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Potential Impact of micro RNA-146a Gene Polymorphisms in Oxidative Stress of Diabetic Mellitus Type 1

Fulla Abd Alsattar Alriyahee[1], Noora M. Hameed [2], Israa Harjan Mohsen[3], Mona N. Al-Terehi*[4]

[1]University of Basrah, College of Education for Pure Sciences, Iraq,
[2] Anesthesia Techniques / Al-Nisour University College/ Iraq,
[3]University of Babylon, College of Nursing/Iraq
[4]University of Babylon /College of Science/Iraq
Email: monanajah1981@gmail.com

ABSTRACT

Micro-RNA is small non coding molecules discovered recently and it has main role in different cell process and its deregulation contributed in different diseases etiology, the present study aims to investigate effect of micro-RNA 146a gene polymorphism in oxidative stress in diabetes mellitus type 1 patients, samples and data collection implemented for fasting blood glucose (FBG), total antioxidant (TOT) and reactive oxygen species (ROS) in addition to DNA extraction for gene amplification and genotyping by single strand conformation polymorphism technique, the results of present study show significant elevation in FBG (194.29, 65.00)and TOT (1.433, 0.566) for patient and control respectively, also significant differences in ROS level (4.848, 4.98). the genotyping show (A, B, C and D) genotypes, a significant differences in C and D (P 0.0004), C was found in all patients but didn't appeared in control, D found in all control while didn't found in patients, A found in 61.53% and 100% in patients and control in non –significant (p 0.0643), B found in 73.07% in DM1 patients and in all control. The effect of genotyping in oxidative stress shows all genotyping effect in ROS and total antioxidant level, A, B and C show significant differences in total antioxidant (P 0.017, 0.017, and 0.008), but no significant differences were observed in ROS for all genotyping. The present study concluded that the microRNA-146a associated with oxidative modulator in diabetes mellitus type 1.

Keywords: diabetes mellitus type 1, total antioxidant, reactive oxygen species, micro-RNA146a

Correspondence:
Mona N. Al-Terehi
University of Babylon /College of Science/Iraq
Email: monanajah1981@gmail.com

INTRODUCTION

In the last decades the diabetes mellitus disease was recorded in a high incidence rate in Iraqi population the diabetes mellitus type 1 (DM2) is an autoimmune destruction of pancreatic beta-cells damage by macrophages and T lymphocytes (American Diabetes Association, 2015). About 80-90% of beta-cells damage by the infiltrating immune system. The development of DM2 is slow, that give potentially long time for possibility to identify and theoretically treat individuals at risk (Guay and Regazzi, 2013; Atkinson, Eisenbarth 2001). Investigations focused on the oxidative stress role in disease incidence and development, they found ROS and NOS are contributed in different disease, such as cancer, autoimmune disease, cardiovascular disease and diabetes mellitus types, the accumulation of ROS effects in DM causes different complications like neuropathy, nephropathy, cardiovascular disease and retinopathy (Asmat et al., 2016; Liguori et al., 2018). Other axis in present study deal with microRNAs (miRNAs) that discovered recently, as a results of its role in several disease and biological processes it can be used as biomarker for health evaluation and disease progression (Guay and Regazzi, 2013). miRNAs are large number of noncoding RNAs molecules consist of 20-22 n contributed in gene regulation and stopped translation by different mechanisms (Carrington and Ambros, 2003; Esteller, 2011, Butz et al., 2016) the changes in changes in microRNA expressions have been correlated with diseases (Lin and Gregory, 2015; Zalts and Shomron, 2011; Pauley et al., 2009). The present study aims to linked oxidative stress in DM1 with microRNA 149 gene polymorphism using single strand changes polymorphisms.

METHODOLOGY

The study groups consist of 30 patients with diabetes mellitus type 1 were attended to Margan hospital city in Hilla, Controls included 30 apparently healthy individuals. Blood samples and other data were collected based on the ethical approval of environmental and health ministry of Iraq and All subjects were taken written consent for participation in study.

Exclusion Criteria: complication of diabetic disease, cancer, thyroidsim and autoimmune diseas were exclusion from present study.

