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Synuclein-γ Polymorphisms and radiotherapy for breast cancer: a retrospective study

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► ***Original article***

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ABSTRACT

Background: Synuclein γ (SNCG), a member of the brain protein synuclein family, is highly expressed in pervasive human breast carcinoma and was associated with poor outcomes in radiotherapy treatment. So, the present study was conducted to assess the C243G and A377T polymorphisms of this gene in relationship with the success of radiotherapy. ***Materials and Methods:*** In this retrospective study, 114 patients who had undergone a radical modified mastectomy and referred for radiation therapy were studied. Genomic DNA was extracted from peripheral blood cells. Genotyping was done for C243G and A377T polymorphisms. Differences in allele or genotype frequencies were evaluated. ***Results:*** The median overall Survival (OS) Rate of all patients was 44 months (IQR: 5.09). Genotyping the patients, there were 25 (%) GG, 34 (%) CG, and 55 (%) CC patients for exon 3 polymorphism (C243G). Comparison of three genotypes effect on OS was not significant (X^2 (2) =2.25, P=0.323). Hazard Ratio of CC vs. GG was 1.042 (95%CI:0.5360-2.027), which was not statistically significant. There were 60 (%) AA, 37 (%) AT, and 17 (%) TT patients for exon 4 polymorphism (A377T). Comparison of three genotypes effect on OS was not significant (X^2(2) =0.441, P=0.802). Comparing only patients with AA and TT genotypes, Hazard Ratio of AA vs. TT was 1.26 (95%CI:0.5123-3.118), which was not statistically significant. ***Conclusion:*** There is a possibility of the role of GG and TT genotypes as a genetic risk factor in breast cancer which should be evaluated in a study with higher sample size.

Keywords: *Synuclein γ, overall survival, breast cancer, radiotherapy.*

INTRODUCTION

Radiation therapy (also called radiotherapy, X-ray therapy, or radiation) is the use of a certain type of energy (ionizing radiation) to kill cancer cells and shrink a tumor [1]. Radiation therapy damages cancerous cells (target tissue) or kills them by destroying genetic structures, making it impossible for cancerous cells to grow and divide. Although radiation may kill both cancer cells and healthy cells, most healthy cells recover completely from the effects of radiation. The goal of radiation therapy is to destroy the cells as much as possible while minimizing damage to healthy tissues [2]. There are different types of radiation and there are different ways to irradiate. The choice of treatment for breast cancer depends primarily on the stage of the disease, which includes surgery history, radiation therapy, chemotherapy, hormone therapy, and biological therapy [3]. The need for radiation therapy varies from person to person, depending on the type of surgery performed, the condition of the tumor margin after surgery, and the extent of axillary lymph node involvement; However, in patients who have undergone breast surgery, it is an important and necessary treatment method in which the remaining breast mass, chest wall and in some cases axillary and neck lymph nodes are also irradiated [4,5]. Many

genetic factors are known to be associated with breast cancer and its progression. Recently, scientists have focused on genetical disturbances that may alter treatment progress [6]. Synucleins are a family of small proteins consisting of 3 members, α-synuclein α (SNCA), β-synuclein (SNCB) and Synuclein γ (SNCG) [8]. Synoclines are specifically implicated in neurological diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD). Mutations in SNCA are genetically linked to Parkinson's disease [8,9]. Synuclein γ (SNCG), a member of the brain protein synuclein family, is highly expressed in pervasive human breast carcinoma but not in normal or benign breast tissue. Synocline γ (SNCG) was first identified as a breast cancer-specific gene 1 (BCSG1) [9]. This gene is highly expressed in advanced and metastatic breast tumors but is not normal in breast epithelial tissues [9]. Min *et al.* have recently shown that overexpression of SNCG predicts lack of benefit from radiotherapy for breast cancer patients [10]. Also, in various other cancers SNCG expression was associated with response to cancer treatment [11]. while, previous studies have examined the increased expression of synuclein-γ in breast cancer and shown the role of this factor in reducing response to treatment [11]; there are not enough evidences about this issue in clinical settings and the present study investigated the possible relationship between genetic differences in individuals in terms of polymorphisms of this gene and its possible effect on radiotherapy response.. So, the present study was performed to determine the polymorphisms of this gene in a population of women with breast cancer and its relationship with the success of radiotherapy.

