Tıp Fakültesi Klinikleri Cilt 2 • Sayı 2 • Nisan 2019 (23-27)

Evaluation of the Relationship between eNOS and Breast Cancer

Dilek ERDEM[1], Mahmut BUYUKSIMSEK[1], Meral GUNALDI[2], Nilgun ISIKSACAN[3], Sacide PEHLIVAN[4]

Abstract

Objective: The aim of this study was to determine the relationship between polymorphisms of endothelial nitric oxide synthase (eNOS) gen and development of breast cancer.

Material and methods: Thirty-seven cases with breast cancer and seventy healthy controls were enrolled in the present study. The study focused on three functional variants; a variant in a variable number of 27 bp tandem repeats in intron 4 (VNTR) of eNOS gene. We genotyped these variants using the polymerase chain reaction (PCR) and/or PCR-restriction fragment length polymorphism (RFLP) method. The distribution of allele and genotype in eNOS was compared between cases with breast cancer and healthy controls using chi-square test.

Results: With regard to the eNOS (VNTR) variant, significantly decreased breast cancer risk was found for eNOS BB polymorphism (OR=0.56; 95%CI:0.463-0.676; p=0.001). No statistical association were found between the eNOS AA and AB polymorphisms and breast cancer risk (p=0.223 and 0.487).

Conclusion: In the current study, eNOS BB, was found to be associated with breast cancer. In contrast, eNOS AA genotype was not associated with breast cancer. Further studies are needed to determine whether these gene polymorphisms have a place in diagnosis or determining the risk of disease.

Keywords: Breast cancer, Endothelial nitric oxide synthase, Risk factor

[1]Department of Medical Oncology, Bahcesehir University, Samsun Medical Park Hospital, Samsun, Turkey
[2]Department of Medical Oncology, Istanbul Aydin University, Florya WM Medical Park Hospital, Istanbul, Turkey.
[3]Department of Biochemistry, Bakirkoy Dr. Sadi Konuk Hospital, Istanbul, Turkey.
[4]Department of Medical Biology, Istanbul University, Istanbul Faculty of Medicine, Istanbul, Turkey.
Corresponding autor: Meral GUNALDI, Florya WM Medical Park Hospital, Beşyol Mahallesi İnönü Cad., 34295 Küçükçekmece/İstanbul, Tel: 444 2873, e-posta: meralgunaldi@gmail.com
Geliş Tarihi: 05.01.2019 Kabul Tarihi: 27.01.2019

Öz

Amaç: Çalışmanın amacı, endotelyal nitrik oksit sentaz (eNOS) gen polimorfizmleri ile meme kanseri gelişimi arasındaki ilişkiyi belirlemektir.

Gereç ve yöntem: Çalışmaya meme kanseri olan 37 olgu ve 70 sağlıklı kontrol dahil edildi. Çalışmada; eNOS geninin intron 4'ünde (VNTR) tekrar eden 27 bp tandem değişken sayısındaki bir varyant araştırıldı. Polimeraz zincir reaksiyonu (PCR) ve/veya PCR-kısıtlama fragman uzunluğu polimorfizmi (RFLP) metodunu kullanarak bu varyantları genotiplendirdik. eNOS'ta allel ve genotip dağılımı meme kanserli olgular ile ki-kare testi kullanılarak sağlıklı kontroller arasında karşılaştırıldı.

Bulgular: eNOS (VNTR) varyantı ile ilgili olarak, eNOS BB polimorfizmi için önemli ölçüde azalmış meme kanseri riski bulundu (OR = 0.56; %95CI:0.463-0.676; p=0.001). eNOS AA ve AB polimorfizmleri ile meme kanseri riski arasında istatistiksel bir ilişki bulunmadı (*p*=0.223 ve 0.487).

Sonuç: Bu çalışmada, eNOS BB'nin meme kanseri ile ilişkili olduğu bulundu. Buna karşılık, eNOS AA genotipi meme kanseri ile ilişkili değildi. Bu gen polimorfizmlerinin tanı veya hastalık riskini belirlemede bir yeri olup olmadığını belirlemek için ileri araştırmalara ihtiyaç vardır.

Introduction

Breast cancer is the most common cancer type in females. Although survival rates have increased within the last decade due to early diagnosis and advanced treatment methods, breast cancer still remains the leading cause of death for females (1,2).

The mechanism of the development of breast cancer is not clearly understood, although environmental factors and complex genetic changes have been proposed (3). Oxidative stress-related data have recently come onto the agenda regarding the relationship between genes and breast cancer. One of the most important mechanisms leading to tissue damage is oxidative stress, which is defined as excessive production of reactive oxygen species (ROS) (4).

