



Prognostic Prediction of BRCA Mutations by ^{18}F -FDG PET/CT SUV_{max} in Breast Cancer

Meme Kanserlerinde BRCA Mutasyonlarının ^{18}F -FDG PET/CT SUV_{max} Değeri ile Prognostik Tahmini

İD Semra Özdemir¹, İD Fatma Silan², İD Mehmet Yılmaz Akgün³, İD Nilgün Aracı⁴, İD İsmail Çırpan⁵, İD Fulya Koç Öztürk¹, İD Öztürk Özdemir²

¹Çanakkale Onsekiz Mart University Faculty of Medicine, Department of Nuclear Medicine, Çanakkale, Turkey

²Çanakkale Onsekiz Mart University Faculty of Medicine, Department of Medical Genetics, Çanakkale, Turkey

³Çanakkale Onsekiz Mart University Faculty of Medicine, Department of General Surgery, Çanakkale, Turkey

⁴Çanakkale State Hospital, Clinic of Nuclear Medicine, Çanakkale, Turkey

⁵Çanakkale State Hospital, Clinic of Pathology, Çanakkale, Turkey

Abstract

Objectives: This study aimed to investigate the prognostic prediction of germline BRCA1 and BRCA2 mutations by comparing the maximum standardized uptake value (SUV_{max}) obtained from ^{18}F fluoride-fluorodeoxyglucose positron emission tomography/computed tomography (^{18}F -FDG PET/CT), which is considered a prognostic factor in breast cancer (BC).

Methods: Retrospective interdisciplinary laboratory results of 92 patients with BC who had germline BRCA1 or BRCA2 mutation profiles and underwent ^{18}F -FDG PET/CT were compared. Genotyping was made by next-generation sequencing, and PET/CT scans were re-evaluated. The histopathological data, genetic results, and clinical demographics of all patients were recorded. Patients were divided into two groups in accordance with the presence of germline BRCA1 and/or BRCA2 mutations. Between-group statistical comparison was performed.

Results: In PET/CT performed for primary staging, patients with BRCA-positive BC had significantly higher SUV_{max} ($p=0.039$), larger tumor size ($p=0.025$), and presence of axillary nodal metastases ($p=0.023$) than patients with BRCA-negative BC. Although the Ki-67 index was higher in the BRCA-positive group than BRCA-negative group, this difference was not significant ($p=0.157$). Moreover, in the BRCA-positive and negative groups, SUV_{max} , Ki-67 index, and tumor size, grade, and stage were significantly correlated with each other.

Conclusion: The results of this study showed a strong association between BRCA mutations and SUV_{max} , which indicates the poor prognosis of BC.

Keywords: BRCA1-2 mutation, SUV_{max} , ^{18}F -FDG PET/CT, breast cancer

Öz

Amaç: Bu çalışmanın amacı, meme kanserlerinde (MK) germline BRCA1-BRCA2 mutasyonlarının tahmini prognostik değerini, ^{18}F -florodeoksiglikoz pozitron emisyon tomografi/bilgisayarlı tomografi (^{18}F -FDG PET/CT) tetkikinden elde edilen ve prognostik bir faktör olduğu kabul edilen maksimum standardize tutulum değeri (SUV_{maks}) ile karşılaştırarak araştırmaktır.

Yöntem: Germline BRCA1 veya BRCA2 mutasyon profilleri olan ve ^{18}F -FDG PET/CT taraması yapılan MK'li 92 hastanın retrospektif olarak laboratuvar sonuçları karşılaştırıldı. Genotipleme yeni nesil sıralama tekniği ile yapıldı ve PET/CT taramaları yeniden değerlendirilerek tüm hastaların histopatolojik, genetik sonuçları ve klinik demografik özellikleri not edildi. Hastalar germline BRCA1 ve/veya BRCA2 mutasyonlarının varlığına göre iki gruba ayrıldı ve gruplar arasında istatistiksel karşılaştırma yapıldı.

Address for Correspondence: Semra Özdemir Prof. MD, Çanakkale Onsekiz Mart University Faculty of Medicine, Department of Nuclear Medicine, Çanakkale, Turkey

Phone: +90 286 263 59 50 **E-mail:** semozdemir@yahoo.com ORCID ID: orcid.org/0000-0003-1302-9630

Received: 11.04.2021 **Accepted:** 26.05.2021

©Copyright 2021 by Turkish Society of Nuclear Medicine
Molecular Imaging and Radionuclide Therapy published by Galenos Yayınevi.

Bulgular: BRCA pozitif MK hastalarının, primer evreleme için yapılan ^{18}F -FDG PET/BT görüntülemesinde, SUV_{maks} değerleri ($p=0,039$), tümör boyutu ($p=0,025$) ve aksiller nodal metastaz varlığı ($p=0,023$) BRCA negatif MK hastalarından önemli ölçüde daha yüksek bulundu. Ki-67 değeri, BRCA pozitif grupta BRCA negatif gruba göre daha yüksek olmasına rağmen, bu fark istatistiksel olarak anlamlı değildi ($p=0,157$). Ayrıca BRCA pozitif ve negatif gruplarında; SUV_{maks} , Ki-67, tümör boyutu, grade ve evre değerlerinin birbirleriyle anlamlı korelasyon gösterdiği bulundu.

Sonuç: Mevcut çalışma sonuçlarına göre, BRCA mutasyonları ile SUV_{maks} değerleri arasında MK için kötü prognozu gösteren güçlü bir ilişki vardır.

Anahtar kelimeler: BRCA1-2 mutasyonu, SUV_{maks} , ^{18}F -FDG PET/BT, meme kanseri

Introduction

Breast cancer (BC) is one of the most common types of cancer in women and has a variable spectrum of phenotypic and clinical behaviors, which are caused by genetics, lifestyle, and environmental factors (1,2). One of the main genetic risk factors is the presence of BRCA1-2 mutations. BRCA1-2 germline mutations contribute to 5-10% of BC in most populations. BRCA1 and BRCA2 provide instructions for making a protein that acts as a tumor suppressor (3,4). Tumor suppressor proteins prevent cancer formation by preventing the uncontrolled growth and division of cells or by promoting apoptosis. Hence, mutations in *BRCA* genes can lead to irregular cell growth and tumor development.