MATERIALS AND METHODS

Study population

In this retrospective study, which was based on the records of radiation 114 patients, who were treated from 2014 to 2019 were studied. These were patients who had undergone a radical modified mastectomy and had been referred for radiation therapy. Conditions that led to the choice of radiation therapy for these patients include near or positive margins of surgery, involvement of at least 3 axillary lymph nodes, lesions larger than 5 cm, high-grade malignant chest involvement, invasion of lymphatic vessels and other vessels. According to the pathology report.

Radiation therapy protocol

In radiotherapy of these patients, two tangential fields were used to treat the chest wall and the supraclavicular and axillary corneas were treated with two separate fields. The mean follow-up time in these patients was 69 months. The treatment results in these two groups were compared in terms of local-regional recurrence time.

Genetic assessment

DNA was extracted from peripheral blood samples by standard techniques (RayBio® kit of SNCG PCR, RayBiotech Life, US). Flanking primers were used for genotyping and amplification of exon 3 and 4 as shown in table 1.

Table 1. Flanking primers.

	Primers
Exon 3	PSYN3F (5' TGCGAGCCTGACTCCAGCAG 3')
	PSYN3R (5' GGTGTGGAGTGGAGTGATGC 3')
Exon 4	PSYN4F (5' TTGAGGCCAGGGTAGACAAG 3')
	PSYN4R (5' CCACTCAGGTTCAGGGTTAG 3)

Reactions were performed based on Krüger study using a volume of 10 ul containing 200 nanograms genomic DNA (DNA), 4 pmol of each oligonucleotide, 0.2 millimoles of each of dATP, DGTP, STTP and dCTP, 3 mM MgCl, 1 unit Taq polymerase and I ul of polymerase chain reaction (PCR) buffer w/o MgCly. PCR was conducted with a temperature of 94 ° C for 1 min, a temperature of annealing 64 ° C for 1 min and an extension temperature of 72 ° C for 1 min, performed for 30 cycles.PCR items have been digested with the endonuclease control Hphl. In the case of exon 3 polymorphism (C243 G) a new phl-restriction site was generated by G allele. So Hphl-digestion culminated in 2 extra segments interpreted on agarose gels of 2 per

cent. The T allele was observable by Hphl-digestion for the A377 T polymorphism in exon 4, as a Hphl restriction site was lacking [12].

Statistical analysis

Differences in allele or genotype frequencies were evaluated by chi-square analysis and Mantel-Cox method. A P-value less than 0.05 was accepted as statistically significant. All statistical analysis were performed in SPSS version 19 and visualized by Graphpad Prism software, version 8.

RESULTS

Mean age of our study participants was 59±12.5. Among patient's size of the tumor was higher than 2 cm in 49.12% (n=56). The median overall Survival (OS) Rate of all patients was 44 months (IQR: 5.09). Genotyping the patients, there were 25 (%) GG, 34 (%) CG, and 55 (%) CC patients for exon 3 polymorphism (C243G), as shown in table 2. The median OS of patients with CC genotype was 42 months. The median OS of patients with CG genotype was 51 months and 39 months in GG patients. Comparison of three genotypes effect on OS was not significant based on the Log-rank (Mantel-Cox) test (X2(2) =2.25, P=0.323). Comparing only patients with CC and GG genotypes, Hazard Ratio (Mantel-Haenszel) of CC vs. GG was 1.042 (95% CI of ratio 0.5360 to 2.027), which was not statistically significant (figure 1).

