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Impact of $SIRT_3$ Gene Polymorphism on Acute Myocardial Infarction Susceptibility.

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ABSTRACT

Worldwide, Myocardial infarction is a common presentation of coronary artery disease, The World Health Organization estimated in 2017, that 12.2% of worldwide deaths were from ischemic heart disease, few resent study reports of SIRT3 gene promoter with many polymorphisms are as risk factor of coronary artery disease .Thus the aim present study istors12293349,rs11246029 SIRT3 gene promoter variant among myocardial infarction patients to find out which of them have valuable role in disease susceptible. Material and Methods where is two ml of blood samples were collected from three groups (myocardial infarction patients, patients who have risk for myocardial infarction) , this sample used for DNA extraction and $SIRT_3$ promoter(rs11246029, rs12293349) gene polymorphism with ARMC-method(Amplification refractory mutation system) then electrophoresis was done. present study included 24 patients with ST elevation myocardial infarction (MI), 24 persons who have risk factors of ischemic heart disease and 24 healthy control subjects. Result: rs11246029There were highly significant difference in frequency distribution of CC genotype between MI and control groups (p=0.022) with an EF (0.56). While in case of rs12293349 genotype the significant difference was seen in CT variant, between MI and control (p=0.05) group with an EF= 0.55. Conclusion: The individuals who have(CT variant) of rs12293349 while (CC variant) of rs11246029 SIRT3 gene promoter are more susceptible for myocardial infarction.

Keywords: Gene polymorphism, myocardial infarction, artery.

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INTRODUCTION

Myocardial infarction is a common presentation of coronary artery disease, The World Health Organization estimated in 2017, that 12.2% of worldwide deaths were from ischemic heart disease(1). AMI is the most prevalent cause of death(2). with it being the leading cause of death in high- or middle-income countries and second only to lower respiratory infections in lower-income countries(1). The European Society of Cardiology guidelines suggest that every sixth male and every seventh woman in Europe will die due to an AMI (3).Ischemic heart diseases IHD is becoming a more common cause of death in the developing world. In Iraq WHO2016 reports 33% death because of cardiovascular diseases(4). There are many genetic factors are contributed in this disease, one of these is SIRT3 gene promoter polymorphism.

SIRT3 regulates the enzymatic activity of the key enzymes of oxidative phosphorylation via deacetylation, thereby regulating mitochondrial energy metabolism . SIRT3 is a NAD+-dependent protein deacetylase that is a member of the silent information regulator 2 (SIR2) family(5). SIRT3 can exert controls on a wide range of important biological activities including regulation of nuclear gene expression , metabolic control(6)**.**The human SIRT3 gene is localized to the chromosome 11p15.5, and encodes an NAD-dependent mitochondrial deacetylase of 399-amino acids containing an N-terminal mitochondrial targeting signal and a central catalytic domain.

There are twenty-three DNA sequence variants (DSVs) are identified with SIRT3 gene promoter , (rs71019893) , (rs3817629),(rs56312618),(rs1045288), (rs12293349),(rs369344513),(rs2272563),(rs369178836) (rs185277566)and (rs71019893)(7).

The aim of present study is recognizing (**rs12293349** ;**rs11246029** SIRT3 gene promoter variant among myocardial infarction patients to find out which of them have valuable role in disease susceptible.

MATERIALS AND METHODS

Present study conducted January 2017 to February 2018 a blood sample were collected from three groups . The first group was include 24 patients with MI which include (16male and 8female) ,who were observation in CCU of Al-Diwaniyha teaching hospital(Iraq) , Second group was 24 patients who have risk factor for MI (Hypertension, Hyperlipidemia, Diabetes mellitus), Third group was include 24 healthy volunteers(non coronary artery diseases). Blood sample were collected by venipuncture from these groups. Each blood sample of three groups were collected to 2 ml of blood collected directly in a sterile tube containing EDTA for DNA extraction ,(Amplification refractory mutation system)ARMS-method have been done to studySIRT3 gene polymorphism by use specific primers as in table (1,2) then electrophoresis was done .