These mutations are inherited in an autosomal dominant manner and show a high degree of penetrance. A meta-analysis indicated that BRCA1 and BRCA2 carriers have 57-65% and 45-49% probability of developing BC throughout their life, respectively (5). BRCA1-associated BC types often have high histological grade and are triple-negative. BRCA2-associated breast tumors are usually high-grade, estrogen receptor positive, and human epidermal growth factor receptor-2 (HER-2)-negative (3,6). However, the effect of BRCA mutation carrier on prognosis is still controversial. While some studies have claimed that patients with BC who are BRCA carriers have reduced overall survival compared with patients with sporadic BC, some studies have also stated that it does not affect surveillance or even results in better surveillance (7,8,9,10).

In BC, ^{18}F fluoride-fluorodeoxyglucose positron emission tomography/computed tomography (^{18}F -FDG PET/CT) is frequently performed for staging to obtain long-term prognostic information, evaluation of recurrent disease, and estimation of therapeutic response. Through radiolabeled glucose with ^{18}F -FDG PET/CT, the presence of the primary tumor, nodal involvement, and distant metastasis can be displayed simultaneously; as a result, tumor-node-metastasis (TNM) classification can be most accurately performed (11,12,13). The maximum standardized uptake value (SUV_{max}) obtained from ^{18}F -FDG PET/CT, which is a metabolic indicator, is an accepted parameter in determining BC prognosis. Many studies have shown that

high SUV_{max} is an indicator of poor prognosis (13,14,15). Therefore, this study aimed to investigate the relationship between SUV_{max} and BRCA positivity to predict the effect of BRCA mutation on prognosis.

Materials and Methods

Study Groups

A total of 92 female patients were selected retrospectively among patients with BC who had germline BRCA1 or BRCA2 analysis and who underwent PET/CT between 2017 and 2020. All patients received routine PET/CT imaging protocol. Imaging was completed with a Biograph Duo LSO ^{18}F -FDG PET/CT scanner (Siemens, Germany). Patients who were pathologically diagnosed with BC and underwent PET for primary staging were included in this study. Multidisciplinary laboratory results from histopathology, genetics, and PET/CT and some clinical demographics of all patients were noted. All patients had a family history of BC. None of the patients had undergone surgery, and *BRCA* gene analyses were made before surgery. Patients were divided into two groups in accordance with the presence of germline BRCA1 and/or BRCA2 mutations. Group 1 (BRCA-positive group) was composed of patients who had BRCA1 and/or BRCA2 mutation ($n=18$), while group 2 (BRCA-negative group) comprised patients who had no germline BRCA1 and BRCA2 mutations ($n=74$). In the BRCA-positive group (mean age, 50.11 ± 11.98 years), 11 women were 50 years old or younger. In the BRCA-negative group (mean age, 51.36 ± 10.86 years), 37 women were 50 years old or younger. The clinical characteristics, histopathological features, and PET/CT parameters of the cohorts are shown in Table 1. Only patients who underwent PET/CT for primary staging were included in this study. Patients whose pathology results could not be obtained and who did not undergo PET/CT for primary staging were excluded from the study.

All patients provided written informed consent. The archives of the university and state hospital were used with the permission of the institution. The study was approved by the Çanakkale Onsekiz Mart University Ethics Committee (protocol no: 2020-04, date: 26.02.2020).

Table 1. Laboratory and clinical characteristics of patients with breast cancer			
Clinical characteristics	Patient cohort (n=92)		
	BRCA1-2-positive group (n=18)	BRCA1-2-negative group (n=74)	p value
Mean age (years)			
Mean ± SD	50.11±11.98	51.36±10.86	
Median	49	51	0.629
Min-max	31-74	32-78	
≤50, n (%)	11 (61.1)	37 (50.0)	0.397
>50, n (%)	7 (38.9)	37 (50.0)	
Smoke (+)	8 (44.4)	20 (27.0)	0.248
Alcohol (+)	1 (5.6)	8 (10.8)	0.817
Ki-67			
Mean ± SD	32.89±19.16	26.17±16.72	
Median	32.50	20.00	0.157
Min-max	(5.00-70.00)	(2.00-70.00)	
Histopathologic feature	n (%)	n (%)	p value
Tumor histotype			
Invasive ductal carcinoma (IDC)	14 (77.8)	62 (83.8)	
IDC (papillary type)	0 (0.0)	2 (2.7)	
IDC (mucinous type)	1 (5.6)	2 (2.7)	
IDC (medullary type)	1 (5.6)	0 (0.0)	0.411
IDC (tubular type)	0 (0.0)	1 (1.4)	
Invasive lobular carcinoma	2 (11.1)	5 (6.8)	
Progesterone receptor status			
Positive	8 (44.4)	45 (60.8)	0.320
Negative	10 (55.6)	29 (39.2)	
Estrogen receptor status			
Positive	9 (50.0)	56 (75.7)	0.063
Negative	9 (50.0)	18 (24.3)	
Hormone receptor status			
Positive	9 (50.0)	61 (82.4)	0.010**
Negative	9 (50.0)	13 (17.6)	
Molecular subtypes			
HR+/HER-2- (luminal A)	4 (22.2)	41 (55.4)	0.024**
HR-/HER-2- (triple-negative)	4 (22.2)	11 (14.8)	0.578
HR+/HER-2 (luminal B)	5 (27.7)	20 (27.2)	0.949
HR-/HER-2+ (HER-2 enriched)	5 (27.7)	2 (2.7)	0.002
Grades			
1	4 (22.2)	31 (41.9)	
2	13 (72.2)	35 (47.3)	0.165*
3	1 (5.6)	8 (10.8)	
Stage			
I	1 (5.6)	24 (32.4)	
II A	5 (27.8)	17 (23.0)	
II-B	5 (27.8)	11 (14.9)	
III A	3 (16.7)	7 (9.4)	
III B	1 (5.6)	4 (5.4)	0.080**
III C	0 (0)	6 (8.1)	
IV	3 (16.7)	5 (6.8)	
PET/CT parameters			p value
Primary tumor size (max) (mm)			
Mean ± SD	30.11±10.90	24.69±11.31	0.025*
Min-max	15-51	8-60	

Table 1. Continued

Clinical characteristics	Patient cohort (n=92)		
	BRCA1-2-positive group (n=18)	BRCA1-2-negative group (n=74)	p value
Primary lesion SUV_{max} Mean ± SD Min-max	10.93±8.29 2.60-30.72	6.76±3.90 2.0-17.3	0.039**
Axillary nodal metastasis, n (%) Positive Negative	15 (83.3) 3 (16.7)	40 (54.1) 34 (45.9)	0.023*
Distant metastasis, n (%) Positive Negative	3 (16.7) 15 (83.3)	6 (8.1) 68 (91.9)	0.371***

*Pearson chi-square, **Mann-Whitney U test, ***Fisher's Exact test, SD: Standard deviation, min: Minimum, max: Maximum, HER-2: Human epidermal growth factor receptor-2, SUV_{max}: Maximum standardized uptake value

¹⁸F-FDG PET/CT and Data Analysis

Before PET/CT was performed, patients were instructed to fast for at least 6 h, and serum glucose levels should be <160 mg/dL. All images were acquired approximately 1 h later by a PET/CT scanner after intravenous injection of 3.7 MBq/kg of ¹⁸F-FDG. Initial guideline scout images were obtained, and non-contrasted CT images were taken for the body regions from the vertex to 1/3 proximal thigh, followed by PET. PET/CT images were taken with mean 7-8 bed positions and 2 mm slices. PET/CT images were re-evaluated independently by two nuclear medicine physicians. The SUV_{max} of the primary tumor lesion was automatically calculated according to the region of interest. The TNM classification was made according to PET/CT data.

