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Study the CAT-21 A/T (rs7943316) gene polymorphism and its correlation with physiological antioxidant index in the Iraqi patients with T2DM

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Abstract---Background: A multi-gene inherited metabolic illness in which the body is unable to produce enough insulin and is characterized by abnormal glucose homeostasis has been now called Type 2 diabetes mellitus (T2DM). Objectives: This study aimed to evaluate the effect of CAT-21 A/T (rs7943316) gene polymorphism and antioxidant index (AOI) in the Iraqi patients with T2DM. Methods: In general, 135 individuals, 90 T2DM patients and 45 healthy control were employed in this research. The spectrophotometer method were used to assessment of TAO-C and MDA levels (antioxidant index TAO-C/MDA), while CAT-21 A/T (rs7943316) SNPs was an estimate by PCR-RFLP and restriction fragments by *Hinf1* enzyme restriction. Results: In this study, the results propose profoundly tremendous contrasts in TAO-C, MDA, and AOI levels among T2DM and the benchmark group ($p\text{-value} < 0.05$). Then again, the outcomes propose the profoundly bad relationship ($r = -0.765$) between the periods of the T2DM bunch with TAO-C as well as, we tracked down an exceptionally certain connection ($r = 0.556$) between the times of the T2DM bunch

with MDA levels. Furthermore, in the genetic part of this work, the results indicated that the AA homozygote genotype implied a statistically significant effect (P-value=0.000) (OR=2.66(1.23-4.12)) for the risk of T2DM. In addition, we observed a significant relation between AT genotype T2DM (OR= 2.99 (1.22-4.98). Moreover, the results suggesting statistical differences (OR) in CAT-21 A and CAT-21 T alleles between T2DM and CONT groups. Conclusion: According to the results, CAT-21 A/T gene polymorphism is a risk factor to the incidence and promote more complication in patients with T2DM.

Keywords---T2DM, antioxidant index, CAT-21 SNP, TAO-C.

Introduction

One of the most disease common in all parts of the world is called Diabetes mellitus (DM) (Abate account & Chandalia, 2003). The main disorders characterized of this disease such as hyperglycemia, altered metabolism of lipids, carbohydrates and proteins (Adom & Liu, 2002). The major initiator of diabetic complications is chronic hyperglycemia (Agil et al., 1999) that's causes damage to many organs such as eyes, kidneys, nerves, heart and blood vessels (Ahmad & Beg, 2001). DM is turning into the third mortality reason for the soundness of humanity alongside malignant growth cardiovascular and cerebrovascular illnesses (Akinpelu et al., 2008). The data of many epidemiological studies suggest that at least one out of twenty deaths is attributable to DM and related complications, a proportion which increases to at least one in ten deaths in adults aged range 35-64 years (Akyol et al., 2002). The anomaly of digestion of carbohydrates is linked to low blood insulin stage or cold-heartedness of goal organs to the insulin that prompts the frequency of DM (Alarcon et al., 2002). Many elements such as growing of the age population, consumption of calorie-rich diet, obesity, and sedentary way of life may also be to a high-quality amplify in the wide variety of diabetics international (Al-Hashem et al., 2009). DM is often promoting by increased production of free radicals and or impaired antioxidant defenses (American Diabetes Association, 2018). The complicated pathogenesis and differed show and any characterization of this problem of DM that affected by the physiological circumstances present at the hour of appraisal and conclusion. The principle DM order as of now utilized depends on both the etiology and the pathogenesis of infection and is helpful in the clinical appraisal of illness and for choosing the expected treatment. DM relying upon this order can be isolated into four primary sorts or classes, type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM), gestational diabetes mellitus (GDM), and DM brought about by specific explicit circumstances, pathologies, as well as problems (International Diabetes Federation, 2019, Rawshani et al., 2017, Amalesh et al., 2011). NIDDM (Non insulin-dependent diabetes mellitus or "adult-onset diabetes Type 2 Diabetes Mellitus (T2DM) (Ameenah et al., 2006). The commonest form of DM is also referred to as type 2 that accounts percentage for 80 –90% of all cases of diabetes in most region on the world (Andersen et al., 2006) that's characterized by disorders in pancreas gland with changes in insulin secretion and insulin resistance. Several factors contribute in T2DM pathogenesis, including environmental and lifestyle factors,

positive family history, ethnicity, and genetics (Ripsin et al., 2009). The goal of current work is to assessment the effect of CAT-21 A/T (rs7943316) gene polymorphism and antioxidant index (AOI) in all subjects involved in this work.

