Tropical Journal of Pharmaceutical Research February 2018; 17 (2): 319-323
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v17i2.17

Original Research Article

Association of single nucleotide polymorphisms in miR-499 and miR-196a with susceptibility to breast cancer

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Sent for review: 5 January 2016 *Revised accepted: 2 January 2018*

Abstract

Purpose: *To investigate the relationship between miR-499 rs3746444 and miR-196a rs11614913 polymorphisms, and susceptibility to breast cancer in an Iranian population.*
Methods: *This case-control study was performed on a population of 200 subjects comprising 100 breast cancer patients (case/observation group) and 100 healthy individuals (control group). Amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) was used to genotype these polymorphisms. P-values and odd ratios were determined, and p-values < 0.05 and odd ratios > 1 were considered statistically significant.*
Results: *There were no significant differences between observation and control groups with respect to rs11614913 T/C polymorphism. The rs11614913 T allele was not identified as a risk factor for susceptibility to breast cancer (OR = 0.86, 95 % CI = 0.85 - 1.3, p = 0.46). However, there were significant differences between observation and control groups with respect to rs3746444 T/C polymorphism. It was observed that cytosine-cytosine (CC) (OR = 4.5, 95 % CI = 1.3 - 15.4, p = 0.06), and cytosine-thymine (CT) (OR = 1.9, 95% CI = 1- 3.6, p = 0.04) genotypes had protective influence against susceptibility to breast cancer.*
Conclusion: *These results indicate that CC and CT genotypes are associated with reduced risk of breast cancer. In particular, the presence of C allele is significantly associated with a low risk of breast cancer. These findings may provide useful information for prevention and early diagnosis of breast cancer.*

Keywords: *Breast cancer, Early diagnosis, miR-196a rs11614913, miR-499 rs3746444, Polymorphism, ARMS-PCR*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Breast cancer is the most common malignancy among women: about one million new patients are added annually to the world population of female breast cancer patients. This cancer accounts for 10 % of all cancers and 23 % of female cancers in developed countries [1].

There are more than 1000 microRNA (miRNA) genes in the human genome which regulate the translation or degradation of human messenger

RNA (mRNA) by a complement sequence. Genetic variations in a miRNA may affect its maturation and biogenesis, and these are linked to the pathogenesis of a lot of diseases, which include breast cancer [2]. Researchers have shown that genetic polymorphisms are one of the main causes of individual differences in cancer prevalence. Single nucleotide polymorphisms (SNPs) in miRNAs can be used as genetic markers for prognosis breast cancer susceptibility or prediction [3].

Several miRNAs are abnormally expressed in breast cancer tissue when compared with normal breast tissue. These include miR-196a and miR-499. Indeed, miR-196a is exceedingly expressed in breast cancer tissue [4]. Moreover, several studies have recognized genetic variants in the precursor miRNA sequence of miR-499 rs3746444 as possible biomarkers related to the etiology of breast cancer [5].

There are a few molecular and epidemiological studies on the association between breast cancer and polymorphisms in miR-499 rs3746444 and miR-196a rs11614913 [6].

To the best of our knowledge, there are no reports on the association between miR-196a rs11614913 and miR-499 rs3746444 polymorphisms, and the risk of breast cancer in an Iranian population.

In order to investigate if such association exist, a hospital-based case-control study on an Iranian population was carried out in this study, based on genotyping analysis applying amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) assay.

EXPERIMENTAL

Patients

Whole blood samples were collected from healthy females and females with breast cancer in the teaching hospital of Mashhad University of Medical Sciences, Iran. A total of 100 control samples were chosen from different ages (18 – 61), while 100 breast cancer cases were selected from female patients aged 45 – 64 years. Each patient included in the study gave informed consent. The study protocol conformed with the ethical codes of the 1975 Declaration of Helsinki [7], and was approved by the local Research and Medical Ethics Committee (approval ref. no. IR.IAU.5106930121003). All samples were collected in the hospital according to Iranian medical ethics.