Tumor size, presence of axillary node metastasis, and distant metastasis parameters, which are obtained from PET/CT images used for TNM classification, were statistically compared. The obtained SUV_{max} values of the two groups were also compared.

Genotyping of Target BRCA Genes

Targeted next-generation sequencing (NGS) of candidate BC-associated genes was performed on blood samples from patients with BC. Total genomic DNA was extracted from whole-blood samples and submitted at recruitment for BRCA1 and BRCA2 genotyping. The mutational profiles of 43 patients with primary breast tumors were correlated with clinicopathological data and compared with individuals without BC (non-BC controls). DHS-102Z Human BRC1 and BRCA2 multiplex amplicon-based library preparation panel and Qiagen Illumina NGS run system (Qiagen, QIAseq, Germany) were used for target gene profiling of breast-cancer-susceptibility genes including BRCA1 and BRCA2. For sequencing, an Illumina HiSeq2500 NDS Platform

(Illumina, Little Chesterford, UK; appendix pp. 20-21) was used. In this study, predicted variants of missense, silent, frameshift, non-sense, and other splice site exchanges were defined and confirmed by Sanger sequencing. DNAs were isolated by sample preparation nucleic acid kit (Qiagen, MiniseqMN00813), and amplifications of target breast-cancer-susceptibility genes were performed by QIAseq targeted DNA panel Illumina NGS run and evaluated by QIAseq targeted DNA panel analysis pipeline. All data were interpreted by ingenuity variant analysis. Some variants of uncertain significance (VUS) and/or specific variants were evaluated by ExAC, GnomAD, ClinVAR, Varsome, GERP, LRT, MetaLR, MetaSVM, MutationAssesor, MutationTaster, DANN, dbNSFP, FATHMM, and Provean.

Statistical Analysis

All data analysis was performed using statistical package software SPSS (Statistical Package for Social Sciences) version 20.0. Descriptive data were presented as frequency, percentage, mean, standard deviation, median, minimum, and maximum values. According to the number of patients in the groups, the compatibility of variables to normal distribution was examined using the Shapiro-Wilk test. Non-parametric tests were preferred as the analysis method by examining the sample size and compliance tests with normal distribution. Mann-Whitney U test was used to compare age and continuous variables between groups. Pearson chi-square and Fisher's exact test were used to compare categorical variables between groups. A value of p<0.05 was considered significant.

Relationships between PET/CT and histopathological parameters were analyzed with Spearman correlation analysis. Correlation was interpreted as follows: 0.00-0.24, weak; 0.25-0.49, medium; 0.50-0.74, strong; 0.75-1.00, very strong relationship.

Results

The study population consisted of 92 female patients with BC who underwent ^{18}F -FDG PET/CT for primary staging and who also had germline BRCA1 or BRCA2 analysis. The mean age of the patients was 51.13 ± 11.01 (minimum-maximum: 32-78) years. The mean ages of patients in the germline BRCA1 or BRCA2-positive ($n=18$) and BRCA-negative ($n=74$) groups were 50.11 ± 11.98 and 51.36 ± 10.86 years, respectively (Table 1). Although the number of patients aged <50 years was higher in the BRCA-positive group (61.1%) than in the BRCA-negative group (50%), the difference was not significant. All patients who were evaluated in this study had a family history of BC. No significant difference was found between both groups in terms of smoking and alcohol use. Other details about the demographic and clinical characteristics of the cohorts are summarized in Table 1.

Table 1 also shows the histopathological types, hormone receptor status, and molecular subtypes for the BC cohort in this study. The mean Ki-67 index was lower in the BRCA-positive group than in the BRCA-negative group, but the difference was not significant ($p=0.157$). While no significant difference was found between the two groups in terms of histopathological type, grade, and stage of the tumor, differences were found in the molecular subtypes. The rate of hormone receptor (estrogen and/or progesterone) positivity was significantly higher in the BRCA-negative group (82.4%) than in the BRCA-positive

group (50%), ($p=0.010$). The most common subtype was Luminal A (HR+/HER-2-) in 55.4% of the BRCA-negative group, and the difference between the two groups of patients was significant ($p=0.024$). The least common subtype was HER-2 enriched (HR-/HER-2+) in 2.7% of the BRCA-negative group, and this difference was also significant ($p=0.002$) (Table 1).

Considering the PET/CT parameters between the groups, the size and SUV_{max} of the primary breast tumor lesion were significantly higher in the BRCA-positive group ($p=0.025$, $p=0.039$) according to the TNM criteria. After the diagnosis of cancer, results of PET/CT performed for staging purposes revealed that axillary node involvement was significantly higher in the BRCA-positive group than in the BRCA-negative group (Table 1). However, the presence of distant metastases at the time of primary staging was not significantly different in both groups.

Correlation analyses among SUV_{max} , Ki-67 index, tumor size, tumor grade, tumor stage, and age were performed in all groups. All parameters, except for age, showed a significant correlation with each other at a medium-strong level. These correlation rates were nearly comparable in both groups, so the difference was not significant (Table 2, 3).

In this study, we found a strong correlation between SUV_{max} and Ki-67 values (Table 1, Figure 1). In addition, SUV_{max} correlated moderately with tumor size and highly correlated with tumor grade and stage (Table 1).

