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Polymorphism of *tumor suppressor* gene (*p53*) Codon 72 in Iraqi patients with acute myocardial infarction

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Objectives According to previous studies, the aim of the presented work is to investigate the possible association between the codon 72 polymorphism (Pro72Arg, rs1042522) of the tumor suppressor gene (*p53*) with acute myocardial infarction (AMI) in Iraqi patients of Karbala province.
Methods A total of 58 smokers and non-smokers patients with AMI diagnosed by clinical history, electrocardiography and troponin I level and another 35 healthy controls were included in this study. The (Pro72Arg) polymorphism of the *p53* gene was evaluated by molecular techniques.
Results The genotype distribution for the Pro72Arg variant of the *p53* gene in AMI non-smokers patients (PP: $n = 9$, 47.4%; RP: $n = 2$, 10.5%; RR: $n = 8$, 42.1%) and controls (PP: $n = 9$, 50.0%; RP: $n = 9$, 50.0%; RR: $n = 0$, 0.0%) was significantly different ($p = 0.002$). and the genotype distribution for the Pro72Arg variant of the *p53* gene in AMI smokers patients (PP: $n = 20$, 51.3%; RP: $n = 1$, 2.6%; RR: $n = 18$, 46.2%) and controls (PP: $n = 14$, 82.4%; RP: $n = 2$, 11.8%; RR: $n = 1$, 5.9%) was significantly different ($p = 0.009$).
Conclusion These findings suggest that the Pro72Arg polymorphism of tumor suppressor *p53* gene is associated with AMI patient studies.
Keywords AMI, PCR, RFLP, polymorphism

Introduction

Different genes including the tumor suppressor (*p53*) gene have been implicated in the etiology of coronary artery diseases.[1] By regulating gene expression and other indirect means *p53* participates in the regulation of glucose, fatty acid, amino acid and purine metabolism, in addition it influences mitochondrial integrity and oxidative phosphorylation, insulin sensitivity, antioxidant response.[2] Furthermore *p53* gene plays an important role in regulating vascular smooth muscle cell growth and may mediate and abnormal occurrence of apoptosis in atherosclerotic lesions by attenuating or accelerating the apoptotic death process.[3] On the other hand *p53* gene could regulate cell division and apoptosis within atherosclerotic plaque depending on the level of *p53* gene expression induced by DNA damage and cell type. Affected mutations in *p53* gene can induce dysfunction of *p53* and inhibit apoptosis and loss of gene activity could which play a relevant role in the pathogenesis of atherosclerosis.[4]

A common *p53* tumor suppressor gene polymorphisms occur at codon 72 of exon 4, with two alleles encoding either arginine (CGC) or proline (CCC). The distribution of the three genotypes (R/R, R/P and P/P) depends largely on the ethnic composition of the studied population.[5]

The Pro or Arg of codon 72 has variants reported to differ in functional activity because this polymorphism is located in the proline rich domain of *p53*, which is necessary for the *p53* protein to fully induce apoptosis.[6]

The aim of the presented work is to investigate the possible association between the codon 72 polymorphism (Pro72Arg, rs1042522) of the tumor suppressor gene (*p53*) with (smoker and non-smoker) acute myocardial infarction in Iraqi patients of Karbala province.

Materials and Methods

Subjects

The study was conducted during the period from Nov., 2014 till Sep., 2015. Fifty eight patients presented with typical chest pain to the cardiac care unit (CCU) in Al-Hussein Teaching Hospital, Al-Hussein Medical City/ Kerbela Health Directorate and Department of Biochemistry- college of Medicine/University of Karbala. Thirty five persons age – matched healthy volunteers were selected as a control group. Both groups were divided into smokers and nonsmokers (39 AMI smokers patient and 17 smoker control) and (19 non-smokers AMI patient and 18 nonsmoker control). The diagnosis was based on the clinical history, presentation confirmed by ECG and various investigations of cardiac biomarker.

DNA extraction

About 10 ml of venous blood sample was drawn from each patient and control groups. Two ml of blood sample collected in EDTA tube for genomic DNA extraction used for molecular analysis. The DNA extraction kit was purchased from BIONEER, South Korea.

The remaining sample was transferred into another tube at room temperature and stand for 20 min for clotting and then centrifuged at 3000 rpm for serum collection used for various cardiac biomarkers determinations.