Table 1: Primer of rs11246029 SIRT$_3$gene

Primer	Sequence	
rs11246029	T allele	CCCGGTCCCGCCTCCGAGT
	C allele	AAGGAGGCGGGGGCGTGG
	F	CGACCCGTTCAACTACCCGGCC
	R	TCACCGCCATCCGGGTTGAAAA

NCBI-SNP: **rs11246029**

Table 2: Primerofrs12293349SIRT$_3$gene

Primer	Sequence	
rs12293349	T allele	CATCCCGGTTGTTCTTCTGGGT
	C allele	ATGACAGCAGGAAGACCCCAGG
	F	AGAGACGCGCTGTAACCGAGC
	R	CGGCGCTCACTTCTTCGTGTAG

NCBI-SNP: **rs12293349**

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Statistical Analysis

Data were summarized , presented and analyzed using statistical package for social science (SPSS version 23)and Microsoft office Excel 2016.Numeric data were presented as mean ,standard deviation, Odd ratio and 95% confidence interval was estimated to measure risk.

RESULTS

The mean age of patients with MI was 58.83±11.04 years, the mean age of risk group was 53.04± 9.65 years and that of control group was 60.00±9.87 years. There was no significant difference in mean age among study and control groups enrolled in the present study (P=0.058), which ensures age matching that is mandatory for such a study**.**

Regarding to gender distribution about 16 patients (66.7%) were male, and 8 patients(33.3%) were female, while the risk group included 10 (41.7%) male and 14 (58.3%) female and the control group included 16 (66.7%) male and 8 (33.3%) women. There was no significant difference in mean age among the three groups regarding distribution of patients according to gender (P=0.121), which ensures gender match that is mandatory for such a study.

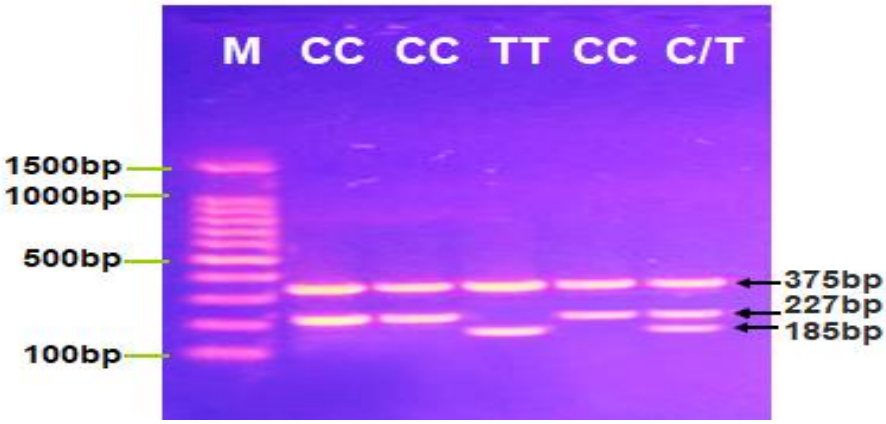

Figure 1: Agarose gel electrophoresis image that show the ARMS-PCR product analysis of SIRT3 gene (rs11246029) blood patients and healthy control samples.

ARMS-PCR product was analysis by 1% agarose gel. Where M: marker (150-100bp), lane (CC) wild type homozygote was show at two bands (375bp internal control band and 227bp C allele bands), lane (TT) mutant type homozygote was show at two bands (375bp internal control band and 185bp T allele bands), and lane (C/T) heterozygote was show at three band digested at (375bp internal control band, 227bp C allele and 185bp T allele bands).

A gel electrophoresis was done as in figure(1) .The frequency distribution of genotype and allele belonging to rs11246029 according to groups is shown in table (3). There were highly significant difference in frequency distribution of CC genotype between MI and control groups (p=0.022) with an EF (0.56).

On the other hand, both of CT, and TT genotype show no significant differences between MI and control group (p=0.065, and P=0.065) respectively. As shown in Table (3)

Allele C was more frequently seen in patients with MI than in control group (*P* = 0.031), with EF (0.40). Patients are more likely to have allele C by an Odds ratio of 2.7, as shown in table (3). While allele T was more frequently seen in control than in MI group (*P* = 0.031), with PF (0.40). Patients are less likely to have allele T, as shown in table (3).

Regarding to Risk and control group, There were no significant difference in frequency distribution of theses genotype between Risk and control groups (p=5 20.8, p= 0.759, and p= 0.637) respectively. However,

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both CC, and CT genotype were more frequently seen in risk group than in control group, with EF (0.22, 0.10 respectively). Risk group are more likely to have genotype CC, by an Odds ratio of 1.55, as shown in table (3).Regarding to allelic distribution, Allele C was more frequently seen in risk group than in control group, but statistically it seem not significant (*P* = 0.469), with EF (0.16).