Table 2. Significant relationship between SUV_{max} values of histopathological variables such as Ki-67 value, primary tumor size, tumor stage, and age at diagnosis of patients with mutated breast tumors (group 1)

Spearman's correlation analysis group 1 ($n=18$)	SUV_{max}	Ki-67	Tumor			Age
			Size	Grade	Stage	
SUV_{max} rho p value						
Ki-67 rho p value	0.678 <0.001*					
Tumor size rho p value	0.462 <0.001*	0.308 0.003*				
Tumor grade rho p value	0.533 <0.001*	0.551 <0.001*	0.357 <0.001*			
Tumor stage rho p value	0.594 <0.001*	0.364 <0.001*	0.692 <0.001*	0.514 <0.001*		
Age rho p value	-0.069 0.513	0.150 0.154	0.127 0.226	0.034 0.747	0.086 0.415	

*Correlation is significant at the 0.05 level (two-tailed). Rho: Correlation coefficient, p: Spearman correlation analysis, SUV_{max} : Maximum standardized uptake value

Various structural point mutations were detected in *BRCA1* and *BRCA2* genes in the current cohort. In addition, 4 (4.3%) patients showed *BRCA1* mutation, 11 (11.9%) showed *BRCA2* mutation, and 3 (3.2%) showed mutations in both *BRCA1* and *BRCA2*. No point mutation was detected in the remaining patients (80.5%) with BC

(Table 4). Moreover, 2 frameshift and 3 missense point mutations were detected in *BRCA1*, and 4 frameshift and 12 missense point mutations were detected in *BRCA2* (Table 4). All missense and frameshift mutations were located in various exonic frames for both genes. The detected point mutations showed different levels of clinical significance

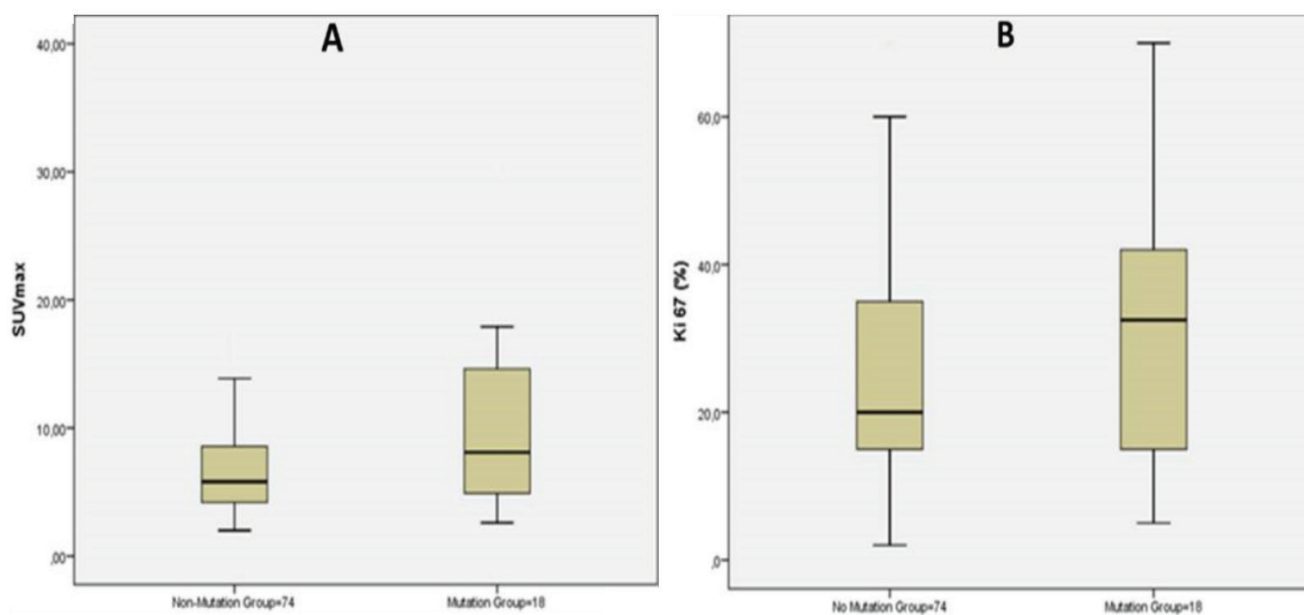


Figure 1. Median SUV_{max} (A) and Ki-67 (B) values between mutated and non-mutated groups

SUV_{max} : Maximum standardized uptake value

Table 3. Significant relationship between the SUV_{max} values of histopathological variables such as Ki-67 value, primary tumor size, tumor stage, and age at diagnosis in patients without mutated breast tumors (group 2)

Spearman's correlation analysis group 2 (n=74)	SUV_{max}	Ki-67	Tumor			Age
			Size	Grade	Stage	
SUV_{max} rho p value						
Ki-67 rho p value	0.731 <0.001*					
Tumor size rho p value	0.379 <0.001*	0.397 0.003*				
Tumor grade rho p value	0.512 <0.001*	0.564 <0.001*	0.294 <0.011*			
Tumor stage rho p value	0.579 <0.001*	0.408 <0.001*	0.676 <0.001*	0.495 <0.001*		
Age rho p value	-0.092 0.438	-0.147 0.213	0.051 0.664	-0.027 0.820	0.008 0.947	

*Correlation is significant at the 0.05 level (two-tailed). Rho: Correlation coefficient, p: Spearman correlation analysis, SUV_{max} : Maximum standardized uptake value

when compared with the latest literature findings. One missense point mutation in exon 27 of BRCA2 was likely benign, and five missense point mutation in various exons in BRCA2 were of VUS. Two pathogenic and 16 pathogenic point mutations were detected in the present breast tumor cohort (Table 4).

An example of case of BRCA1-positive BC is shown in Figure 2, 3. Figure 2 shows the NGS mutated profiles for BRCA1 for this case. Figure 3 shows the ^{18}F -FDG PET/CT of axial slices of the same case. In this BRCA1-positive case, the high SUV_{max} and the presence of a conglomerate of metastatic axillary LAP are observed as indicators of poor prognosis.

Discussion

This study was conducted to investigate the prognostic value of BRCA1-2 germline mutations in patients with BC by comparing ^{18}F -FDG PET/CT findings. Various clinical and meta-analytic studies indicate that patients with cancer having a high primary SUV_{max} may have a worse prognosis (16,17,18). Moreover, SUV_{max} has been reported to be a prognostic factor of BC (16,19,20). For example, Kitajima et al. (21) performed a prospective study to compare PET/CT and magnetic resonance imaging findings and reported that the preoperative SUV_{max} of primary BC lesion is a prognostic factor, but not apparent diffusion coefficient. In addition, Ravina et al. (12) claimed that the pretreatment

tumor SUV_{max} could be used as an independent imaging biomarker of poor prognosis. In a review study, Caresia Aroztegui et al. (22) revealed that baseline tumor glycolytic activity is associated with biology and prognosis.