Materials and methods

Study design

The study covered of 140 subjects (90 patients with T2DM (56 male and 34 female) at range of age (29-59 Y) and 45 subjects as control group) with matching in age and genders.

Determination of TAO-C, MDA and AOI levels

The strategy for assessment of MDA depends on the colorimetric response with thiobarbituric corrosive (TBA) at 90-100°C and pH 2-3 for 15 minutes to frame pink shading item, which can be estimated by spectrophotometer at a frequency of 532 nm. The samples of subjects serum to measure of malondialdehyde (MDA) is determined by such procedure with new modification in which two groups of tubes are prepared as shown table 1:

Table-1: Summary method of estimation of MDA

No.	Reagent	Sample	Blank
1	Serum	150µl	-
2	TCA (17.5 %)	1 ml	1 ml
3	TBA (0.67 %)	1 ml	1 ml

Genotyping analysis

The DNA was removed relying upon the salting-out technique and was measured utilizing an UV spectrophotometer and the respectability of DNA was checked by settling 2 µl genomic DNA tests on 1% agarose gel electrophoresis. PCR Analysis: PCR was performed to enhance CAT - 21A/T (rs7943316) SNP. The accompanying preliminaries (forward and switch) were planned utilizing on the web programming (www.simgene.com/primer3). PCR-RFLP was performed by involving interesting introductions for CAT - 21A/T (rs7943316) SNPs examination of genotyping and Hinf1as limitation compound (RE), as displayed in table 2:

Table-2: primers of PCR-RFLP method of CAT-21 A/T(rs7943316) SNP

Primer sequence (5'→3')	Amplicon length	RE bands (<i>Hinf1</i>)
CAT-21 F: AATCAGAAGGCAGTCCTC	250 bp	117 and 73 bp
CAT-21 R: CGGGGAGCACAGAGTGT		

The PCR was acted in an all out volume of 50 µl of the response combination with Taq polymerase and conveyed by the thermocycler (bio-rad) and exposed to

denaturation at 95 C° for 4 min, trailed by 30 patterns of 94 C° for 45 sec, the strengthening temperature of 60.6 C° for 450 second and the last augmentation stage at 72 C° for 5 min. After PCR, the item was electrophoresed on 1.5% gel, and the PCR items were seen with 250 bp band which were kept immunized by the RE (Hinf1) chemical at 37°C for short-term and afterward electrophoresed on 2% gel (Figures 1 and 2). The consequences of the underlying PCR decided the site of SNP position 250 bp of A/T allele, and the finish of the Hinf1enzyme after PCR item uncovered SNP position 117 and 73 bp of two A and T alleles. The last PCR item was photograph documentation on the UV analyzer.

Statistical Analysis

In the study, frequency and percentage, as well as mean and standard division (SD) were applied to comparison of results. The Hardy-Weinberg equation was used for performed the genotype analysis in T2DM with healthy group.

Results

T2DM group medicinal properties as shown in table 3:

Table-3: T2DM clinical properties

Clinical variables	No = 90	Percentage (%)	p-value
Age			
29-49	50	56	0.230
49-59	40	44	
Gender			
M	56	53	0.113
F	34	47	
BMI			
≥30	45	52	0.315
<30	40	48	

The results of this study conducted that profoundly tremendous contrasts in TAO-C, MDA, and AOI levels among T2DM and control bunch (p-value< 0.05), as showing in table 4:

Table-4: TAO-C and MDA levels in study group

Groups	TAO-C (U/ml) mean± SD	P-value	MDA(U/l) mean± SD	P-value
T2DM n=90	19±7	0.001	7.19±2.1	0.001
CONT n=45	29±4		2.6±0.8	

Also the results showing the highly negative correlation ($r=-0.765$) between ages of T2DM group with TAO-C as well as, we found highly positive correlation ($r=0.556$) between ages of T2DM group with MDA levels , as shows in figure1:

