Acta Scientific MEDICAL SCIENCES (ISSN: 2582-0931)

Volume 8 Issue 3 March 2024

Research Article

Association of the MiR-499 Gene rs-3746444 A/G Polymorphism with the Myocardial Infarction in the Tunisian Population

Amani Kallel[1,2]*, Daouda Dia[1,2], Manel Ben Halima[3], Mohamed Bessem Hammami[1,2], Haifa Sanhaji[1,2], Mohamed Sami Mourali[3] and Moncef Feki[1,2]

[1]University of Tunis El Manar, Faculty of Medicine of Tunis, LR99ES11, Tunis, Tunisia

[2]Laboratory of Biochemistry, Rabta Hospital, Tunis, Tunisia

[3]Department of Cardiology, Rabta Hospital, Tunis, Tunisia

***Corresponding Author:** Amani Kallel, University of Tunis El Manar, Faculty of Medicine of Tunis, LR99ES11, Tunis, Tunisia.

Received: October 12, 2023

Published: February 06, 2024

Abstract

MicroRNA-499 (miR-499) is thought to play a cardioprotective role by inhibiting myocyte apoptosis. The study aimed to determine the allelic and genotypic frequencies of miR-499 rs3746444 A/G polymorphism and to investigate its association with myocardial infarction (MI) in the Tunisian population. The SNP was genotyped in 160 patients with myocardial infarction (MI) and 168 healthy controls using PCR-RFLP. Both AG (0.46 *vs.* 0.34; P = 0.02) and GG (0.15 *vs.* 0.06; P = 0.01) genotypes and G allele (0.38 *vs.* 0.23; P < 0.001) of the miR-499 rs3746444 A/G polymorphism were significantly more frequent in MI patients than controls. Carriers of the minor G allele (i.e., AG+GG genotypes) showed lower plasma triglyceride levels (1.86 ± 0.64 mmol/L vs. 2.24 ± 1.13, mmol/L; P = 0.002). The study showed that miR-499 gene rs3746444 A>G polymorphism is associated with MI in the Tunisian population. The minor G allele could be considered as a biomarker for MI susceptibility in this population.

Keywords: Coronary Artery Disease; Myocardial Infarction; miR-499 Gene; rs3746444 Polymorphism; Epigenetics

Abbreviations

BMI: Body Mass Index; CI: Confidence Interval; CVD: Cardiovascular Disease; DNA: Deoxyribonucleic Acid; EDTA: Ethylene Diamine Tetra Acetic Acid; HDL-C: HDL Cholesterol; LDL-C: LDL Cholesterol; MI: Myocardial Infarction; miRNA/miR: Micro-RNA; mRNA: Messenger RNA; Myh7b: Myosin Heavy Chain 7B; OR: Odds-Ratio; bp: Base Pair; PCR: Polymerase Chain Reaction; RFLP: Restriction Fragment Length Polymorphism; RNA: Ribonucleic Acid; TC: Total Cholesterol; TG: Triglyceride.

Introduction

Cardiovascular diseases (CVD), particularly myocardial infarction (MI), are among the leading causes of mortality, worldwide [1]. They are multifactorial diseases resulting from genetic predisposition and gene-environment interactions [2]. There is a growing recognition that epigenetic factors such as microRNAs (miRNAs/miRs) impact cardiovascular health [3]. The miRs are non-coding single-stranded small RNA of 21-23 nucleotides, acting as negative regulators of the expression of a multitude of genes by binding to 3'UTR of mRNA [4,5]. The

miRs are involved in a variety of biological processes such as cell differentiation, cell proliferation, and apoptosis [4]. Due to their easy detection in nearly all biological fluids, circulating miRs can be used as potential biomarkers for various diseases, such as cancer [6], autoimmune diseases [7] and CVD [8].

Some miRs were proven to regulate gene expression in cardiac tissue, and apoptosis and necrosis of myocardial cells [9]. The miR-499 is specifically expressed in the heart and skeletal muscle. It plays a key role in cardiac development and cardiomyocytes maturation [10]. It been shown to participate in cardiovascular remodeling after cardiomyocyte injury and inhibit apoptosis to and could serve as biomarkers for the diagnosis of CVD. Jayawardena., *et al*. 2012 [11] showed that a combination of 4 miRNAs (miR-combo), including miR-1, miR-133a, miR-208, and miR-499, is capable of reprogramming fibroblasts into cardiomyocyte-like cells in both mouse cultured cardiac fibroblasts and in ischemic myocardium. The rs-3746444 polymorphism located in the precursor of miR-499 has been associated with susceptibility to various CVD including MI [12-15] whereas other studies showed no association [16,17]. Most data on the topic arise from Asian populations particularly Chinese, with only few data come from Caucasians and other ethnic groups. Moreover, previous studies clearly demonstrated that genotypic and allelic distributions of the polymorphism vary according to ethnicity. The G allele is noticeably less frequent in Asians than in Caucasians [13]. In this study, we investigated the association between the polymorphism rs-3746444 A>G at the miR-499 gene and MI in a sample of the Tunisian population.