Table 3: The frequency distribution of genotype and allele belonging to rs11246029 between MI, Risk and control group

Genotype	MI	Control	P	OR (95% CI)	EF	PF
CC	16 (66.6)	6 (31.6)	**0.022**	4.33 (1.2-15.69)	0.56	----
CT	4 (16.7)	8 (42.1)	0.065	0.28 (0.07-1.12)	----	0.47
TT	4 (16.7)	5 (26.3)	1.000	0.99 (0.22-4.33)	----	0.26
Allele	**MI**	**Control**	**P**	**OR (95% CI)**	**EF**	**PF**
C	36 (75.0)	20 (52.6)	**0.031**	2.70 (1.08-6.72)	0.40	----
T	12 (25.0)	18 (47.4)	**0.031**	0.37 (0.15-0.92)	----	0.40
Genotype	**Risk**	**Control**	**P**	**OR (95% CI)**	**EF**	**PF**
CC	10 (41.7)	6 (31.6)	0.497	1.55 (0.44-5.47)	0.22	----
CT	9 (37.5)	8 (42.1)	0.759	0.83 (0.24-2.82)	0.10	----
TT	5 (20.8)	5 (26.3)	0.637	0.74 (0.18-3.05)	----	0.15
Allele	**Risk**	**Control**	**P**	**OR (95% CI)**	**EF**	**PF**
C	29 (60.4)	20 (52.6)	0.469	1.37 (0.58-3.25)	0.16	----
T	19 (39.6)	18 (47.4)	0.469	0.73 (0.31-1.72)	----	0.16

Table 4: The frequency distribution of genotype and allele belonging to rs12293349between MI, Risk, and control group

Genotype	MI	Control	P	OR (95% CI)	EF	PF
CC	7 (29.2)	10 (52.6)	0.118	0.37 (0.11-1.131	----	0.41
CT	12 (50.0)	4 (21.1)	**0.051**	3.75 (0.96-14.65)	**0.55**	----
TT	5 (20.8)	5 (26.3)	0.953	0.74 (0.44-1.72)	----	0.15
Allele	**MI**	**Control**	**P**	**OR (95% CI)**	**EF**	**PF**
C	26 (54.2)	24 (63.2)	0.401	0.69 (0.29-1.65)	----	0.19
T	22 (45.8)	14 (36.8)	0.401	1.45 (0.61-3.46)	0.19	----
Genotype	**Risk**	**Control**	**P**	**OR (95% CI)**	**EF**	**PF**
CC	9 (37.5)	10 (52.6)	0.474	0.64 (0.19-2.16)	----	0.29

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CT	6 (25.0)	4 (21.1)	1.000	1.25 (0.30-5.27)	0.12	----
TT	9 (37.5)	5 (26.3)	0.437	1.68 (0.45-6.25)	0.26	----
Allele	**Risk**	**Control**	**P**	**OR (95% CI)**	**EF**	**PF**
C	24 (50.0)	24 (63.2)	0.222	0.58 (0.24-1.39)	----	0.26
T	24 (50.0)	14 (36.8)	0.222	1.71 (0.72-4.09)	0.26	----

The frequency distribution of genotype and allele belonging to rs12293349 figure (2) according to groups is shown in table (4). Genotype CC was seen in 7 (29.2%), and 10 (52.6%) of MI, and control groups respectively. Genotype CT was seen in 12 (50.0%), and 4 (21.1%) of MI, and control groups respectively. Genotype TT was seen in 5 (20.8%), and 5 (26.3%) of MI, and control groups respectively. The only significant difference was seen in frequency distribution of CTgenotypes between MI and control groups (P=0.05), and EF (0.55). Thus, genotype CT was more frequently seen in risk group than in control group with OR (3.75).

