Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(5), 83-90 | ISSN:2251-6727

Polymorphism of Telomerase Reverse Transcriptase (TERT) Gene Rs2853669 in Iraqi Patients with Type2 Diabetes Mellitus

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(Received: 02 September 2023 Revised: 14 October Accepted: 07 November)

KEYWORDS
susceptibility, transcriptase, genotyping, polymorphism, diabetes

ABSTRACT:

The aim of this study was to investigate the association of polymorphism of telomerase reverse transcriptase (TERT) rs2853669 to the susceptibility of type2 diabetes mellitus in Iraqi patients. Forty five patients with type 2 diabetes mellitus (20 males and 25 females) and 35 healthy controls (17 males and 18 females) were genotyped for telomerase reverse transcriptase (TERT) rs2853669 using TaqMan custom SNP genotyping assay .In both patients and control groups , the distribution frequencies of genotypes and alleles of the rs2853669 A/G gene were in Hardy-Weinberg equilibrium (p>0.05) . The most common genotype in both control and type2 diabetes mellitus patients was AG with a percentage of 91.12% and 74.28% respectively. The genotypes AG and AA were higher in type2 diabetes mellitus patients than healthy control 91.12% and 8.88% in patients vs. 74.28% and 22.85% in controls . A allele was more predominate comparing to G allele with percentage of 60% and 54.44% for A, 40% and 45.56% for G in control and patients groups respectively with no significant differences .

The association analysis of (TERT) rs2853669 with susceptibility to type2 diabetes mellitus showed that the individuals carrying the heterozygous AG genotype and homozygous AA genotypes were more likely to have a significantly increased risk of type2 diabetes mellitus with OR=3.5481 (CI95%0.9902 to 12.7128), and OR= 0.3293 (CI95% 0.0902 to 1.2020)respectively, p= 0.0518 and 0.0927 for each genotype respectively. The GG genotype decreases the association with type2 diabetes mellitus OR=0.2527 (CI95%0.0100 to 6.3964), p =0.4041. These results suggested that G allele might play a protective role against type2 diabetes mellitus whereas the A allele might consider a risk factor in type2 diabetes mellitus . The subgroup analysis revealed that the type2 diabetes mellitus risk of females with (TERT) gene AG genotype was 2.6250 times higher than that in controls OR= 2.6250 (CI 95% 0.6155 to 11.1954), p= 0.1922 . GG genotype decreases the probability of contracting the disease significantly with OR = 0.2288 (CI 95% 0.0088 to 5.9470), p = 0.3749. AA genotype decreases also the association with type 2 diabetes mellitus with OR= 0.4952 (CI95% 0.1121 to 2.1879), p = 0.3539 . Among males, the AG genotype increased type2 diabetes mellitus risk among patients OR=9.8966 (CI95%0.4741 to 206.6018) p=0.1393. The homozygous AA genotype decreases the association with the disease OR=0.1010 (CI95%0.0048 to 2.1094) p=0.1393. There were no males with the homozygous genotype GG in patients and controls.

Introduction

Type 2 diabetes (T2DM) is a multifactorial disorder that affects multi-organ and can alter telomerase (encoded by *hTERT* gene) activity and thus, may affect telomere length (Huda and Yasmin and Nabi ,2021) . Telomerase, a ribonucleoprotein complex containing a template RNA subunit, a telomerase-associated protein, and a *telomerase reverse transcriptase (TERT)*, extends telomeres length by adding telomeric repeats to the chromosome ends (Lewis and Wuttke ,2012). In most cells, *TERT* is the critical rate-limiting component responsible for the catalytic activity of telomerase (Seol *et al*.,2008).

Numerous evidence suggests that telomeres and telomerase have important roles in senescence *in vitro* and *in vivo* (Chen *et al*.,2013) . The production of high levels of reactive oxygen species results in a disturbance of the redox balance and shifts cells into a state of oxidative stress, which subsequently leads to premature senescence

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JCHR (2023) 13(5), 83-90 | ISSN:2251-6727

with the shortening of telomeres (Concetti *et al*.,2013). Therefore, diabetes may be associated with the loss of function of telomerase activity. Telomerase activity may decrease upon having diabetes (Huda and Yasmin and Nabi ,2021) .

Single nucleotide polymorphisms (SNPs) in the *TERT* region have been linked to telomer biology disorders , including, but not limited to, bone marrow failure (Vulliamy *et al*.,2005 ; Yamaguchi *et al*.,2005) , aplastic anemia (Yamaguchi *et al*.,2005) , myeloid dysplastic syndrome (a type of haematopoietic stem cell deficiency disorder) (Rollison *et al*.,2011) , combined pulmonary fibrosis and emphysema (George *et al*.,2015) , and cancers of hematopoietic or epithelial origins (Kachuri *et al*.,2016 ; Zhang *et al*.,2014 ; Donaires *et al*.,2017) . Recently, two *TERT* gene SNPs, namely *rs2736100* and *rs2853669*, previously associated with myeloproliferative neoplasms (Trifa *et al*.,2018 ; Trifa *et al*.,2016) . However, there are rare data on the relationship between type2 diabetes mellitus and *TERT* gene polymorphism .