DNA isolation and amplification

A 5-mL sample of peripheral whole blood was collected from each subject into an EDTA anticoagulant tube. Genomic DNA was extracted using High Pure PCR Template Preparation Kit (Roche Diagnostics, GmbH, Mannheim, Germany) according to the manufacturer's protocol. The miR-196a and miR-499 gene SNPs were analyzed using ARMS-PCR. In this method, three primers were used to amplify the rs11614913 and rs3746444 SNPs (Table I). The PCR reactions were prepared using Taq DNA Polymerase (Ampliqon, Denmark) according to the manufacturer's manual. The conditions of PCR were: initial denaturation at 95 °C for 5 min, 33 cycles of denaturation (95 °C for 30 s), annealing (60 °C for 30 s), extension (72 °C for 40 s), and a final extension step (72 °C for 10 min). The PCR products were mixed by power load stain and ran in a 1 % agarose gel.

Table 1: Primer sequences for the detection of mir-146a and miR-499 gene polymorphisms

Gene	SNP	Primer Sequence(5' → 3')
miR-196a	rs11614913	TCTCTAgTCCTTAgggAggT ACCgACTgATgTAACTCTgg ACCgACTgATgTAACTCTgA
miR-499	rs3746444	TCTATgAgAATTATgCgggC AgCACAgACTTgCTgTCAC AgCACAgACTTgCTgTCAT

Statistical analysis

To examine the differences in allele frequencies, chi-square (χ^2) analysis was used and the distribution of genotypes between case and control samples was calculated using (SPSS v. 22). The association between genotype and risk of breast cancer was evaluated by calculating the odds ratio (OR) and their 95 % confidence intervals (95 % CI) with logistic regression models. All statistical tests were two-sided. Values of OR > 1, and p values < 0.05 were considered statistically meaningful, indicating the effect of allele distribution on breast cancer probability.

RESULTS

The frequency distribution of miR-196a rs11614913 T/C genotypes in breast cancer patients and normal subjects is presented in Table 2. There were no significant differences in rs11614913 T/C polymorphism between the breast cancer patients and controls. Thus, miR-196a rs11614913 T/C polymorphism was not identified as a risk/protection factor for susceptibility to breast cancer. In addition, the

rs11614913 T allele was not identified as a risk factor for susceptibility to breast cancer (OR = 0.86, 95 % CI = 0.85- 1.3, p = 0.46).

The frequency distributions of miR-499 rs3746444 T/C genotypes in breast cancer patients and normal subjects are presented in Table 3. There were significant differences between the breast cancer group and the control group samples with respect to genotypes or alleles according to the (χ^2) analysis of data. The CC (OR = 4.5, 95% CI = 1.3- 15.4, p = 0.06) and CT (OR = 1.9, 95% CI = 1- 3.6, p = 0.04) genotypes had protective effect on breast cancer. In addition, individuals with C allele presented a low risk for breast cancer development (OR = 2.03, 95% CI = 1.3-3.3, p = 0.003).

Table 2: Genotype and allele frequencies of rs11614913 (C/T) polymorphism for breast cancer patients and healthy subjects

Genotype	Case (%)	Control (%)	OR(95%CI)	*P*-value
CC	33(33.7)	25(25)	1 (reference)	-
CT	51(52)	62(62)	0.62(0.33 - 1.18)	0.15
TT	14(14.3)	13(13)	0.82(0.33 - 2)	0.66
Allele				
C	117(60)	112(56)	1(reference)	-
T	79(40)	88(44)	0.86(0.58 - 1.3)	0.46

Table 3: Genotype and allele frequencies of rs3746444 (T/C) polymorphism for breast cancer patients and healthy subjects

Genotype	Case (%)	Control (%)	OR(95%CI)	*P*
TT	35(43.8)	63(63)	1 (reference)	-
CT	35(43.8)	33(33)	1.9 (1 - 3.6)	0.04
CC	10(12.5)	4(4)	4.5 (1.3 - 15.4)	0.02
Allele				
T	105(66)	159(79.5)	1 (reference)	-
C	55(34)	41(20.5)	2.03(1.3 - 3.3)	0.003

DISCUSSION

The present research was conducted to assess the association between polymorphisms in miR-499 rs3746444 and miR-196a rs11614913, and breast cancer development, in view of absence of such studies in an Iranian population [8]. Numerous associations between miRNAs and incidence of breast cancer have been demonstrated experimentally. An understanding of the mechanisms involved in these associations is crucial for the use of miRNAs markers as strong tools for the blockage and treatment of breast cancer [9].

