24.3)	0 (0)	18 (100)	
Stage	I	13 (17.6)	2 (15.4)	11 (84.6)	
	II	37 (50)	0 (0)	37 (100)	
	III	24 (32.4)	0 (0)	24 (100)	0.029
	IV	0 (0)	0 (0)	0 (0)	
Lymph node status	Positive	52 (70.3)	0 (0)	52 (100)	
	Negative	22 (29.7)	2 (9.1)	20 (90.9)	
ER status	Positive	41 (55.4)	0 (0)	41 (100)	
	Negative	28 (37.8)	2 (7.1)	26 (92.9)	0.161
	*Unknown	5 (6.8)	0 (0)	5 (100)	
PR status	Positive	41 (55.4)	0 (0)	41 (100)	
	Negative	28 (37.8)	2 (7.1)	26 (92.9)	0.161
	*Unknown	5 (6.8)	0 (0)	5 (100)	
Her2/neu status	Positive	27 (36.5)	0 (0)	27 (100)	
	Negative	42 (56.7)	2 (4.8)	40 (95.2)	0.367
	*Unknown	5 (6.8)	0 (0)	5 (100)	
Distant metastasis	Positive	0 (0)	0 (0)	0 (0)	
	Negative	74 (100)	2 (2.7)	72 (97.3)	

* Unknown hormone receptor status were excluded from chi-squar test. ER, Estrogen Receptor; PR, Progestrone; Her2, Human epidermal growth factor receptor2

RASS1A and *RARβ2* genes in the white blood cells of breast cancer patients using MS HRM. Although methylation was found in DNA derived from peripheral blood cells, no significant difference was reported in the frequency of methylation between case and control groups (Wojdacz et al., 2011). In addition, Cho et al. in their study examined the promoter methylation of *BRCA1*, *CDH1*, and *RARβ* genes in the blood samples and tumor tissues on Long Island counties' population using the Methylight quantitative method. Similarly, they have reported that no relationship exists between DNA methylation of tumor tissue and DNA methylation in white blood cells (Cho et al., 2015). In study conducted by Bosviel et al., the association between *BRCA1* gene promoter methylation in peripheral blood cells and the risk of developing breast cancer in 908 cases and 990 controls has been evaluated. Moreover, *BRCA1* promoter methylation was observed in peripheral blood cells of 47.1% of breast cancer patients as well as 45.9% of controls; however, the difference was not statistically significant (P: 0.08). Nonetheless, it was reported that the methylation of the *BRCA1* promoter in patients aged more than 70 years old is higher than that of controls (P: 0.017) (Bosviel et al., 2012). On the other hand, our study revealed no significant relationship between *BRCA1* promoter methylation status in peripheral blood cells and patients' demographic characteristics such as age and menopausal status. In contrast, several studies have previously reported significant differences in terms of *BRCA1* gene promoter methylation levels between peripheral blood cells of breast cancer patients and controls (Gupta et al., 2014; Iwamoto et al., 2011; Wong et al., 2011). Overall, it seems that the differences in the types of used method, sample size, study populations, and selected breast cancer subtypes have led to controversy in the final reported data. In a study by Prajzendanc et al. the peripheral blood cells of 942 patients and 500 controls along with 262 tissue samples have been assessed. As a result, findings demonstrated that the *BRCA1* gene promoter methylation in peripheral blood cells is strongly associated with the risk of developing triple-negative breast cancer subtype ($P<0.001$), whereas it has no significant relationship with estrogen receptor-positive breast cancer (0.46). Furthermore, *BRCA1* gene methylation showed a significant coordination between peripheral blood cells and tumor samples ($P<0.001$) (Prajzendanc et al., 2020). Similarly, Gupta et al., (2014) have stated that a significant relationship exists between *BRCA1* gene promoter methylation in peripheral blood cells and the risk of developing triple-negative and medullary breast cancer. Based on the Wong et al.,'s (2011) report, the precise selection of the type of breast cancer subtype and the accuracy towards clinical-pathological characteristics seem to affect the final results. Patients with no mutation in the *BRCA1* gene and similar clinicopathological features as well as patients carrying the *BRCA1* mutation illustrated a higher percentage of methylation of this gene in peripheral blood cells compared to patients with less clinical similarity. Additionally, they showed high levels