The present study was designed to analysis the polymorphism of *telomerase reverse transcriptase(TERT) rs2853669* and to determine whether there is an association between this polymorphism and type2 diabetes mellitus phenotype in an Iraqi population from Wasit province.

Materials and Methods

This is a case –control study, The participants included 45 of type2 diabetes mellitus patient (20 males and 25 females) their age 40 to 70 years (mean ± standard deviation: 57.95 ± 52.04 years, median= 53 years). The control group comprised of 35 healthy individuals (17 males and 18 females), their age 40-70 years (mean ± standard deviation: 51.88 ± 46.38 years, median=45 years). Data collection encompassed a range of factors, including demographic details, medical history, and sample collection date, gathered from participants who met global diagnostic criteria. samples were collected from Al-Azizia General Hospital and blood bank in Al-Azizia , Wasit province , Iraq . For blood sample collection, 3 ml of blood was obtained via vein puncture from each participant, with the collected blood then transferred to sterile ethylenediaminetetraacetic acid –k3 (EDTA) tubes, labelled, and stored at -20°C for subsequent DNA extraction and genotyping.

Genomic DNA Extraction:

Genomic DNA was extracted from whole blood utilizing the Quick-DNA™ Blood MiniPrep kit (Zymo, USA) Catalogue Nos. D3024 & D3025. The quality of the extracted genomic DNA was assessed via Nanodrop, measuring the A260/A280 absorbance ratio within the range of 1.8 to 2.0, indicative of high quality.

SNPs Genotyping:

The TaqMan custom SNP genotyping assay from Thermo Fisher Scientific was utilized for genotyping the SNP *rs2853669* in the *telomerase reverse transcriptase(TERT) gene*. Real-time PCR was employed for the allele-specific discriminating approach. The reference and alternative alleles for *rs2853669* were referred to from NCBI.

Statistical Analysis:

The data analysis was carried out using SPSS 21.0 software. The significance level (P-value) was categorized as follows: Sig. denoting Significant ($P<0.05$), and NS representing non-Significant. Analysis of variance (ANOVA) was employed to assess group differences.

Results

In both patients and control groups, the distribution frequencies of genotypes and alleles of *telomerase reverse transcriptase (TERT) rs2853669* A/G was not consistent with Hardy-Weinberg equilibrium (Chi sq. 4.512< 3.84) ($P<0.05$) that the genetic and genotype frequency is not constant from generation to generation . The allele and genotype frequencies of *rs2853669* A/G gene polymorphisms were used to estimate the odds ratio (OR), confidence intervals (95% CIs) and p-value . The results showed that the frequency of the heterozygous AG genotype was the main genotype in patients compared to the control group with a percentage of 91.12% in patients, while its percentage in control was 74.28% . The AA genotype displayed nonsignificant difference when comparing patients with control type2 patients (8.88 %) compared to control (22.85 %). While, the GG genotype was less frequent with a percentage of (0.00%) in patients which means that this genotype did not appear in the sample under study and (2.87%) in control . The A and G allele frequencies in SNP *rs2853669* A/G were not significantly different between the two groups ($P>0.05$). The G allele was the major one in studied groups with a percent of 60% and 54.44% in control and patients groups respectively, whereas the A allele was the minor

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one with a percent of 40% and 45.56 % in these groups respectively .

Table (1) Distribution of genotypes and allele frequencies of *telomerase reverse transcriptase (TERT) rs2853669* in Type 2 diabetes mellitus patients and controls

Groups	Genotype (%)			Allele frequency (%)	
	AA	AG	GG	A	G
Control	8(22.85)	26(74.28)	1(2.87)	42(60%)	28(40%)
Patients	4(8.88)	41(91.12)	0.00	49(54.44%)	41(45.56%)
Chi square	4.512				
P-value	0.104				
Significance	Ns.				

Ns: non-significant P>0.05

Susceptibility analysis of *telomerase reverse transcriptase (TERT) rs2853669* polymorphism with type 2 diabetes mellitus

Table (2) shows the association of each genotype of *telomerase reverse transcriptase (TERT) rs2853669* with susceptibility to type 2 diabetes mellitus. This further analysis showed that the individuals carrying the homozygous AG genotype were more likely to have increased risk of type 2 diabetes mellitus significantly with OR= 3.5481 (CI95% 0.9902 to 12.7128) ,p=0.0518 . The genotypes GG and AA decreases the association with type 2 diabetes mellitus with OR= 0.2527 (CI95% 0.0100 to 6.3964), and p= 0.4041 and OR=0.3293 (CI95% 0.0902 to 1.2020) and p= 0.0927 for each genotype respectively.