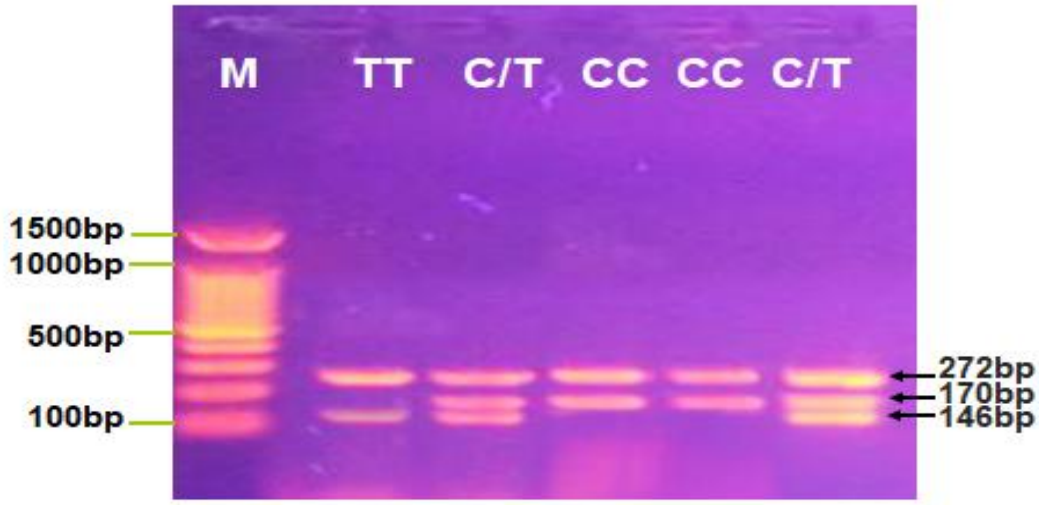

Figure 2: Agarose gel electrophoresis image that show the ARMS-PCR product analysis of SIRT3 gene (rs12293349) blood patients and healthy control samples.

ARMS-PCR product was analysis by 1% agarose gel. Where M: marker (150-100bp), lane (CC) wild type homozygote was show at two bands (272bp internal control band and 170bp C allele bands), lane (TT) mutant type homozygote was show at two bands (272bp internal control band and 146bp T allele bands), and lane (C/T) heterozygote was show at three band digested at (272bp internal control band, 170bp C allele and 146bp T allele bands).

In addition to that, allele C was seen in 26 (54.2%), and 24 (63.2%) of MI, and control groups, respectively. Allele T was seen in 22 (45.8%), and 14 (36.8%) of MI, and control groups, respectively. Neither allele C nor allele T showed significant difference in their frequency distribution among MI, and control groups (P>0.05), as shown in table (4).

Concerning Risk and control group, Genotype CC was seen in 9 (37.5%), and 10 (52.6%) of risk, and control groups respectively. Genotype CT was seen in 6 (25.0%), and 4 (21.1%) of risk, and control groups respectively. Genotype TT was seen in 9 (37.5%), and 5 (26.3%) of risk, and control groups respectively. There were no significant difference was seen in frequency distribution of **CC, CT, and TT** genotypes between risk and control groups (P=0.474, p= 1.00. p= 0.437) respectively, and EF (0.55). Thus, genotype CT was more frequently seen in risk group than in control group with OR(3.75).as shown in table (4).

In addition to that, allele C was seen in 24 (50.0%), and 24 (63.2%) of risk, and control groups, respectively. Allele T was seen in 24 (50.0%), and 14 (36.8%) of risk, and control groups, respectively. Neither allele C nor allele T showed significant difference in their frequency distribution among risk, and control groups (P>0.05), as shown in table (4).

DISCUSSION

To date, few studies have linked the SIRT3 gene polymorphsim with coronary artery disease. Keeping in mind this is the first locally conducted study concerning such subject.

According to data of the above studded genotype , the only significant correlation concerning rs11246029 genotype was seen in CC variant between MI and control group with an EF= 0.56 While in case of rs12293349 genotype the significant difference was seen in CT variant, between MI and control (p=0.05) group with an EF= 0.55 as mentioned previously.

Accordingly, this SNPs were found in MI patients with significantly higher frequencies compared to controls SNPs, might be significantly decrease the transcriptional activities of the SIRT3 gene promoter in cells. Therefore, these SIRT3 gene promoter SNP may reduce SIRT3 levels, contributing to the MI development as risk factors. The human SIRT3 gene promoter contains high GC contents and lacks the TATA box sequence and there are binding sites for activator protein 1 (AP1), GATA-binding factor,) and transcription factor ZF5, as well as multiple specificity protein 1 (SP1) binding sites(8). Nuclear respiratory factor 2, a transcription factor that regulates mitochondrial genes, binds to the promoter of SIRT3 gene and induces its expression(9). In this study, the SNP reduced the SIRT3 promoter transcriptional activity cells to different extents. Therefore, expression of the human SIRT3 gene may be manipulated for therapeutic purposes.

A series of downstream substrates of SIRT3, as well as histone, have been identified. SIRT3 deacetylates and activates several enzymes that are critical in maintaining cellular ROS levels and promote resistance to oxidative stress, including superoxide dismutase 2 (SOD2) and) (10, 11)(12). Increased ROS levels and oxidative stress have been demonstrated to contribute to the atherogenesis(13, 14).