Genomic DNA extraction: DNA was extract according to manufacturer leaflet concentration and purity were detected in addition to genomic DNA electrophoresis (Geneaid / Taiwan).

Primers sets: MiR-146a primer was F: GGGTCTTTGCACCATCTCTG, R-TCCAGTCTTCCAAGCTCTTCA. PCR annealing temperature was 57C for 20 sec was amplified at 60.5 annealing TM (Vinci et al., 2013).

PCR conditions and size products: MiR-149. 30cycles (30 s / 94˚C, 20 s / 60.5˚C, 50 s /72˚C, finally 10 min / 72˚C) for MiR-149, PCR products were electrophoresis in agarose gel (1.5% agarose, 70 V, 20 mA for 45 min) with ethidium bromide staining, the PCR size product was (178 bp) for MiR-149 . The results were analysis using odd ratio at (CI 95%, p value <0.05). SSCP technique and electrophoresis according to Al-Terehi *et al*., (2016).

RESULTS AND DISCUSSION

The results of present study show that the age of patients and control non -significant differences (0.469), significant differences (0.002) observed in BMI that were 30.89 and 26.24 for patient and control respectively, fasting blood glucose was high in patients 194.29 mg/dl than control 65.00 mg/dl, significant differences was observed in total antioxidant and ROS for patients and control (p 0.019, 0,000) respectively in addition to 51% of patients were smokers and 92.3% have family history of disease incidence (table 1).

Type 1 diabetes is insulin-dependent diabetes mellitus (DM2) the autoimmune disorder of β-cells by immune mediated pathways that liberated ROS and some types of cytokines lead to pancreatic β-cells damage and insulin secretion losing (Meghan et al., 2011). The results of present study show high level of TOT in diabetic patients than control group while significant differences appeared in ROS level, the elevation in TOT is a result of redox modulation and oxidative balance between ROS and TOT, in present study the total antioxidant causes decreased in ROS level in patients group, the Oxidative stress is elevated in diabetes mellitus result from increasing in the production of ROS and a decline in the mechanism of antioxidant defense. In DM The ROS are formed disproportionately by glucose oxidation, non-enzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins (Rodiño-Janeiro et al., 2010).

Table 1. mean differences of some parameters of study groups.

Items	Patients	control	P value
age	48.33±12.68	50.47±6.22	0.469
BMI	30.89±6.23	26.24±3.36	0.002
FBG **mg/dl**	194.29±86.88	65.00±47.69	0.011
TOT	1.433±4.84	0.566±0.93	0.019
ROS	4.848±0.126	4.98±0.123	0.000
Smoker	51%	49%	
Family history	92.3%	0	

Micro RNA-146a gene polymorphism in study groups

The results of microRNA gene polymorphism show that the PCR size products were 195 bp according to virtual amplification (table 2)

Table 2. Virtual amplification of microRNA-146a

Accession number	PCR product
Homo sapiens isolate P4 miR-146a miRNA gene, SNP|rs2910164 alleles='C/G	195 bp product from linear template Untitled, base 417 to base 611 (GGGTCTTTGCACCATCTCTG - TCCAGTCTTCCAAGCTCTTCA). GGGTCTTTGCACCATCTCTGAAAAGCCGATGTGTATCCTCAGCTTTGAGAACTGAATTCC ATGGGTTGTGTCAGTGTCAGACCTSTGAAATTCAGTTCTTCAGCTGGGATATCTCTGTCA TCGTGGGCTTGAGGACCTGGAGAGAGTAGATCCTGAAGAACTTTTTCAGTCTGCTGAAGA GCTTGGAAGACTGGA