Table (2) Odds ratio of *telomerase reverse transcriptase (TERT) rs2853669* in patients with Type 2 diabetes mellitus and controls

Genotypes	Control	Patients	OR	OR95%CI	P-value	Significance
AA	8(22.85)	4(8.88)	0.3293	0.0902 to 1.2020	0.0927	Ns.
AG	26(74.28)	41(91.12)	3.5481	0.9902 to 12.7128	0.0518	Sig. *
GG	1(2.87)	0.00	0.2527	0.0100 to 6.3964	0.4041	Ns.
Allelic A vs G	42A (%60) 28G (%40)	(%54.44) 49 (%45.56)41	1.2551	0.6664 to 2.3638	0.4818	Ns.

***P< 0.05 ,Ns: non-significant P>0.05**

The odds ratio among females of *telomerase reverse transcriptase (TERT) rs2853669* in studied groups

Association analysis showed that the type 2 diabetes mellitus risk of females with *telomerase reverse transcriptase (TERT) gene* AG genotype was 2.6250 times higher than that in controls OR= 2.6250 (CI 95% 0.6155 to 11.1954), p= 0.1922 . Table (3) . GG genotype decreases the probability of contracting the disease significantly with OR = 0.2288 (CI 95% 0.0088 to 5.9470), P = 0.3749. AA genotype decreases also the association with type 2 diabetes mellitus with OR= 0.4952 (CI95% 0.1121 to 2.1879), P = 0.3539.

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JCHR (2023) 13(5), 83-90 | ISSN:2251-6727

Table (3) Odds ratio of *telomerase reverse transcriptase (TERT) rs2853669* in females with type 2 diabetes mellitus patients and control

Genotypes	Control	Patients	OR	OR95%CI	P value	Significance
AA	5	4	0.4952	0.1121 to 2.1879	0.3539	Ns.
AG	12	21	2.6250	0.6155 to 11.1954	0.1922	Ns.
GG	1	0.00	0.2288	0.0088 to 5.9470	0.3749	Ns.

Ns: non-significant P>0.05

The odds ratio among males of *telomerase reverse transcriptase (TERT) rs2853669* in studied groups

Type 2 diabetes mellitus patients particularly with AG genotype increases the association about 9 times among males patients than that in controls OR= 9.8966 (CI95% 0.4741 to 206.6018), P = 0.1393 . Genotype AA reduce the likelihood of type 2 diabetes mellitus with OR= 0.1010 (CI95% 0.0048 to 2.1094),P= 0.1393 . There were no males with the homozygous genotype GG in patients and controls.

Table (4) Odds ratio of *telomerase reverse transcriptase (TERT) rs2853669* among males with Type 2 diabetes mellitus patients and controls

Genotypes	Control	Patients	OR	OR95%CI	P value	Significance
AA	3	0.00	0.1010	0.0048 to 2.1094	0.1393	Ns.
AG	14	20	9.8966	0.4741 to 206.6018	0.1393	Ns.
GG	0.00	0.00	no	no	no	

Ns: non-significant P>0.05

Discussion

The most important result of the present study is the association of type2 diabetes mellitus and *TERT gene* polymorphism. The TERT gene is located on chromosome 5p15.33 (Cong and Wen and Bacchetti ,1999) and encodes the catalytic subunit of telomerase (Kilian et al.,1997) . In the present study, SNPs have investigated : *rs2853669* in the *TERT* promoter located within intron 2 of *TERT* in patients with type2 diabetes mellitus and healthy controls. Although a growing body of evidence supports an association between short telomeres and type 2 diabetes mellitus (T2DM), most studies have been cross-sectional by nature, trying to answer whether the metabolic disturbances of T2DM cause telomere attrition or if the shorter telomeres lead to higher risk of T2DM. Biological hypotheses address both scenarios. Short telomeres may lead to premature cell senescence, resulting in the reduced cell mass and subsequently impaired insulin secretion and glucose tolerance (Elks and Scott ,2014) . Conversely, elevated blood glucose concentrations increase oxidative stress and potentially interfere with telomerase function leading to shorter telomeres. On the other hand, high concentration of blood glucose alone, as shown in cultured human fibroblasts, did not cause telomere shortening. However, it was significantly accelerated in cell cultures containing the pro-inflammatory cytokine, interleukin 1 beta (Salpea et al.,2013) .