A recent study has shown that SIRT3 targets human verylong-chain acyl-CoA dehydrogenase, a key fatty acid oxidation enzyme(15). Therefore, decreased SIRT3 levels may contribute to MI development by: 1) affecting lipid metabolism, inflammation and other pathways, initiating the atherosclerosis; and 2) interfering with fatty acid oxidation, ROS generation and mitochondrial functions, leading to death of cardiomyocytes. Exact molecular mechanisms need further investigated and elucidated.

A previously conducted study found that, A SNP rs11555236 (C>A) in intron 5 of the SIRT3 gene, which increases expression of SIRT3 gene, has been associated with extended lifespan of humans(16).

CONCLUSION

The individuals with SIRT3 gene promoter; rs12293349 genotype (CT variant);rs11246029 (CC variant) were seem at risk for myocardial infarction.

REFERENCES

[1] Mestl HE, Edwards R. Global burden of disease as a result of indoor air pollution in Shaanxi, Hubei and Zhejiang, China. Science of the total environment. 2011;409(8):1391-8.
[2] Nymark C. Afflicted by an acute myocardial infarction: patients' thoughts, feelings and actions prior to care-seeking: Inst för kliniska vetenskaper, Danderyds sjukhus/Dept of Clinical Sciences, Danderyd Hospital; 2015.
[3] Cardiology TFotMoS-SEAMIotESo. Steg PG, James SK, Atar D, Badano LP, Blömstrom-Lundqvist C, et al. ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation. Eur Heart J. 2012;33(20):2569-619.
[4] Organization WH. Noncommunicable diseases (NCD) country profiles 2014: Indonesia. 2014. 2016.
[5] Giralt A, Villarroya F. SIRT3, a pivotal actor in mitochondrial functions: metabolism, cell death and aging. Biochemical Journal. 2012;444(1):1-10.

[6] Shi T, Wang F, Stieren E, Tong Q. SIRT3, a mitochondrial sirtuin deacetylase, regulates mitochondrial function and thermogenesis in brown adipocytes. Journal of Biological Chemistry. 2005;280(14):13560-7.
[7] Yin X, Pang S, Huang J, Cui Y, Yan B. Genetic and functional sequence variants of the SIRT3 gene promoter in myocardial infarction. PloS one. 2016;11(4):e0153815.
[8] Bellizzi D, Dato S, Cavalcante P, Covello G, Di Cianni F, Passarino G, et al. Characterization of a bidirectional promoter shared between two human genes related to aging: SIRT3 and PSMD13. Genomics. 2007;89(1):143-50.
[9] Satterstrom FK, Swindell WR, Laurent G, Vyas S, Bulyk ML, Haigis MC. Nuclear respiratory factor 2 induces SIRT 3 expression. Aging Cell. 2015;14(5):818-25.
[10] Merksamer PI, Liu Y, He W, Hirschey MD, Chen D, Verdin E. The sirtuins, oxidative stress and aging: an emerging link. Aging (Albany NY). 2013;5(3):144.
[11] Chen Y, Zhang J, Lin Y, Lei Q, Guan KL, Zhao S, et al. Tumour suppressor SIRT3 deacetylates and activates manganese superoxide dismutase to scavenge ROS. EMBO reports. 2011;12(6):534-41.
[12] Tao R, Coleman MC, Pennington JD, Ozden O, Park S-H, Jiang H, et al. Sirt3-mediated deacetylation of evolutionarily conserved lysine 122 regulates MnSOD activity in response to stress. Molecular cell. 2010;40(6):893-904.
[13] V Goncharov N, V Avdonin P, D Nadeev A, L Zharkikh I, O Jenkins R. Reactive oxygen species in pathogenesis of atherosclerosis. Current pharmaceutical design. 2015;21(9):1134-46.
[14] Li H, Horke S, Förstermann U. Vascular oxidative stress, nitric oxide and atherosclerosis. Atherosclerosis. 2014;237(1):208-19.
[15] Verdin E, Zhang Y, Bharathi S, Rardin M, Uppala R, Gibson B, et al. SIRT3 and SIRT5 regulate the enzyme activity and cardiolipin binding of very long-chain acyl-CoA dehydrogenase. 2015.
[16] Albani D, Ateri E, Mazzuco S, Ghilardi A, Rodilossi S, Biella G, et al. Modulation of human longevity by SIRT3 single nucleotide polymorphisms in the prospective study “Treviso Longeva (TRELONG)”. AGE. 2014;36(1):469-78.