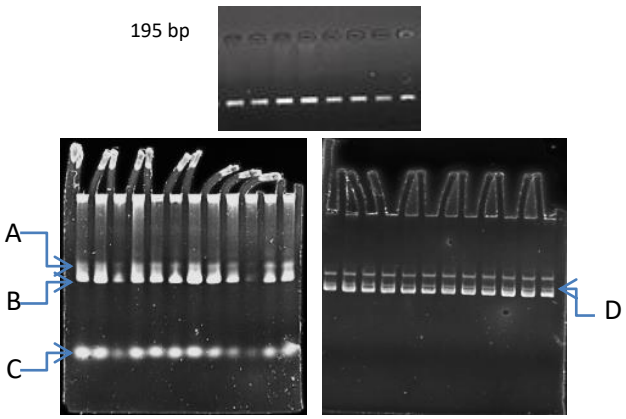

Figure 1. electrophoresis pattern of microRNA-146a, size products of amplification (195) bp, left pattern SSCP for patients, right pattern SSCP for control group.

The polymorphism of microRNA-146a

The results of present study show four genotyping represented by A, B, C and D. (Figure 1), different genotyping appearances in patients and control, significant differences in genotype C and D (P 0.0004), C appearances in all patients but didn't appeared in control, D appeared in all control while didn't found in patients. Other genotypes diverted between patient and control, A found in 61.53% and 100 & in patients and control respectively in non –significant (p 0.0643), B found in 73.07% in DM1 patients and in all control contributors in present study in non-significant differences also (table 3).

Table 3 genotyping of micro-RNA 146a in DM1 and control group.

Pattern	DM1%	Ctr%	Odd ratio	P value
A+	61.53%	100	15.9091	0.0643
A-	38.46	0	CI 95% 0.8489 -298.1592	
B+	73.07	100	9.6154	0.1326
B-	26.92	0	CI95% 0.5035 -183.6254	
C+	100	0	1325.0000	0.0004
C-	0	100	CI95% 24.8261-70716.9044	
D+	0	100	1325.0000	0.0004
D-	100	0	24.8261-70716.9044	

Effect micro-RNA 146a gene polymorphism in oxidative stress in DM1 and control group

The Effect micro-RNA 146a gene polymorphism in oxidative stress in DM1 and control group was studied, the results show that all genotyping effect in ROS and total antioxidant level, A, B and C show significant differences in total antioxidant (P 0.017, 0.017, and 0.008), no significant differences were observed in ROS for all genotyping (table 4).

Table 4. effect of microRNA-146a gene polymorphism in the total antioxidant level and ROS

	Total antioxidant			ROS		
Pattern	DM1	Ctr	P value	DM1	Ctr	P value
A+	1.573	0.568	0.017	4.8605	4.854	0.973
A-	1.106	0		4.815	0	
B+	1.232	0.568	0.017	4.868	4.854	0.992
B-	1.7783	0		4.781	0	
C+	1.415	0	0.008	4.844	0	0.943
C-	0	0.568		0	4.85	
D+	0	0.568	0.995	0	4.854	0.906
D-	1.411	0		4.844	0	

The difference of genotyping in present study was diverted according to the SNPs rs2910164 which enrolled in present amplification (table 1) that proved its contribution in risk of diabetes mellitus type 2 in Chinese population (Li et al., 2015) and this SNP effect in miR-146a expression that reduced pre and mature molecules (Jazdzewski et al., 2008; Mann et al., 2016) this types of microRNA contributed in inflammatory events by attenuated NF-kappa B which is important in pathophysiology and complication of diabetes (Patel and Santani, 2009; Ndisang, 2010; Suryavanshi and Kulkarni, 2017). Other study reported association rs2910164 with diabetic nephropathy in T1DM patients (Kaidonis et al., 2016). The rs2910164 SNP is found within the seed sequence of the pre-miR-146a, at the stem-loop, C allele reduced the level of miR146a and has protective activity (Ciccacci et al., 2014; Assmann et al., 2017).

Many studies prove contributed of micro RNA types in oxidative stress in diabetic disease Wan and Li, (2018) concluded that the miR-146a/Nox4 decreases generation of ROS and inflammation and prevents diabetic nephropathy, they found that excessive production of miR-146a blocked Nox4 protein expression and lowering ROS production, inflammation and oxidative stress.The present study concluded that the microRNA-146a associated with oxidative modulator in diabetes mellitus type 1.

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