In previous studies that have investigated the association of polymorphisms of several factors and other biomarkers among patients with type2 DM from Wasit province, Balal and Ghali,2021 revealed that IL-10 is a major contributor to the onset of type 2 diabetes mellitus and there may be a correlation between low levels of interleukin-10 and type two diabetes. Al-Sarray and Ahmed ,2021 found that may be a correlation between high levels of TNF-α and type 2 diabetes mellitus. Shamkhi and Ahmed, 2021 displayed that levels of SIRT1 may be not associated with type2 diabetes mellitus. Furthermore, the cell free mitochondrial

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DNA increases significantly in patients with type2 diabetes mellitus (Hussein and Ghali,2022). COX-1 is a major contributor to the onset of type 2 diabetes and there may be an association between low levels of cyclooxygenase-1and type 2 diabetes (Jebil and Ghali2021). The association analysis of IL-17AG197A gene polymorphism with T2DM displayed that heterozygous AG genotype of IL-17AG197A showed a risk association among T2DM with OR=1.24 CI95% (0.31 - 5.01) p-value =1.00 and the G allele was associated with an increased risk ofT2DM (Khadhum and Ahmed ,2022). Mahmood and Ghali,2022 a revealed that there was an association between the polymorphism of Osteoprotegerin (OPG) polymorphism and susceptibility to type2 diabetes mellitus. Mahmood and Ghali,2022 b found also that there may be a correlation between high levels of OPG and T2DM.

There are no studies that investigated the association of *telomerase reverse transcriptase (TERT) rs2853669* variant A>G in type 2 diabetes mellitus patients, as most of the previous studies dealt with this gene in patients with different types of cancer or investigated the *TERT rs2853669* variant T > C, in type 2 diabetes mellitus patients . Therefore, this work is the first study that investigates the genetic polymorphism of *TERT* A <G *rs2853669* in patients with type 2 diabetes.

The polymorphism of *TERT rs2853669* in the current study showed significant deviation from the Hardy-Weinberg distribution, with higher proportion of heterozygotes in T2DM subjects. The association analysis of each genotype *TERT rs2853669* with susceptibility to Type 2 diabetes mellitus showed that the individuals carrying the homozygous AG genotype were more likely to have increased risk of type 2 diabetes mellitus .These results are in consistent with (Goswami et al.,2021) which their results suggested a possible role of telomere biology in T2DM. In a study with Polish T2DM cases and controls, the researchers did not find any association with the *rs2853669* TC genotype and T2DM (Gutmajster et al.,2018) Several cancers have been associated with *rs2853669* polymorphism, such as breast cancer and lung cancer (Savage et al.,2007 ; Zhong et al.,2013) , though relevant results remain inconclusive. Significant association has also been detected between *rs2853669* polymorphism and AML susceptibility in a Swedish population (Mosrati et al.,2015) , but no significant association was detected in Chinese Han population. Vinothkumar etal., 2020 (Vinothkumar et al.,2020) displayed that significantly high frequency (41%) of homozygous variant allele *rs2853669* (GG) in patients with cervical cancer compared with control samples [Recessive allele model odds ratio (OR)=1.71; 95% CI=1.20-2.43; P=0.003]and the variant allele 'G' showed a significantly increased association with cancer risk in the dominant model. These results were consistent with previous studies reported from other human cancer types (Spiegl-Kreinecker et al.,2015 ; Yoo et al.,2015 ; Shadrina et al.,2015 ; Helbig et al.,2017) . The difference might be caused by different genetic backgrounds. rs2853669 is located upstream of the *hTERT* transcription starting site and within a specificbinding site for Ets2 (Cawthon ,2002) . This variant has been suggested to be a positive regulator of the *hTERT gene* that is required for telomerase activation. A transition mutation from ancestral T to mutant C of *rs2853669* results in the damage of Ets2 core binding sequence 'GGAA/T', which may affect the binding ability of Ets2 and therefore, the transcriptional efficiency and expression level of the *hTERT gene*. It is interesting to note that whether wild type T allele or mutant C allele containing *hTERT* promoter sequence will be considered as a better choice because Ets2 interacts with c-Myc and together they binds to *hTERT* promoter that ultimately leads to proliferation of breast cancer (Cheng et al.,2020) . The subgroup analysis by gender showed that the risk genotype AG of *TERT rs2853669* was not found to be correlated with the gender in patients with T2DM that needs further exploration in larger sample size for conclusions. The subgroup analysis by gender showed interesting results.

Conclusion

The polymorphism of *telomerase reverse transcriptase rs2853669* variant A>G are associated with the susceptibility of type2 diabetes mellitus. This effect is comparable in males and females. The polymorphism of *(TERT) rs2853669* variant A>G suggest the importance of telomere maintenance pathway in T2DM susceptibility. The genotype AG of *(TERT) rs2853669* variant A>G increases the risk of T2DM and the A allele is a risk factor in T2DM.

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