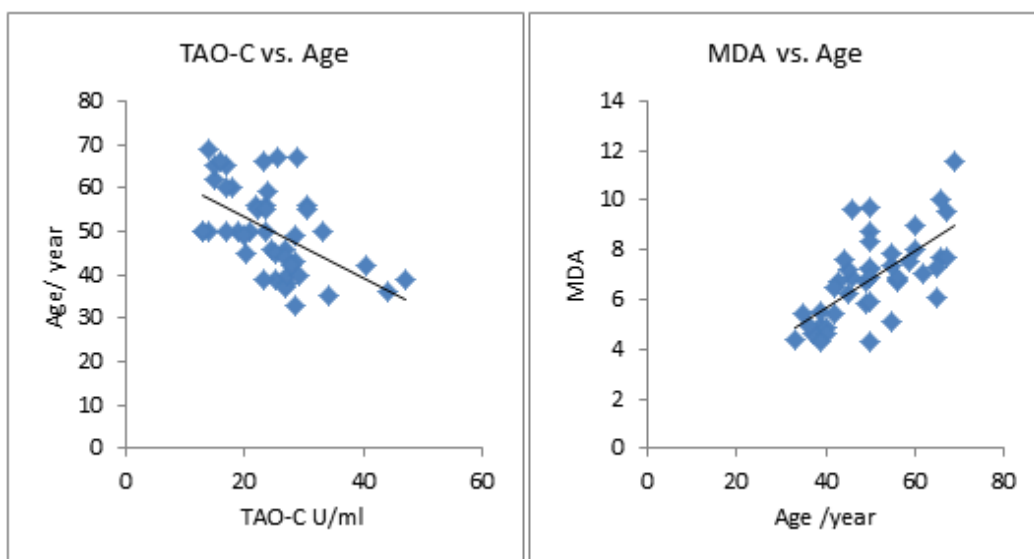


Figure-1: Correlation between TAO-C and MDA ($\mu\text{mol/l}$) levels with Age (year) in T2DM group

The results of our study showing negative correlation between BMI of T2DM group with BMI as shown in fig.3:

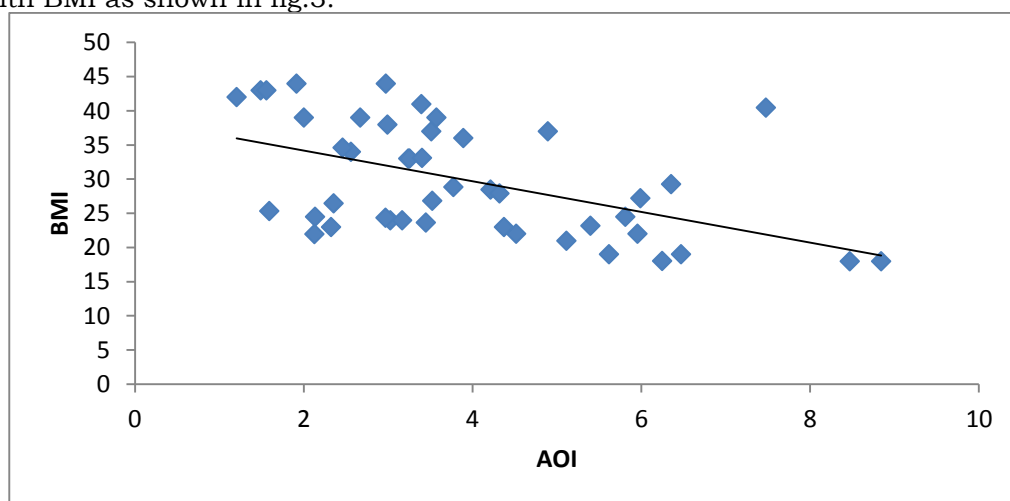


Figure-2: Correlation between BMI and AOI levels in T2DM group

Polymer chain reaction-restriction fragment length polymorphism was used to assessment the genotyping analysis and restriction of target sequence of CAT-21 A/T gene to explain the genotyping analysis,, as showing in figures 3 and 4:

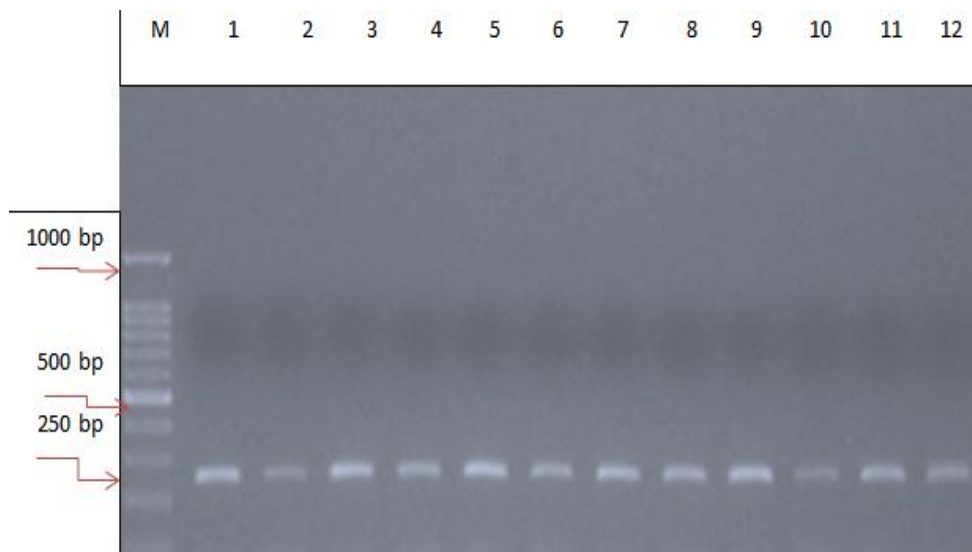


Figure-3: Electrophoresis of the CAT-21 genotype. Lane M is DNA marker

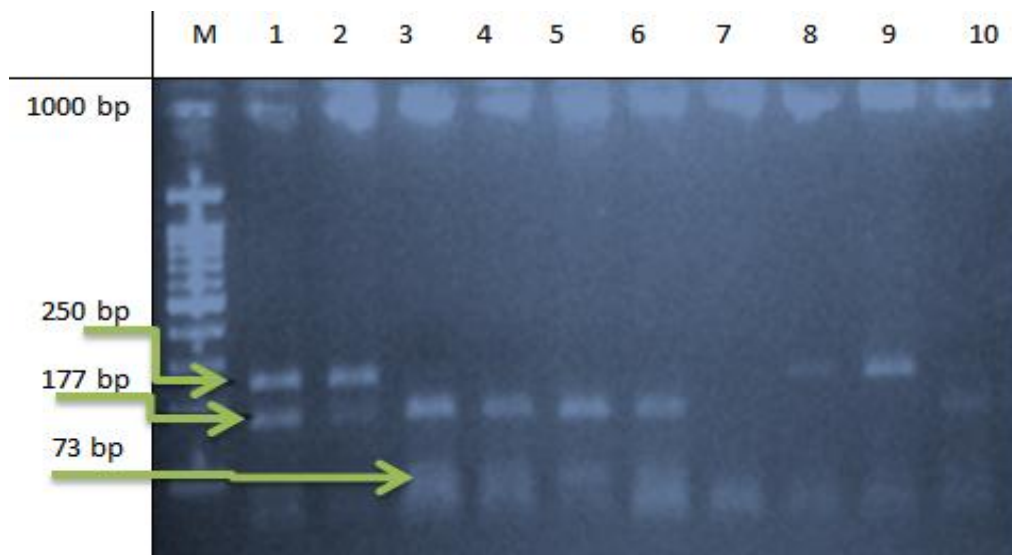


Figure-4: Electrophoresis Results after Incubation with the *HinfI* Enzyme

The results of present study suggesting frequency of genotype polymorphism and allele frequency of CAT -21A/T (rs7943316) as shown in figure 5:

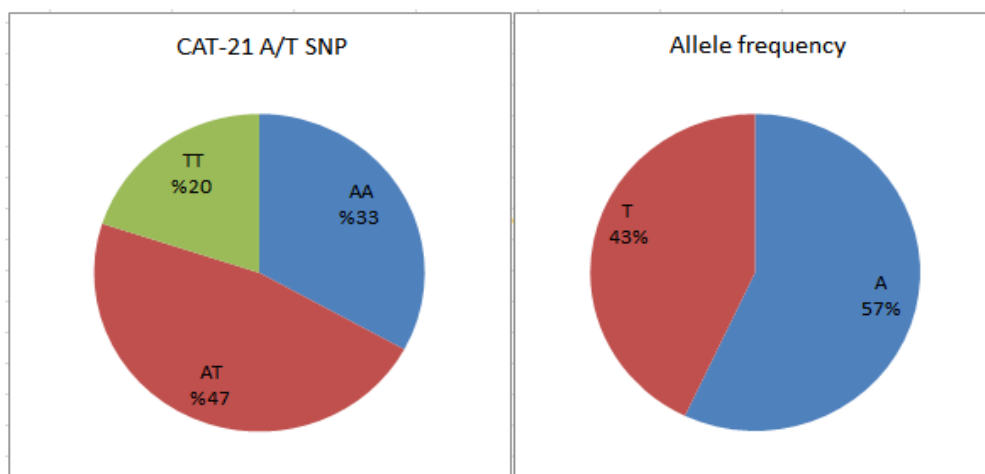


Figure-5: Frequency of genotype polymorphism (left) and allele frequency (right) of CAT -21A/T (rs7943316)

By the odd ratio statistical analysis test, the results show differences in CAT-21 A/T SNP, as listed in table 5::

Table-5: Comparison of CAT -21A/T genotypes incidence in T2DM and control groups

	CONT n=45	T2DM n=90	Total	OR (CI 95%)	p-value
Genotypes (%)	(%)	90(%)			
AA	15 (33)	27 (30)	42	1.0 (reference)	
AT	21 (47)	33 (37)	54	2.66 (1.23-4.12)	
TT	9 (20)	30 (33)	39	2.99 (1. 22-4.98)	0.000*
TOTAL	45	90	135		
HWE(Alleles frequency) (p+q=1)					
A	57%	48%	2.33 (1.44-3.39)		0. 000*
T	43%	52%			

The present results of this work indicating that the AA homozygote genotype implied a statistically significant effect (P-value=0.000) (OR=2.66(1.23-4.12)) for the risk of T2DM. In addition, we observed a significant relation between AT genotype T2DM (OR= 2.99 (1.22-4.98). Moreover, the results suggesting statistical differences (OR) in CAT-21 A and CAT-21 T alleles between T2DM and CONT groups.

Discussion

The non-insulin dependent diabetes mellitus (NIDDM) that known by insulin resistance that which may be combined with relatively reduced insulin secretion is called DM (Fujioka,2007) . The people who are older, sedentary or obese, or

have a family history of the disease are more features common in DM (John & Marek, 2005). Other factors such as strong inheritable genetic connection in NIDDM, having relatives (especially first degree) with type 2 increases risks of developing NIDDM very substantially (Kzar et al., 2020). In this work, we evaluated the CAT-21 A/T SNPs in patients with T2DM and its correlation with AOI. By results indicated that no significant differences in the age, gender, and BMI between T2DM and control groups ($p\text{-value}>0.05$). The end product of peroxidation of poly unsaturated fatty acids and related esters is MDA that consider of biological marker of lipid peroxidation caused by oxidative stress (Al-Gazally et al., 2018). The common data of many epidemiological studies indicated that at least one out of twenty deaths is attributable to DM and related complications, a proportion which increases to at least one in ten deaths in adults aged range 35-64 years. In this work, the outcomes demonstrated that the AA homozygote genotype inferred a measurably huge impact ($P\text{-value}=0.000$) ($OR=2.66(1.23-4.12)$) for the gamble of T2DM. In addition, we observed a significant relation between AT genotype T2DM ($OR= 2.99 (1.22-4.98)$). Moreover, the results suggesting statistical differences (OR) in CAT-21 A and CAT-21 T alleles between T2DM and CONT groups. The results of current work was agreement with other studies (Kzar et al., 2019- Al-Charak et al., 2020) that suggested of the gene polymorphism is risk factor to incidence many of diseases. The creators recognize specific restrictions of this review. Affirmation of normal variations in the human genome with unobtrusive impacts on normal sickness hazard like T2DM, regardless of whether genuine, need huge example sizes to defeat the impact of numerous hereditary and natural modifiers. Likewise, our review subjects comprised of Iraqi identity, and along these lines, the generalizability to different nationalities is obscure.

Conclusion

According to the results, CAT-21 A/T gene polymorphism is a risk factor to the incidence and promote more complication in patients with T2DM.

Disclosures and confluc interest: Non

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