The increase in ROS is considered to be mutagenic and carcinogenic. ROS contributes to the development of cancer as a result of changes in cell proliferation and inhibited apoptosis (5).In contrast, nitric oxide (NO) plays an important role in many metabolic processes such as vasodilation, immune response, platelet and leukocyte adhesion, yet, high concentrations of NO are thought to be effective in carcinogenesis. The catalyst in endothelial NO synthesis is endothelial nitric oxide synthase (eNOS). A variable number of tandem repeats (VNTR, 27 nt) in intron 4 is a functional variant which accounts for >25% of basal plasma NO production (6).

Material and Methods

Study Population

The study group included 37 breast cancer patients subjects and 70 healthy controls. All of the healthy controls were systemically healthy and non-smokers. The patients and controls were informed of the study's purpose and method and they all agreed to participate. The exclusion criteria were malignancy history, intake of antibiotics or anti-inflammatory drugs in the previous 6 months. A detailed medical history was taken, followed by a complete whole body examination. The patients and controls were from the same geographic areas. We genotyped the eNOS (intron 4 VNTR) polymorphism. Informed consent was obtained from each participant before blood sampling, and the study was approved by the local Ethical Committee with 2015/12/01, (13.07.2015) protocol number in terms of the study methods and protocols.

Genotyping analysis

The DNA of the participants was isolated from peripheral blood mononuclear cells using the Plus Blood Genomic DNA Purification Kit (GeneMark,). eNOS (intron 4 VNTR) gene variants was genotyped by polymerase chain reaction (PCR) restriction fragment length polymorphism (RFLP) analysis. For the internal quality control, twenty percent of the samples were duplicated in order to prevent sample or reading errors.

eNOS (VNTR) variant genotyping: eNOS intron 4 variant was analyzed by PCR using following primer: F:5'-AGGCCCTATGGTAGTGCCTTT-3', and R:5'-TCTCTTAGTGCTGTGGTCAC-3'. The PCR product (393 bp and/or 420 bp) was obtained. The products were then separated on 4% NuSieve GTG agarose gel. The experimental process was repeated twice for each sample (12). The amplified products of 363 bp were digested with MluI restriction enzyme (MBI Fermentas, St Leon-Rot, Germany) at 65°C producing fragments of 291 and 72 bp for the A Allele or fragments of 363 bp for the G allele. The insertion variant contains a duplication of a fragment shown in upper case as follows: 5′ -CCCTCTTTCCCCACC TCTTCCTTCCGCTCCTTTACCTACCACCTT-3′ .The polymorphic region was amplified with PCR, yielding products of 457 and 502 bp for the deletion and insertion, respectively (7). All PCR and/or digested products were separated on 2% ethidium bromide-stained agarose gels and visualized under ultraviolet transilluminator.

Statistical analysis

Data were analyzed using the computer software SPSS for Windows (version 13.0; SPSS, Inc., Chicago, IL, USA). The statistical significance of the differences between the patient and control groups was estimated by logistic regression analysis. Adjusted odds ratios (ORs) were calculated with a logistic regression model that controlled for gender and age and were reported to be at 95% confidence intervals (CI). Differences in allele frequencies between the control group and patients were compared with a chisquare test and when needed, Fisher's exact test was used. The Hardy-Weinberg equation was used to calculate estimated genotype frequency and experienced genotype frequency. For statistical comparisons between groups, a Mann-Whitney U-test was used. A *p* value less than 0.05 was considered statistically significant.

Results

The study group included 37 patients with breast cancer and 70 healthy volunteers. The patients and controls were from the same geographical areas. Demographic and clinical data of patients with breast cancer are given in Table 1.

Table 1: Clinical feature of breast cancer patients

Age	Median 59 (32-103)	
Gender	Female	37
Family History	No	25
	Yes	12
Menopause	No	12
	Yes	25
Molecular subtypes	Luminal A	21
	Luminal B	8
	Her 2 positive	1
	Triple Negative	7
Grade	I	4
	II	22
	III	11
Metastasis	Yes	31
	No	6

The genotype distributions of the eNOS (VNTR) variants were presented in Table 2.

eNOS (VNTR) variant: The distribution of AA, AB and BB genotypes for eNOS3 (VNTR) variant were observed in 47.2%, 21.4% and 31.4% of healthy controls and in 70.1%, 29.9% and 0% of cases with breast cancer, respectively. Significantly decreased breast cancer risk was found for eNOS BB polymorphism (OR=0.56; 95%CI:0.463-0.676; *p*=0.001). No statistical association were found between the eNOS AA and AB polymorphisms and breast cancer risk (*p*=0.223 and 0.487).