Table 2. Subject's genotyping results.

	C243G			A377T		
	CC	CG	GG	AA	AT	TT
Number	25	34	55	60	37	17
%	21.93	29.82	48.25	52.63	32.46	14.91
OS, median	42	51	39	43	43	32

There were 60 (%) AA, 37 (%) AT, and 17 (%) TT patients for exon 4 polymorphism (A377T). The median OS of patients with AA genotype was 43 months and 32 months in TT patients, and OS of AT genotype was 43 months. Comparison of three genotypes effect on OS was not significant based on the Log-rank test (X2(2) =0.441, P=0.802). Comparing only patients with AA and TT genotypes, Hazard Ratio (Mantel-Haenszel) of AA vs. TT was 1.26 (95% CI of ratio 0.5123 to 3.118), which was not statistically significant (figure 2).

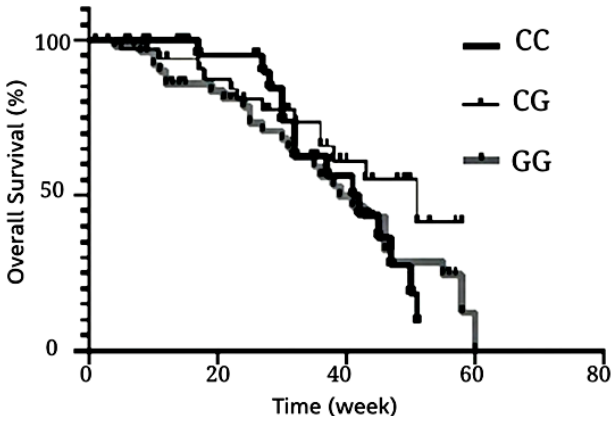

Figure 1. Kaplan–Meier curve of OS based on the C243G polymorphism.

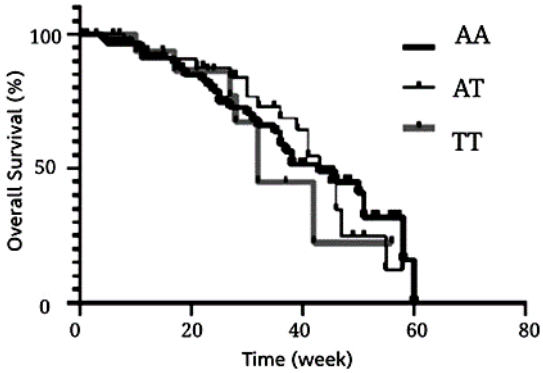

Figure 2. Kaplan–Meier curve of OS based on the A377T polymorphism.

DISCUSSION

Breast cancer in women is a major public health problem worldwide. This disease is the second most common cancer in humans (men and women) and the first most common cancer in women in Iran and the world. Breast cancer accounted for 23% of all new cancers and 14% of all cancer deaths in 2012 [5-7]. Risk factors for breast cancer include: Family history of breast cancer, genetic status, personal history of breast cancer, formation of abnormal cells in the lobules or mammary glands in breast tissue, breast volume, levels of endogenous hormones,

Menstrual cycles, pregnancy, breastfeeding, bone density, lifestyle factors such as postmenopausal hormone use, obesity and overweight, physical activity, diet, alcohol and tobacco use, anti-smoking pills Pregnancy and other risk factors such as radiation, use of certain medications, environmental and occupational pollution, and genetic factors associated with breast cancer [6,7]. Breast cancer is a highly heterogeneous disease caused by the interaction of hereditary and environmental risk factors and leads to the progressive accumulation of genetic and epigenetic changes in breast cancer cells. Although epidemiological evidence suggests that there are specific risk factors (such as age, obesity, and alcohol consumption), a family history of breast cancer is the strongest risk factor for the disease [5-7]. The results of many studies suggest that polymorphisms can increase the susceptibility to cancer by altering the DNA sequence. A limited number of epidemiological studies have evaluated the association between C243G and A377T polymorphisms of the synuclein-γ gene and breast cancer risk. Synuclein-γ (SNCG) is a member of the synuclein family which is a small, soluble, highly conserved group of neuronal proteins that have been implicated in both neurodegenerative diseases and cancer [13]. It was first named breast cancer-specific gene 1 (BCSG1) due to its highly specific expression in advanced stages of breast cancer compared to its undetectable level in normal or benign breast lesions [14].