Material and Methods

Study population

We conducted a case–control study involving 328 unrelated individuals living in the City of Tunis (Tunisia). Consecutive patients admitted to the Department of Cardiology (Rabta Hospital of Tunis) for MI from January 2021 to December 2022 were enrolled. Diagnosis of MI was confirmed according to the European Society of Cardiology criteria [18]. During the same period, hospital employees and their relatives were invited to participate to the study as controls. The criteria of eligibility for both patients and controls were as follows: consenting subject of both sexes, age above eighteen, no current or past malignancy, inflammatory, autoimmune, or systemic disease (except CVD for patients), and no renal or liver function impairment, breastfeeding, or pregnancy. Unrelated controls were selected by matching with patients by gender, five-year age class and neighborhood income. No one among the controls has history of cardiovascular event or sign of cardiovascular system dysfunction (except of hypertension). After applying of these criteria, 160 patients with MI and 168 controls were retained in the study. Demographic data, personal and familial history of CVD, as well as risk factors related to cardiovascular diseases were collected for all participants. Weight and height were measured for the subjects barefooted and lightly clothed. A clinical exam (blood pressure, cardiac frequency) and a biological exam were realized for all participants. Twelve hours-fasting venous blood sample (10 mL) was collected from each participant, into EDTA-tube for genetic analyses and into lithium heparin-tube for biochemical analyses. In patients, the blood sample was collected at least one week following the MI (after patient's stabilization). Individuals who smoked once a day for over 1 year were defined as smokers. Diabetes mellitus was defined as hyperglycemia, requiring antidiabetic drugs or fasting blood sugar > 7.0 mmol/L. Hypertension was defined as systolic blood pressure ≥ 140 mm Hg and/or diastolic blood pressure ≥ 90 mm Hg, or the use of antihypertensive drug treatment. Obesity was defined as body mass index (BMI) > 30 kg/m^2. Dyslipidemia was defined as total cholesterol > 6.47 mmol/L and/or triglyceride > 2.26 mmol/L. The study was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. The protocol was approved by the Ethics Committee of Rabta hospital, and all subjects gave written informed consent to participate to the study.

Genetic analyses

DNA was extracted from peripheral blood leukocytes using salting-out method. The miR-499 gene is located in the intron 20 of the Myh7b gene, which encodes for the heavy chain myosin 7b [19]. Genotype of miR-499 gene rs-3746444 A>G polymorphism was determined using polymerase chain reaction (PCR) restriction fragment length polymorphism (RFLP). The primers used to amplify the DNA segment including the polymorphic site were 5′-AGGACAAGTTCTCTGAGTTCT-3′ (forward), and 5′-ATAGCAGCTTCTTGTCCAGCTT-3′ (reverse). They delimit a fragment of 146 bp length. The PCR products were digested with the restriction enzyme *BclI* which yields 120 and 26 bp fragments

length. The products were separated in 2.5% agarose gel, stained with ethidium bromide.

Biochemical analyses

Plasma glucose, total cholesterol (TC), triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C) levels were determined by Abbott-ALINITY autoanalyzer (Abbot Diagnostics, Abbott Park, IL) using the specific reagent kits. Low-density lipoprotein cholesterol (LDL-C) levels were calculated using the Friedwald formula [20].

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS 22.0 for Windows; SPSS Inc., Chicago, IL, USA). Distributions of continuous variables in groups were expressed as mean± SD and compared with unpaired Student's t-tests. $\chi 2$ tests were used to test for departures from Hardy–Weinberg equilibrium and to compare genotype distributions between groups. We calculated odds ratio (OR) together with their 95% confidence intervals (95% CI) as estimators of the relative risk of MI for the rs-3746444 genotypes. The polymorphism was first assessed in three genotype categories (wild-type, heterozygous variant, and homozygous variant) and then grouped into two categories with heterozygote and homozygote variants combined because of the dominant model of inheritance observed for this polymorphism. A binary logistic regression model was performed to test whether the rs-3746444 polymorphism is associated with MI independently of potential confounders. The regression model was adjusted for the main cardiovascular risk factors (i.e., age, gender, smoking, diabetes, hypertension, obesity, and dyslipidemia). A two-tailed p-value of 0.05 was considered statistically significant.