Many parameters affecting prognosis such as patient age, tumor size, expression of HER-2, and estrogen and progesterone hormone receptors have also been defined in BC (23,24,25,26). However, data about the effect of BRCA mutation on BC prognosis are limited and varied. Many studies have indicated that the presence of BRCA mutations reduces or does not affect overall survival of patients with BC when compared with those having sporadic BC and even leads to good surveillance (8,10,27,28,29,30). A meta-analysis assessing the association of BRCA mutations with survival in patients with BC claimed that BRCA1 and BRCA2 mutations were associated with poor overall survival in patients with BC, but had no significant impact on BC-specific survival or event-free survival (31). Similarly, Taneja et al. (32) reported that BRCA1/2 carriers with BC often show high nuclear grades and, thus, are associated with poor prognosis.

However, De Talhouet et al. (3) reported that the effect of mutation on prognosis depends on BC subtypes; in the non-triple-negative BC group, the BRCA1/2 mutations did not have any impact on survival, whereas in the triple-negative BC group, BRCA1/2 germline mutations are associated with prolonged survival.

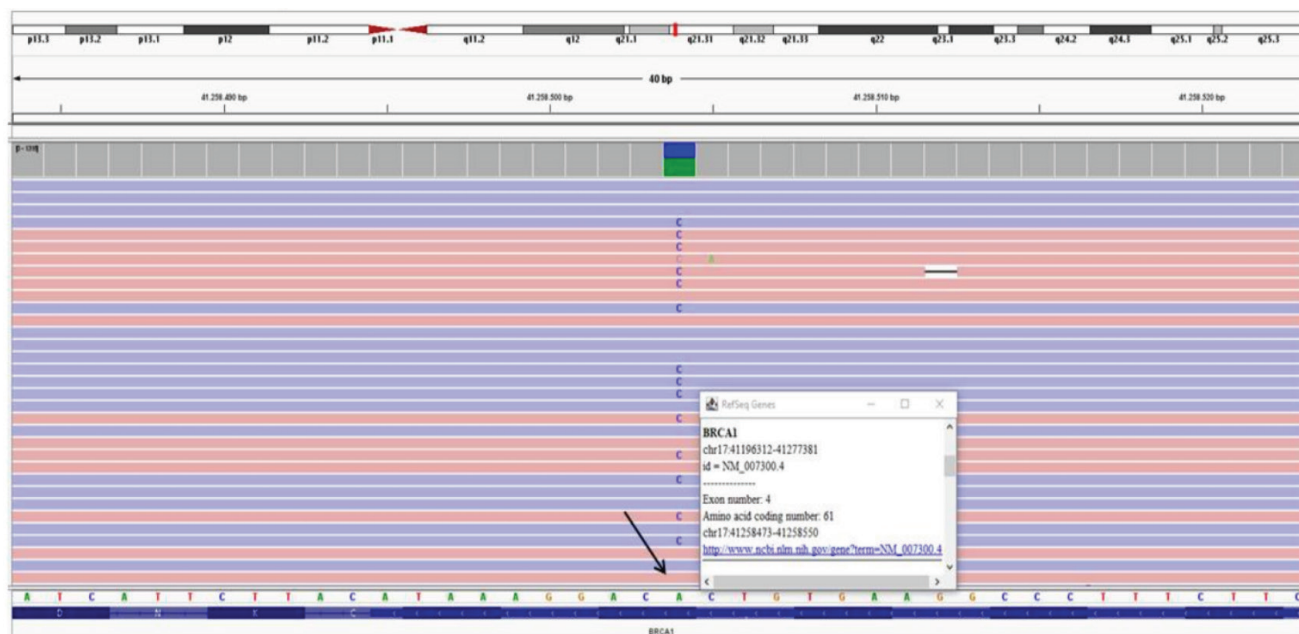


Figure 2. NGS mutated profiles of *BRCA1* gene for a 54-year-old patient with breast cancer. Arrow indicates the germline A>C transversion in codon 61 for exon 4

NGS: Next-generation sequencing

Table 4. Mutated genes, exon, codon, base substitution, mutation type, and clinical significance of germline BRCA1-2 mutation profiles detected in patients with breast cancer

Case no	BRCA gene		Mutation		Clinical significance			
	1	2	Location	Type	LB	VUS	PP	P
1	+		Exon 10 c.3835G>A (p.Ala1279Thr)	Missense				+
		+	Exon 27 c.9934A>G (p.Ile3312Val)	Missense			+	
2		+	Exon 10 c.943T>A (p.Cys315Ser)	Missense		+		
3		+	Exon 27 c.9976A>T (p.Lys3326Ter)	Missense		+		
4	+		Exon 4 c.181T>G (p.Cys61Gly)	Missense				+
		+	Exon 10 c.1792A>G (p.Thr598Ala)	Missense		+		
5	+		Exon 10 c.2800C>T (p.Q934*)	Frameshift				+
6		+	Exon 11 c.44914_4915insA (p.val1639fs)	Missense				+
7		+	Exon 26 c.9586A>G (p.Lys3196Glu)	Missense				+
8		+	Exon 11 c.6613G>A (p.Val2205Met)	Missense		+		
9		+	Exon 27 c.9934A>G (p.I3312V)	Missense		+		
10		+	Exon 22 c.8940delA (p.Glu2981Lysfs*7)	Frameshift				+
11	+		Exon 4 c.182T>G (p.Cys61Gly)	Missense				+
12	+		Exon 11 c.3333delA (p.E1112fs*5)	Frameshift				+
13		+	Exon 19 c.8452G>A (p.Val281Ile)	Missense			+	
14		+	Exon 22 c.8940delA (p.Glu2981Lysfs*8)	Frameshift				+
15		+	Exon 7 c.599C>T (p.Thr200Ile)	Missense				+
16	+		Exon 10 c.3333delA (p.Glu1112AsnfsTer5)	Frameshift				+
		+	Exon 27 c.9976A>T (p.Lys3326Ter)	Missense	+			
17	+		Exon 20 c.5329dupC (p.Gln1777fsTer*74)	Frameshift				+
18		+	Exon 11 c.5427C>A (p.Cys1809ter)	Missense				+

LB: Likely benign, VUS: Variants of uncertain significance, PP: Probably pathogenic, P: Pathogenic