of *BRCA1* promoter methylation in their tumor DNA. In the current study, out of 74 patients, 16 cases were in the triple-negative subgroup, and both of our positive cases in terms of *BRCA1* promoter methylation, were included in this subgroup as well (12.5%). Nevertheless, no significant relationship was found between the *BRCA1* promoter methylation status in peripheral blood cells and the immunohistochemical status of the studied patients (Table 3). By taking everything into account, a question raises as whether a functional link can be established between the promoter methylation of the *BRCA1* gene in peripheral white blood cells and the presence of methylation of the same gene in breast tumor tissue? In other words, how does the methylation of DNA in white blood cells related to the risk of developing breast cancer? Several previous studies have suggested some hypotheses in this regard, which are as follows: It has been suggested that abnormal methylation of genes in peripheral blood DNA might occur due to germline transmission of methylation changes or due to some somatic changes occurring during early development or under certain environmental conditions throughout lifetime. Accordingly, these changes are induced in blood cells and other tissues as well (Gupta et al., 2014; Khakpour et al., 2015; Wojdacz et al., 2011). It has also been suggested that the DNA methylation profile of T cells and mononuclear cells in peripheral blood, are changed while the cancer progresses (Parashar et al., 2018). It is worth mentioning that using the MSP qualitative method and sample size can affect the difference between the results of our study and those of some other studies. In conclusion, despite observing 2 promoter methylation cases, no significant association was found between the *BRCA1* gene promoter methylation in white blood cells and the risk of developing breast cancer. In addition, no significant relationship was observed between *BRCA1* promoter methylation status and the patients' clinicopathological and demographic characteristics. Despite the contradictory results previously reported in various studies, it is suggested that the exact functional and pathophysiological association between DNA promoter methylation in peripheral blood cells and breast cancer tissue should be investigated, as well as the comparison between expression of the *BRCA1* gene in peripheral blood cells and tumor tissue. Performing more large-sample size studies and conducting assessments using different methods in the future are necessary for better evaluation.

Author Contribution Statement

M.B. designed the study. H.H. drafted the manuscript, processed the experimental data, performed the statistical analysis, designed the tables and figures, collected the samples. M.B., A.T. and M.T. processed the experimental data and collected the samples..

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Data are available by request to the corresponding author and there is no conflict of interest.

References

Al-Moghrabi N, Nofel A, Al-Yousef N, et al (2014). The molecular significance of methylated *BRCA1* promoter in white blood cells of cancer-free females. *BMC Cancer*, **14**, 1-12.

Baldwin RL, Nemeth E, Tran H, et al (2000). *BRCA1* promoter region hypermethylation in ovarian carcinoma: a population-based study. *Cancer Res*, **60**, 5329-33.

Bianco T, Chenevix-Trench G, Walsh DC, et al (2000). Tumour-specific distribution of *BRCA1* promoter region methylation supports a pathogenetic role in breast and ovarian cancer. *Carcinogenesis*, **21**, 147-51.

Birgisdottir V, Stefansson OA, Bodvarsdottir SK, et al (2006). Epigenetic silencing and deletion of the *BRCA1* gene in sporadic breast cancer. *Breast Cancer Res*, **8**, 1-10.

Bosviel R, Garcia S, Lavediaux G, et al (2012). *BRCA1* promoter methylation in peripheral blood DNA was identified in sporadic breast cancer and controls. *Cancer Epidemiol*, **36**, 177-82.

Brennan K, Garcia-Closas M, Orr N, et al (2012). Intragenic ATM methylation in peripheral blood DNA as a biomarker of breast cancer risk. *Cancer Res*, **72**, 2304-13.

Catteau A, Harris WH, Xu C-F, et al (1999). Methylation of the *BRCA1* promoter region in sporadic breast and ovarian cancer: correlation with disease characteristics. *Oncogene*, **18**, 1957-65.

Cho YH, McCullough LE, Gammon MD, et al (2015). Promoter hypermethylation in white blood cell DNA and breast cancer risk. *J Cancer*, **6**, 819.

Daniels SL, Burghel GJ, Chambers P, et al (2016). Levels of DNA methylation vary at CpG sites across the *BRCA1* promoter, and differ according to triple negative and "BRCA-like" status, in both blood and tumour DNA. *PLoS One*, **11**, e0160174.

Dobrovic A, Simpfendorfer D (1997). Methylation of the *BRCA1* gene in sporadic breast cancer. *Cancer Res*, **57**, 3347-50.

Ferla R, Calo V, Cascio S, et al (2007). Founder mutations in *BRCA1* and *BRCA2* genes. *Ann Oncol*, **18**, vi93-8.