The miRNAs are involved in several critical biological processes such as development, proliferation, differentiation and apoptosis. Genetic polymorphisms in miRNAs (SNPs) can potentially affect the selection or processing of miRNA targets. Several recent studies have assessed the association between SNPs in miR-196a rs11614913 and miR-499 rs37464, and susceptibility to breast cancer [5, 10]. In view of the important roles of miR-499 rs3746444 and miR-196a rs11614913 in the etiology of breast cancer, the present study evaluated the distribution of these genotypes and assessed the risk of breast cancer development in Iranian women.

The results obtained indicate that rs11614913 polymorphism is not associated with an increased risk of breast cancer in this case-control study in Iranian women. This is in agreement with the results obtained by Jedlinski *et al* who reported that there was no relation between rs11614913 polymorphism and breast cancer in Caucasian case-control cohort in Queensland, Australia [11]. Similarly, a study conducted on a Brazilian female population found no relation between rs11614913 polymorphism and breast cancer [12]. In addition, Catucci *et al* reported that there was no statistical correlation between the existence of miR-196a rs11614913 in women affected by breast cancer and controls in German and Italian populations [8]. However, the results obtained in the present study are at variance with those reported by Hu *et al* and Hoffman *et al* who established an association between rs11614913 and the risk of breast cancer. Some population-specific factors, such as differences in genetic background, environmental factors, or lifestyle might explain these discrepancies in relation between rs11614913 polymorphism and breast cancer [10,13].

Many case-control studies have explored the relation between SNP in miR-499 and risk of different kinds of cancer [14]. Polymorphism in miR-499 rs3746444 has been shown to be distinctly correlated with a variety of diseases, including breast cancer [10,15]. Yet, only a few epidemiological studies have focused on the association between miR-499 rs3746444 polymorphisms and risk of breast cancer.

In the present study, the association between miR-499 rs3746444 polymorphism and breast cancer risk was investigated. The results indicate

an association between rs3746444 polymorphism and breast cancer susceptibility. In addition, CC and CT genotypes were associated with a protective effect against breast cancer. Furthermore, women with C allele had a low risk for breast cancer development. Consistent with these findings, a significant association has been demonstrated between rs3746444 polymorphism and breast cancer risk in an Asian population [5]. Moreover, Xu *et al* reported breast cancer risk among a Chinese population with respect to rs3746444 polymorphism [16]. A recent research by Sun *et al* also showed a statistically significant association between miRNA-499 rs3746444 polymorphism and cancer susceptibility in Asians, but not in Caucasian population [17].

Although the results of this study did not suggest any association between rs11614913 in miR-196a and risk of breast cancer, there are some evidence that miR-196a may play a major role in carcinogenesis. Among the possible targets of miR-196a are genes that take part in various cancer-related pathways. The split of HOX gene products similar to HOXB8 mRNA has been shown to be relatively due to miR-196a.

The HOX genes regulate a number of genes that, in turn, regulate huge networks of alternative genes. Thus, they are involved in some key biological processes. Furthermore, the potential targets of miR-196a are LSP1 and TOX3, which are new breast cancer susceptibility genes recently identified in a whole-genome association research. It is probable that the rs11614913 SNP modulates the acts of these two genes in breast cancer progress. However, this is yet to be established [18].

Limitations of the study

It was not possible to investigate precisely-established risk factors (age at menopause, age at menarche, hormone replacement therapy, and family history) to detect gene-environment interaction effects.

CONCLUSION

The findings of this study indicate that SNP in miR-499 rs3746444 plays a pivotal role in the development of breast cancer in the Iranian population investigated. However, there is no significant association between miR-196a rs11614913 SNP and breast cancer susceptibility. However, well-designed studies involving larger population samples are required to establish more firmly the associations of these miRNA SNPs with risk of breast cancer.

DECLARATIONS

Acknowledgement

The authors acknowledge Islamic Azad University, Ahvaz Branch (Iran) for the financial support for this research.

Conflict of interest

The authors declare that no conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Mojarrad M. and Salehzadeh A. studied the concepts and collected the samples. Doulahand Mojarrad M. collected the data and did laboratory tests. Salehzadeh A. analyzed the data and drafted the article.

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