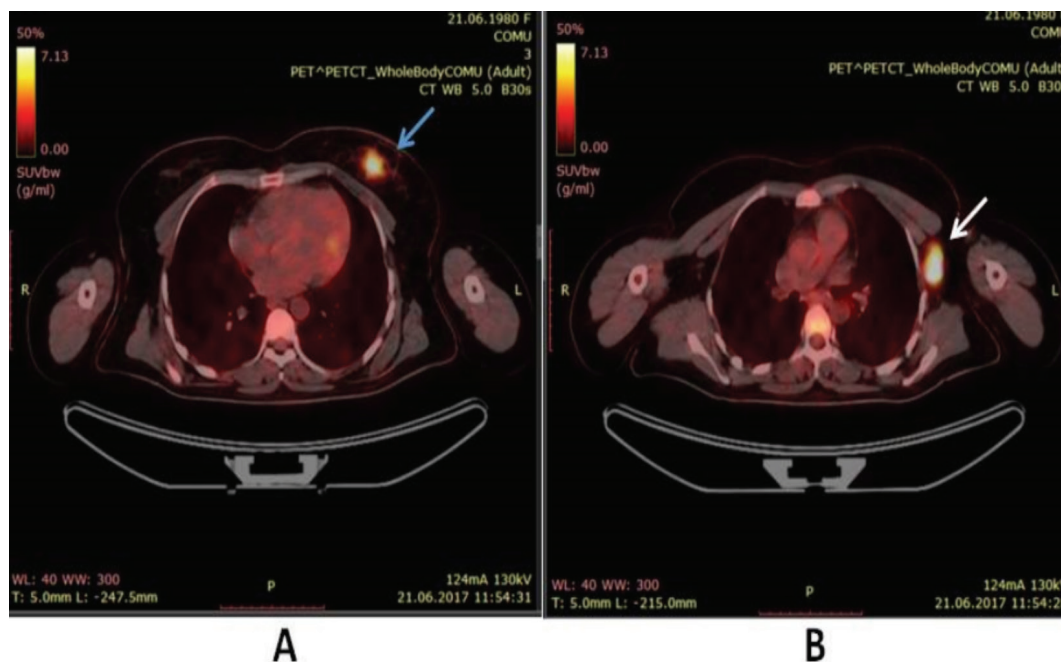


Figure 3. ^{18}F -FDG PET/CT images of axial slices. The blue arrow indicates increased ^{18}F -FDG (SUV_{max} of 8.6) uptake of 25-mm primary breast lesion in the left breast of the patient with germline *BRCA1* gene point mutation shown in Figure 2. The white arrow also indicates increased ^{18}F -FDG (SUV_{max} of 9.7) uptake in conglomerate LAP in left axillary level III in the same case

^{18}F -FDG PET/CT: ^{18}F Fluoride-fluorodeoxyglucose positron emission tomography/computed tomography, SUV_{max} : Maximum standardized uptake value

In this study, BRCA mutations were detected in 19.5% of 92 patients with BC. The mutation rate is higher than that in the normal population because *BRCA* gene analyses were performed in patients with a family history of BC. *BRCA1/2* germline mutations are more common in patients with a family history of BC. The incidence of *BRCA1-2* mutation in unselected patients with BC is 5-10% in most populations. In the literature, the age at BC diagnosis is lower in patients with germline *BRCA* mutation than in those with sporadic cancer (33,34). However, our cohort consisted of *BRCA*-positive and wild-type BC cases with positive BC family histories, and no significant difference was found between the groups in terms of age at diagnosis and the number of patients aged <50 years.

The Ki-67 index, which is a cell proliferation marker in predicting BC prognosis, has also been investigated, and conflicting results have been reported. In addition, correlation levels between Ki-67 index and SUV_{max} varied from low to high (35,36,37). The results of the present study showed a strong correlation between SUV_{max} and Ki-67 values. SUV_{max} also correlated moderately with tumor size and highly correlated with tumor grade and stage. The finding of comparable correlation rates between the groups and the lack of difference was consistent with other results.

Remarkably, in the present study, the rate of hormone receptor (estrogen and/or progesterone) positivity was significantly higher in the *BRCA*-negative group ($p=0.010$). In the literature, *BRCA1* carriers were more likely to be estrogen or progesterone receptor-negative, whereas those with *BRCA2*-mutated BC were more likely to be estrogen-negative or progesterone-positive (33). However, owing to the small number of our patients, we could not analyze the difference between *BRCA1* and *BRCA2* in terms of the presence of hormone receptors. Likewise, the most common subtype was Luminal A (HR+/HER-2-) in 55.4% and the least common subtype was HER-2 enriched (HR-/HER-2+) in 2.7% of the *BRCA*-negative group. Hormone receptor-positive BC has a better prognosis. Therefore, based on this information alone, it would not be wrong to claim that *BRCA*-negative BC will show a better prognosis. Moreover, the SUV_{max} , which is already used routinely to predict prognosis, was higher in the *BRCA*-positive group, which supports the prediction of poor prognosis. In addition, the rate of having a larger tumor size and axillary lymph node involvement was higher in the *BRCA*-positive group than in the *BRCA*-negative group, and this finding support our claim for a worse prognosis.

In a Chinese cohort, *BRCA* mutation carriers were more likely to have lymph node involvement upon BC diagnosis.

Even after adjusting the clinical prognostic factors, the results were significantly worse, suggesting that BRCA mutation is an independent factor of poor prognosis (38). Mori et al. (39) also have published similar results that patients with BRCA tumors having a family history of BC were associated with a poor prognosis. Our results were similar to the results of these two studies.

Study Limitations

The major limitation of this study was the small population size. Thus, the results of this study needs to be confirmed by larger-scale studies to clarify the prognostic value of BRCA mutations. In addition, these results should be supported by studies evaluating the relationship of BRCA mutation status with clinical outcomes such as disease-free survival and overall survival.

Conclusion

The prognostic role of germline BRCA1 or BRCA2 point mutations in patients with BC is unclear. In this retrospective study, we analyzed and compared the interdisciplinary laboratory findings derived from 92 patients with BC of which 18 were BRCA mutation carriers. Various structural point mutations were also found, e.g., 6 missense and 15 frameshift mutations were detected in 19.5% of BRCA mutation carriers, and a strong association was found between SUV_{max} values. The results of this study show a strong correlation between BRCA mutations and SUV_{max}, which indicate the poor prognosis of BC. Risk stratification based on this finding can play a very important role in the management of patients with BRCA-positive BC. Based on the current results, it is possible to estimate the strong association of BC-susceptible BRCA gene variants with SUV_{max} criteria that indicates the poor prognosis of BC. However, the interpretation of the results of this study are limited by the retrospective study design, high risk of selection bias, lack of data about disease treatment related to BRCA mutation carriers and non-carriers, and the relatively small sample size. The results of this study suggest that germline BRCA1 and BRCA2 mutation status has a significant prognostic value and those remarks are strongly supported by SUV_{max}. Clearly, further prospective and/or retrospective studies with larger sample size are needed to clarify the interdisciplinary laboratory corrections in patients with BC.