Our results showed that there is no statistically significant relationship between the studied polymorphisms and the amount of OS. However, individuals with GG and TT genotypes had lower OS levels. Conducting this study in a larger sample size seems to help in the analysis of this issue. The number of patients followed up in this pilot clinical study was relatively small. However, it can be concluded from our study that the observed effect of the synuclein-γ gene relationship and the progression of breast cancer occurs more epigenetically. In our study, other factors such as age, hormone receptors and type of radiotherapy were not considered, but the selected patients were tried to be similar in terms of stage of cancer to eliminate this bias.

Min *et al.* have recently shown that overexpression of SNCG predicts lack of benefit from radiotherapy for breast cancer patients [10]. Thus, our results are in complete agreement with this study and reinforce the idea that SNCG expression may serve as a potential biomarker to identify breast cancer patients who are less likely to benefit from radiotherapy. SNCG expression has been reported in several other human cancers. Since new radiotherapy techniques have expanded the indication of radiotherapy for the treatment of HCC, it would be of interest to determine the potential effect of SNCG in these cells [11].

Kang *et al.* reported that radiotherapy increases synuclein-γ expression in the MCF7 cell line. synuclein-γ can suppress the immune system by inhibiting the differentiation and activation of dendritic cells. However, the association between synuclein-γ and radiotherapy is unclear [15]. In another study, synuclein-γ mRNA was seen in neoplastic breast epithelial cells. This was not the case in healthy cells. The link between synuclein-γ expression and breast cancer progression has interested researchers in investigating the role of synuclein -γ as a prognostic marker for breast cancer [16]. In a study, it was shown that synuclein-γ gene mRNA expression is highly correlated with breast cancer stage [17].

To date, limited studies have been performed on the genetic variants of synuclein-γ genetic polymorphisms associated with breast cancer. According to recent studies, some functional genetic variants in the genes encoding synuclein-γ, through cell defense mechanisms, affect the susceptibility to cancer.

These polymorphisms may cause different functions in synuclein-γ genes in different individuals depending on the type of allele. Therefore, given the important role that synuclein-γ plays in predicting breast cancer cherogenosis, its altered function may be associated with breast cancer risk.

Thus, although the status of lymph node involvement was similar in our patients, factors such as estrogen receptors were not included in our study. Functionally, synuclein-γ was

significantly hormone-dependent in breast cancer cells in vitro and in mice [18].

Different results may be happening if the study get repeated in different populations, due to differences in genetic resources, as well as the influence of genetic factors and the environment. In this study, only two functional polymorphisms were investigated and no environment-gene interactions were investigated. Since breast cancer susceptibility can be affected by interactions between several polymorphisms as well as interactions between polymorphisms and the environment, and also because the frequency of alleles varies in different populations, it is necessary to study this in other populations.

CONCLUSION

In general, based on the results of this study, there was no significant relationship between synuclein-γ gene polymorphisms and breast cancer, but there is a possibility of the role of GG and TT genotypes as a genetic risk factor in breast cancer susceptibility in the study population. This issue was not evaluated in any previous study. A closer look at the issue requires a study with a larger sample size. Considering the limitations of the present study, including the small size of the population and due to the complex etiology of this disease, it is necessary to conduct further studies in larger populations with other genetic and environmental factors to reach an accurate and comprehensive conclusion.

***Conflicts of interest*:** *Declared none.*

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