Tumor suppress gene P53 polymorphism

Four primers (Table 1) were used in a single PCR reaction, P1 and P2 to amplify a 281 bp band (control band) which make sure the success of the amplification, P3 specific to amplify a 193 bp band which indicate the present of proline allele and P4

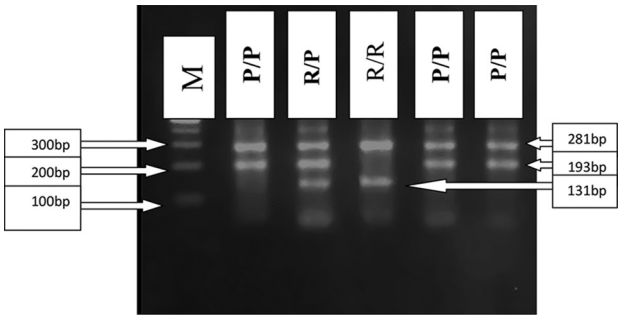

Fig. 1 **Agarose gel electrophoretogram of the *p53* Arg72Pro polymorphism.**

specific to amplify a 131 bp band which indicate the present of arginine allele as shown in (Fig. 1).[7]

Analysis for different subjects (M = molecular weight marker)

PCR premix™ kit was used to amplify. It contained 5 µl of extracted DNA, (0.5 µl from each P1 and P2 and 1µl from each P3 and P4) of 10 pmol/µl primers were mix in total volume of 20 µl. Then the mixture was added to lyophilized PCR premix formula.

The mixture was heated for 5 min at 94°C and underwent 40 cycles of amplification: annealing (65°C for 30 s), extension (72°C for 30 s) and denaturation (94°c for 30 s). The PCR product was analyzed on 1.5% agarose gel stained with ethidium bromide.[7]

Result

The results showed a significant difference in distribution of *p53* (codon 72) allelic polymorphism ($P < 0.01$) as shown in Table 1. Higher percentage in RR(Arginine/Arginine) allele (42.1%) in non-smoker AMI patient in compared (0%) with non-smoker normal control groups, while low percentage in RP (Arginine/Proline) allele (10.5%) in non-smoker AMI patient in compared (50%) with non-smoker normal control groups (Table 2).

Significant difference in distribution of *p53* (codon 72) allelic polymorphism ($P < 0.01$) as shown in Table 3. Higher percentage in RR (Arginine/Arginine) allele (46.2%) in smoker AMI patient in compared to smoker normal control groups (5.9%), while low percentage in PP (Proline/Proline) allele (51.3%) in smoker AMI patient in compared to smoker normal control groups (82.4%) (Table 3).

Discussion

The results showed a significant difference in distribution of *p53* (codon 72) allelic polymorphism, high percentage in RR allele in smoker and non-smoker AMI patient in comparing to smoker and non-smoker normal control group. These findings were agreeing to Chilean study by (José Caamaño et al. 2011). This study showed increasing in RR allele in patients and explained that Apoptosis in endothelial cells have related to plaque instability and thrombus formation.

Table 1. **Sequence of primers used for PCR of *p53* gene polymorphism**

Primer	Sequence
P1	5′-GCCGTCCCAAGCAATGGATGATT-3′
P2	5′-GGCAACTGACCGTGCAAGTCACAG-3′
P3	5′-AGAATGCCAGAGGCTGCTCCACC-3′
P4	5′-CTTCTGGTGCAGGGGCCAAGC-3′

Table 2. **Comparison between non-smoker myocardial infarction patients and non-smoker control groups in the distribution of *p53* (codon 72) allelic polymorphism**

		Codon 72 allelic polymorphism			*p*- value
		RR (*n* =), %	PP (*n* =), %	RP (*n* =), %	
Non-smoker	Patient	(8) 42.1%	(9) 47.4%	(2) 10.5%	$p < 0.01$
	Control	(0) 0.0%	(9) 50.0%	(9) 50.0%	

*RR: Arginine/Arginine, PP: Proline/Proline, RP: Arginine/Proline.

Table 3. **Comparison between smoker myocardial infarction patients and smoker control groups in the distribution of *p53* (codon 72) allelic polymorphism**

		Codon 72 allelic polymorphism			*p*- value
		RR (*n* =), %	PP (*n* =), %	RP (*n* =), %	
Smoker	Patient	(18) 46.2%	(20) 51.3%	(1) 2.6%	$p < 0.01$
	Control	(1) 5.9%	(14) 82.4%	(2) 11.8%	

*RR: Arginine/Arginine, PP: Proline/Proline, RP: Arginine/Proline.

The disturbance in the apoptotic response may lead to accumulation of intimal cells through a therogenesis and functional consequence of the Pro72Arg polymorphism has related to inhibition of p73 function, a member of the *p53* family of nuclear transcription factors, implicated in tumor suppression, Arg polymorphic allele is more efficient in binding to p73, blocking its action and facilitating the proliferation of vascular smooth muscle cells. By this mechanism Arg variant of the *P53* Pro72Arg polymorphism is more susceptible to suffer deregulation of apoptosis during atherosclerosis progression.[8]

Also another Brazilian study by (Disciplina de Genética et al 2007) suggested that Arg72 allele is associated with cardiovascular disease.[9] In additional type 2 diabetes mellitus T2DM which is risk factors for AMI and the fact that the Carriers of genotypes containing Arg72 allele previously associated with susceptibility to type 2 diabetes mellitus T2DM than pro72 genotype carriers.[10]

Conclusion

In this study, we ascertain that a significant association between the codon 72 polymorphism (Pro72Arg, rs1042522) of the tumor suppressor gene (*p53*) with acute myocardial infarction (AMI). ■

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