Acknowledgments

The authors thank the family members who agreed to participate in this study.

Ethics

Ethics Committee Approval: The study was approved by the Çanakkale Onsekiz Mart University Ethics Committee (protocol no: 2020-04, date: 26.02.2020).

Informed Consent: All patients provided written informed consent.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: S.O., Y.A., Concept: S.O., F.S., O.O., Design: S.O., F.S., O.O., Data Collection or Processing: S.O., N.A., F.K.O., I.C., Analysis or Interpretation: S.O., F.K.O., Literature Search: S.O., O.O., F.S., Writing: S.O., O.O.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

References

1. Baglia ML, Cook LS, Mei-Tzu C, Wiggins C, Hill D, Porter P, Li CI. Alcohol, smoking, and risk of Her2-overexpressing and triple-negative breast cancer relative to estrogen receptor-positive breast cancer. *Int J Cancer* 2018;143:1849-1857.
2. Brewer HR, Jones ME, Schoemaker MJ, Ashworth A, Swerdlow AJ. Family history and risk of breast cancer: an analysis accounting for family structure. *Breast Cancer Res Treat* 2017;165:193-200.
3. De Talhouet S, Peron J, Vuilleumier A, Friedlaender A, Viassolo V, Ayme A, Bodmer A, Treilleux I, Lang N, Tille JC, Chappuis PO, Buisson A, Giraud S, Lasset C, Bonadona V, Trédan O, Labidi-Galy SI. Clinical outcome of breast cancer in carriers of BRCA1 and BRCA2 mutations according to molecular subtypes. *Sci Rep* 2020;10:7073.
4. Kemp Z, Turnbull A, Yost S, Seal S, Mahamdallie S, Poyastro-Pearson E, Warren-Perry M, Eccleston A, Tan MM, Teo SH, Turner N, Strydom A, George A, Rahman N. Evaluation of Cancer-Based Criteria for Use in Mainstream BRCA1 and BRCA2 Genetic Testing in Patients With Breast Cancer. *JAMA Netw Open* 2019;2:e194428.
5. Kim EK, Park SY, Kim SW. Clinicopathological characteristics of BRCA-associated breast cancer in Asian patients. *J Pathol Transl Med* 2020;54:265-275.
6. Nicolini A, Ferrari P, Duffy MJ. Prognostic and predictive biomarkers in breast cancer: Past, present and future. *Semin Cancer Biol* 2018;52:56-73.
7. Huszno J, Kolosza Z, Grzybowska E. BRCA1 mutation in breast cancer patients: Analysis of prognostic factors and survival. *Oncol Lett* 2019;17:1986-1995.
8. Copson ER, Maishman TC, Tapper WJ, Cutress RI, Greville-Heygate S, Altman DG, Eccles B, Gerty S, Durcan LT, Jones L, Evans DG, Thompson AM, Pharoah P, Easton DF, Dunning AM, Hanby A, Lakhani S, Eeles R, Gilbert FJ, Hamed H, Hodgson S, Simmonds P, Stanton L, Eccles DM. Germline BRCA mutation and outcome in young-onset breast cancer (POSH): a prospective cohort study. *Lancet Oncol* 2018;19:169-180.
9. van den Broek AJ, Schmidt MK, van 't Veer LJ, Tollenaar RA, van Leeuwen FE. Worse breast cancer prognosis of BRCA1/BRCA2 mutation carriers: what's the evidence? A systematic review with meta-analysis. *PLoS One* 2015;10:e0120189.
10. Baretta Z, Mocellin S, Goldin E, Olopade OI, Huo D. Effect of BRCA germline mutations on breast cancer prognosis: A systematic review and meta-analysis. *Medicine (Baltimore)* 2016;95:e4975.