Gupta S, Jaworska-Bieniek K, Narod SA, et al (2014). Methylation of the *BRCA1* promoter in peripheral blood DNA is associated with triple-negative and medullary breast cancer. *Breast Cancer Res Treat*, **148**, 615-22.

Hosny MM, Sabek NA, El-Abaseri TB, et al (2016). Promoter methylation status of breast cancer susceptibility gene 1 and 17 beta hydroxysteroid dehydrogenase type 1 gene in sporadic breast cancer patients. *Inte J Breast Cancer*, **2016**.

Iwamoto T, Yamamoto N, Taguchi T, et al (2011). *BRCA1* promoter methylation in peripheral blood cells is associated with increased risk of breast cancer with *BRCA1* promoter methylation. *Breast Cancer Res Treat*, **129**, 69-77.

Khakpour G, Pooladi A, Izadi P, et al (2015). DNA methylation as a promising landscape: A simple blood test for breast cancer prediction. *Tumor Biol*, **36**, 4905-12.

Kuchiba A, Iwasaki M, Ono H, et al (2014). Global methylation levels in peripheral blood leukocyte DNA by LUMA and breast cancer: a case–control study in Japanese women. *Br J Cancer*, **110**, 2765-71.
Li L, Choi J-Y, Lee K-M, et al (2012). DNA methylation in peripheral blood: a potential biomarker for cancer molecular epidemiology. *J Epidemiol*, **22**, 384-94.
Matros E, Wang ZC, Lodeiro G, et al (2005). *BRCA1* promoter methylation in sporadic breast tumors: relationship to gene expression profiles. *Breast Cancer Res Treat*, **91**, 179-86.
Niwa Y, Oyama T, Nakajima T (2000). *BRCA1* expression status in relation to DNA methylation of the *BRCA1* promoter region in sporadic breast cancers. *Jpn J Cancer Res*, **91**, 519-26.
Pang D, Zhao Y, Xue W, et al (2012). Methylation profiles of the *BRCA1* promoter in hereditary and sporadic breast cancer among Han Chinese. *Med Oncol*, **29**, 1561-8.
Parashar S, Cheishvili D, Mahmood N, et al (2018). DNA methylation signatures of breast cancer in peripheral T-cells. *BMC Cancer*, **18**, 1-9.
Prajzendanc K, Domagała P, Hybiak J, et al (2020). *BRCA1* promoter methylation in peripheral blood is associated with the risk of triple negative breast cancer. *Int J Cancer*, **146**, 1293-8.
Severi G, Southey MC, English DR, et al (2014). Epigenome-wide methylation in DNA from peripheral blood as a marker of risk for breast cancer. *Breast Cancer Res Treat*, **148**, 665-73.
Starita LM, Parvin JD (2003). The multiple nuclear functions of *BRCA1*: transcription, ubiquitination and DNA repair. *Curr Opin Cell Biol*, **15**, 345-50.
Sturgeon SR, Arcaro KF, Johnson MA, et al (2014). DNA methylation in paired breast epithelial and white blood cells from women undergoing reduction mammoplasty. *Anticancer Res*, **34**, 2985-90.
Tang Q, Cheng J, Cao X, et al (2016). Blood-based DNA methylation as biomarker for breast cancer: a systematic review. *Clin Epigenetics*, **8**, 1-18.
Wojdacz TK, Thestrup BB, Cold S, et al (2011). No difference in the frequency of locus-specific methylation in the peripheral blood DNA of women diagnosed with breast cancer and age-matched controls. *Future Oncol*, **7**, 1451-5.
Wojdacz TK, Thestrup BB, Overgaard J, et al (2011). Methylation of cancer related genes in tumor and peripheral blood DNA from the same breast cancer patient as two independent events. *Diagn Pathol*, **6**, 1-8.
Wong EM, Southey MC, Fox SB, et al (2011). Constitutional methylation of the *BRCA1* promoter is specifically associated with *BRCA1* mutation-associated pathology in early-onset breast cancer. *Cancer Prev Res*, **4**, 23-33.
Yoshida K, Miki Y (2004). Role of *BRCA1* and *BRCA2* as regulators of DNA repair, transcription, and cell cycle in response to DNA damage. *Cancer Sci*, **95**, 866-71.
Zhang L, Long X (2015). Association of *BRCA1* promoter methylation with sporadic breast cancers: Evidence from 40 studies. *Sci Rep*, **5**, 1-12.

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