Results and Discussion

Main demographic, clinical and biochemical characteristics of MI and control groups are summarized in Table 1. MI patients showed higher prevalence of smoking, diabetes, hypertension, dyslipidemia, but lower prevalence of obesity than controls. Age, glucose, and triglyceride were higher, whereas BMI and HDL cholesterol were lower in patients. The study showed that allele frequency of the miR-499 rs3746444 A>G polymorphism in Tunisians fit more with the frequency in Caucasian than Asian populations. The frequency of the minor G allele of 0.23 in Tunisians (control group) is close to that of 0.29 in Egyptians [12], but clearly higher than the frequency of 0.13 to 0.18 in Chinese [16,21,22], of 0.19 in Koreans [23] and of 0.115 in Indians [24]. The frequency of this allele was higher; 0.37 and 0.52 in Iranians [25,26]. The study revealed a significant association between the rs3746444 A>G polymorphism of miR-499 gene and MI in the Tunisian population. Carriers of the G allele have two-fold higher risk for MI. Compared to the patients with AA genotype, those with AG variant and those with GG variant are exposed to two-fold and three-fold higher risk for MI, respectively (Table 2). The association of the polymorphism and MI remained significant after adjusting for the main cardiovascular risk factors (Table 3). Literature yielded inconsistent data on the role of rs3746444 A>G polymorphism in CVD. Some studies showed no association [16,17]. However, data from other studies [12,14,27,28] and from meta analyses suggest there are association of the G allele with CVD. A meta-analysis of nine studies including 5,063 CHD patients and 4,603 controls concluded to an association of rs3746444 polymorphism with CHD risk. The GG genotype was associated with an increased risk for CHD, whereas the A allele may protect against CHD [13]. Another meta-analysis of eight studies with 2507 MI patients and 3796 healthy controls concluded to a significant association between the rs3746444 polymorphism and MI susceptibility; compared to GG variant, AA+AG variants exhibited a two-fold risk for MI [OR (95% CI) = 2.04 (1.37-2.70), $p < 0.001$] [15].

	Controls (n = 168)	MI patients (n = 160)	p-value
Male gender (%)	61.3	68.7	0.0970
Age (year)	49.3 ± 9.59	54.5 ± 7.60	0.026
Body mass index (Kg/m^2)	27.8 ± 5.48	25.3 ± 3.74	<0.001
Smoking (%)	26.7	78.9	<0.001
Diabetes mellitus (%)	6.9	36.6	<0.001

Hypertension (%)	7.7	28.3	<0.001
Obesity (%)	34.3	10.4	<0.001
Dyslipidemia (%)	20.6	32.6	0.025
Glucose (mmol/L)	5.20 ± 1.46	7.11 ± 2.91	<0.001
Total cholesterol (mmol/L)	4.66 ± 0.98	5.05 ± 1.14	0.550
HDL cholesterol (mmol/L)	1.28 ± 0.36	0.90 ± 0.23	<0.001
LDL cholesterol (mmol/L)	2.79 ± 0.83	3.26 ± 1.04	0.081
Triglyceride (mmol/L)	1.28 ± 0.60	2.01 ± 0.85	0.004

Table 1: Main demographic, clinical, and biochemical characteristics of the study population.

Values are expressed as mean ±standard deviation or percent. MI, myocardial infarction.

	Controls (n = 168)	**MI patients (n = 160)**	**OR (95% CI)**	**P-value**
Genotype frequency n (%)				
Codominant model				
AA	101 (0.60)	62 (0.39)	-	
AG	57 (0.34)	74 (0.46)	2.12 (1.32–3.38)	0.02
GG	10 (0.06)	24 (0.15)	3.91 (1.75–8.72)	0.01
Dominant model				
AA	101 (0.60)	62 (0.39)		
AG+GG	67 (0.40)	98 (0.61)	2.38 (1.53–3.71)	<0.001
Allele frequency (%)				
A	0.77	0.62	-	
G	0.23	0.38	2.07 (1.48–2.91)	<0.001

Table 2: Genotype distributions and allele frequencies of miR-499 rs3746444 polymorphism in myocardial infarction patients and controls.

MI, myocardial infarction; OR, odds-ratio; 95% CI, 95% confidence interval.