11. Ulaner GA, Castillo R, Goldman DA, Wills J, Riedl CC, Pinker-Domenig K, Jochelson MS, Gönen M. (18)F-FDG-PET/CT for systemic staging of newly diagnosed triple-negative breast cancer. *Eur J Nucl Med Mol Imaging* 2016;43:1937-1944.
12. Ravina M, Saboury B, Chauhan MS, Jacob MJ, Pandit AG, Sanchety N, Werner TJ, Alavi A. Utility of 18 F-FDG PET/CT in pre-surgical risk stratification of patients with breast cancer. *Hell J Nucl Med* 2019;22:165-171.
13. Kang S, Kim EH, Hwang JE, Shin JH, Jeong YS, Yim SY, Joo EW, Eun YG, Lee DJ, Sohn BH, Lee SH, Lim B, Lee JS. Prognostic significance of high metabolic activity in breast cancer: PET signature in breast cancer. *Biochem Biophys Res Commun* 2019;511:185-191.
14. Has Şimşek D, Şanlı Y, Külle CB, Karanlık H, Kiliç B, Kuyumcu S, Önder S, Özmen V. Correlation of 18F-FDG PET/CT with pathological features and survival in primary breast cancer. *Nucl Med Commun* 2017;38:694-700.
15. Diao W, Tian F, Jia Z. The prognostic value of SUVmax measuring on primary lesion and ALN by 18F-FDG PET or PET/CT in patients with breast cancer. *Eur J Radiol* 2018;105:1-7.
16. Yoo J, Yoon HJ, Kim BS. Prognostic value of primary tumor SUVmax on F-18 FDG PET/CT compared with semi-quantitative tumor uptake on Tc-99m sestamibi breast-specific gamma imaging in invasive ductal breast cancer. *Ann Nucl Med* 2017;31:19-28.
17. Pakk K, Joung C, Kim S. Visceral fat metabolic activity evaluated by preoperative 18F-FDG PET/CT significantly affects axillary lymph node metastasis in postmenopausal luminal breast cancer. *Sci Rep* 2020;10:1348.
18. Refaat R, Abd Alkhalik Basha M, Sobhi Hassan M, Hussein RS, Al-Molla RM, Awad NM, Elkholi E. Is FDG maximum standardized uptake value of primary breast cancer a prognostic factor for locoregional axillary lymph node metastasis? *Acta Radiol* 2019;60:1241-1250.
19. Taghipour M, Wray R, Sheikhbahaei S, Wright JL, Subramaniam RM. FDG Avidity and Tumor Burden: Survival Outcomes for Patients With Recurrent Breast Cancer. *AJR Am J Roentgenol* 2016;206:846-855.
20. Ahn SG, Park JT, Lee HM, Lee HW, Jeon TJ, Han K, Lee SA, Dong SM, Ryu YH, Son EJ, Jeong J. Standardized uptake value of ¹⁸F-fluorodeoxyglucose positron emission tomography for prediction of tumor recurrence in breast cancer beyond tumor burden. *Breast Cancer Res* 2014;16:502.
21. Kitajima K, Miyoshi Y, Yamano T, Odawara S, Higuchi T, Yamakado K. Prognostic value of FDG-PET and DWI in breast cancer. *Ann Nucl Med* 2018;32:44-53.
22. Caresia Aroztegui AP, García Vicente AM, Alvarez Ruiz S, Delgado Bolton RC, Orcajo Rincon J, Garcia Garzon JR, de Arcocha Torres M, Garcia-Velloso MJ; Oncology Task Force of the Spanish Society of Nuclear Medicine and Molecular Imaging. 18F-FDG PET/CT in breast cancer: Evidence-based recommendations in initial staging. *Tumour Biol* 2017;39:1010428317728285.
23. Allison KH, Hammond MEH, Dowsett M, McKernin SE, Carey LA, Fitzgibbons PL, Hayes DF, Lakhani SR, Chavez-MacGregor M, Perlmutter J, Perou CM, Regan MM, Rimm DL, Symmans WF, Torlakovic EE, Varella L, Viale G, Weisberg TF, McShane LM, Wolff AC. Estrogen and Progesterone Receptor Testing in Breast Cancer: ASCO/CAP Guideline Update. *J Clin Oncol* 2020;38:1346-1366.
24. Park C, Park K, Kim J, Sin Y, Park I, Cho H, Yang K, Bae BN, Kim KW, Ahn S, Gwak G. Prognostic values of negative estrogen or progesterone receptor expression in patients with luminal B HER2-negative breast cancer. *World J Surg Oncol* 2016;14:244.
25. Li S, Wu J, Huang Q, He J, Zhu L, Chen W, Li Y, Chen X, Shen K. HER2 positivity is not associated with adverse prognosis in high-risk estrogen receptor-positive early breast cancer patients treated with chemotherapy and trastuzumab. *Breast* 2020;54:235-241.
26. Provencher L, Diorio C, Hogue JC, Doyle C, Jacob S. Does breast cancer tumor size really matter that much? *Breast* 2012;21:682-685.
27. Maksimenko J, Irmejs A, Nakazawa-Miklasevica M, Melbarde-Gorkusa I, Trofimovics G, Gardovskis J, Miklasevics E. Prognostic role of BRCA1 mutation in patients with triple-negative breast cancer. *Oncol Lett* 2014;7:278-284.
28. Ruiz de Sabando A, Urrutia Lafuente E, García-Amigot F, Alonso Sánchez A, Morales Garofalo L, Moreno S, Ardanaz E, Ramos-Arroyo MA. Genetic and clinical characterization of BRCA-associated hereditary breast and ovarian cancer in Navarra (Spain). *BMC Cancer* 2019;19:1145.
29. Song Y, Barry WT, Seah DS, Tung NM, Garber JE, Lin NU. Patterns of recurrence and metastasis in BRCA1/BRCA2-associated breast cancers. *Cancer* 2020;126:271-280.
30. Zhu Y, Wu J, Zhang C, Sun S, Zhang J, Liu W, Huang J, Zhang Z. BRCA mutations and survival in breast cancer: an updated systematic review and meta-analysis. *Oncotarget* 2016;7:70113-70127.
31. Jacot W, Lopez-Crapez E, Mollevi C, Boissière-Michot F, Simony-Lafontaine J, Ho-Pun-Cheung A, Chartron E, Theillet C, Lemoine A, Saffroy R, Lamy PJ, Guiu S. BRCA1 Promoter Hypermethylation is Associated with Good Prognosis and Chemosensitivity in Triple-Negative Breast Cancer. *Cancers (Basel)* 2020;12:828.
32. Taneja P, Maglic D, Kai F, Zhu S, Kendig RD, Fry EA, Inoue K. Classical and Novel Prognostic Markers for Breast Cancer and their Clinical Significance. *Clin Med Insights Oncol* 2010;4:15-34.
33. Foulkes WD, Metcalfe K, Sun P, Hanna WM, Lynch HT, Ghadirian P, Tung N, Olopade OI, Weber BL, McLennan J, Olivetto IA, Bégin LR, Narod SA. Estrogen receptor status in BRCA1- and BRCA2-related breast cancer: the influence of age, grade, and histological type. *Clin Cancer Res* 2004;10:2029-2034.
34. Rosenberg SM, Ruddy KJ, Tamimi RM, Gelber S, Schapira L, Come S, Borges VF, Larsen B, Garber JE, Partridge AH. BRCA1 and BRCA2 Mutation Testing in Young Women With Breast Cancer. *JAMA Oncol* 2016;2:730-736.
35. Surov A, Meyer HJ, Wienke A. Associations Between PET Parameters and Expression of Ki-67 in Breast Cancer. *Transl Oncol* 2019;12:375-380.
36. Soliman NA, Yussif SM. Ki-67 as a prognostic marker according to breast cancer molecular subtype. *Cancer Biol Med* 2016;13:496-504.
37. Deng SM, Zhang W, Zhang B, Chen YY, Li JH, Wu YW. Correlation between the Uptake of 18F-Fluorodeoxyglucose (18F-FDG) and the Expression of Proliferation-Associated Antigen Ki-67 in Cancer Patients: A Meta-Analysis. *PLoS One* 2015;10:e0129028.
38. Chen L, Fu F, Huang M, Lv J, Zhang W, Wang C. The spectrum of BRCA1 and BRCA2 mutations and clinicopathological characteristics in Chinese women with early-onset breast cancer. *Breast Cancer Res Treat* 2020;180:759-766.
39. Mori H, Kubo M, Kai M, Velasquez VV, Kurata K, Yamada M, Okido M, Kuroki S, Oda Y, Nakamura M. BRCAness Combined With a Family History of Cancer Is Associated With a Poor Prognosis for Breast Cancer Patients With a High Risk of BRCA Mutations. *Clin Breast Cancer* 2018;18:e1217-e1227.