Table 2: Comparison of frequencies of antioxidan gene variants between patients with breast cancer and healthy controls.

	Genotype	Breast cancer n (%)	Healthy Control n (%)	OR	95% CI	*p*
eNOS VNTR	AA	26 (70.1)	33 (47.2)	1.739	0.771-3.922	0.223
	AB	11 (29.9)	15 (21.4)	1.428	0.581-3.509	0.487
	BB	0 (0)	22 (31.4)	0.560	0.463-0.676	**0.001**

Discussion

It is well known that oxygen free radicals in oxidative stress have an important role in the pathogenesis of cancer although studies on genes related to oxidative stress have yielded conflicting results. In the present study, it was aimed to determine the relationship between the risk of breast cancer and the variants of eNOS (VNTR) gene variants.

There are studies in literature about the relationship between eNOS polymorphism and the risk of cancer development. The relationship between eNOS-786T>C, 894 G>T and intron 4A/B polymorphisms was investigated in a meta-analysis. eNOS intron 4A/B polymorphism was found to have a significant association with all cancers and the relationship was found to be stronger in prostate cancer patients. eNOS 786T>C polymorphism was significantly associated with all cancer types and subgroup analyses showed a stronger association with prostate cancer, bladder cancer, and breast cancer.

No significant association was found in respect of eNOS 894 G>T polymorphism regardless of cancer type but there was a significant relationship with breast cancer when the subgroup analyses were performed. In the evaluation of pathological subtypes, it was found that eNOS 786T>C polymorphisms were associated with infiltrating ductal carcinomas and other carcinomas whereas eNOS 894G>T polymorphisms were associated with invasive ductal carcinomas only (8). A case-control study of 873 patients with breast cancer and 1034 healthy subjects showed an association between eNOS786T>C or 894G>T polymorphism and breast cancer (8). In another meta-analysis evaluating the relationship between eNOS and cancer, the polymorphisms of eNOS E298D and 786T>C were investigated. In that study, it was found that eNOS e298 and 786T>C polymorphisms were associated with a decrease in the risk of breast cancer development (9). In the current study, eNOS VNTR polymorphism was investigated. The BB genotype was found to be associated with decreased risk of breast cancer and genotypes AA and AB were not significantly associated with breast cancer.

In conclusion, the role of oxygen free radicals in tumor development is well known. However, previous studies which have focused on genes that have roles in the formation and metabolism of oxygen free radicals have revealed different findings and the majority of the studies have been preclinical studies. In the current study eNOS BB genotype was not associated with breast cancer. Further studies are needed to determine whether these gene polymorphisms have a place in diagnosis or determining the risk of disease.

REFERENCES

1.Torre LA, Siegel RL, Ward EM et al. Global cancer incidence and mortality rates and trends-an update. Cancer Epidemiol Biomark Prev 2016;25(1):16-27.

2. Kelly KM, Shah N, Shedlosky-Shoemaker R et al. Living post treatment: Definitions of those with history and no history of cancer. J Cancer Surv 2011;5:158-166.

3. Lichtenstein P, Holm NV, Verkasalo PK. Environmental and heritable factors in the causation of cancer. N Engl J Med 2000;343:78-85.

4. Kehrer JP, KlotzLO. Free radicals and related reactive species as mediators of tissue injury and disease: implications for health. Crit Rev Toxicol 2015;45(9):765-798.

5. Steenport M, Eom H, Uezu M et al. Association of polymorphisms in myeloperoxidase and catalase genes with precancerous changes in the gastric mucosa of patients at inner-cityhospitals in New York. Oncol Re. 2007;18(1):235-240.

6. Dosenko VE, Zagoriy VY, Haytovich NV et al. Allelic polymorphism of endothelial NO synthase gene and its functional manifestations. Acta Biochim Pol 2006;53:299-302.

7. Wang X, Axelsson J, Nordfors L et al. Changes in fat mass after initiation of maintenance dialysis is influenced by the uncoupling protein 2 exon 8 insertion/deletion polymorphism. Nephrol Dial Transplant 2007;22:196-202.

8. Gao X, Wang J, Wang W et al. eNOS Genetic Polymorphisms and Cancer Risk A Meta-Analysis and a Case–Control Study of Breast Cancer. Medicine (Baltimore) 2015;94(26): e972.

9. Yao L, Fang F, Zhong Y et al. The association between two polymorphisms of eNOS and breast cancer risk:a meta-analysis. Breast Cancer Res Treat 2010;124:223-227.