	β coefficient	**SE**	**OR (95% CI)**	**p-value**
Age	0.010	0.026	1.01 (0.96–1.06)	0.700
Obesity	-0.897	0.684	0.40 (0.11–1.56)	0.190
Hypertension	1.621	0.731	5.06 (1.2–21.2)	0.027
Diabetes mellitus	2.446	0.699	11.6 (2.93–45.5)	<0.001
Dyslipidemia	0.235	0.493	1.27 (0.48–3.32)	0.634
Tobacco smoking	3.812	0.586	45.2 (14.3–143)	<0.001
rs3746444 polymorphism	1.245	0.359	3.47 (1.72–7.02)	<0.001
Constant	-5.429	1.654	-	<0.001

ES: standard error; OR: Odds-ratio; 95% CI: 95% confident interval.

Table 3: Binary logistic regression model of the association of myocardial infarction with miR-499 rs3746444 A/G polymorphism.

The exact mechanisms of how miR-499 rs-3746444 A>G polymorphism affects the heart function are still being examined. Some miRs have been proven to intervene in the regulation of gene expression in cardiac tissue, as well as in apoptosis and necrosis of myocardial cells, which are important processes in MI [29]. It has been shown that the minor G allele affects the maturation process of miR-499 and reduces its physiologically active form [30]. The allele decreases the amount of mature miR-499 in cardiomyocytes, alters its anti-apoptotic function, and reduces its inhibitory activity on targeted genes [30,31]. Such effects increase the susceptibility to develop CVD as well as other diseases. Indeed, the polymorphism was associated with increased susceptibility to several other diseases including different types of cancer [32-34], and diverse autoimmune diseases [35,36].

We found no variation in glucose, and total, LDL and HDL cholesterol through the polymorphism variants in either MI patients or controls. However, TG levels were significantly lower in MI patients carrying the G allele (Table 4). Considering that this allele is associated with increased risk for MI, the association could not be explained by its effects on lipid levels. However, the association of G allele with decreased triglyceride levels was documented in literature. A meta-analysis of six studies involving 3,227 subjects reported reduced TG levels in GG genotype compared to the AA wild-type genotype [37]. The mechanism by which this polymorphism induces a decrease in TG remains poorly understood. However, gene-gene interactions could explain the phenomenon. Omics studies identified genes involved in lipid metabolism, such as the gene LIPA and OSBPL1a as potential miR-499 targets [22,38]. LIPA gene encodes for lysosomal acid lipase, an enzyme responsible for the hydrolysis of triglycerides and cholesteryl esters, and OSBPL1a gene is an intracellular lipid receptor.

Variable (mmol/L)	Controls (n = 168)			MI patients (n = 160)		
	AA (n = 101)	AG+GG (n = 67)	p	AA (n = 62)	AG+GG (n = 98)	p
Glycemia	5.32 ± 1.74	4.98 ± 0.78	0.082	6.88 ± 2,91	7.17 ± 2.90	0.803
Triglyceride	1.29 ± 0.62	1.24 ± 0.57	0.463	2.24 ± 1,13	1.89 ± 0.64	0.002
TC	4.82 ± 0.98	4.48 ± 0.93	0.169	5.05 ± 1,40	5.05 ± 0.98	0.194
HDL-C	1.32 ± 0.39	1.24 ± 0.31	0.175	0.83 ± 0,20	0.93 ± 0.23	0.753
LDL-C	2.90 ± 0.83	2.69 ± 0.83	0.579	3.24 ± 1,24	3.29 ± 0.88	0.163

Table 4: Biochemical characteristics of myocardial infarction (MI) patients and controls according to miR-499 rs3746444 T/C polymorphism genotypes.

TC, total cholesterol; HDL-C, HDL cholesterol, LDL-C, LDL cholesterol.

The study has limitations that should be acknowledged and taken into consideration for the data interpretation. The sample size is small, but this did not prevent revealing a significant association of the polymorphism with MI. The patients were unwell matched with controls according to gender, age, body composition and the main cardiovascular risk factors. Thus, one may speculate that the association of rs3746444 polymorphism with MI could result from confounding effects of conditions such as diabetes, hypertension, or dyslipidemia. Finally, the study focused on a single SNP and did not examine the potential variation of the miR-499 levels according to the genetic variants of the polymorphism.

Conclusion

In conclusion, the study provides evidence that the rs3746444 polymorphism of miR-499 gene is associated with MI in the Tunisian population. The minor allele G is associated with a higher risk of MI independently of the main cardiovascular risk factors and thus could be used as a marker genetic susceptibility to CVD and especially MI. These findings have however to be confirmed in other series and further studies should be conducted to elucidate the pathophysiological role of polymorphisms of miR-499 and other miRs in MI.

Acknowledgements

This work was supported by a grant from the "Ministry of Higher Education and Scientific Research" of Tunisia.

Declaration of Interest

The authors declare that they have no conflict of interest.

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