RESEARCH ARTICLE

X-ray Repair Cross-complementing Group 1 (Arg399Gln) Genotyping in Diabetes Mellitus Type 2 with Hypertension

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ABSTRACT

DNA repair genes are one of the important molecules for genome maintains that may be effective by different factors and disease. X-ray Repair Cross-complementing Group 1(XRCC1)gene polymorphism relation with type 2-diabetes mellitus (T2D) with and without hypertension was suggested in present work, polymerase-chain-reaction with the confronting-two-pair primer (PCR-CTPP) was used to detection genotyping of XRCC1, the output shows that (67.5%) was T2D and (32.5%) was T2D with hypertension, genotyping shows two alleles (A, G) and tow genotyping (AG, GG). While AA did not observe in the present study, a non-significant association between T2D and T2D with hypertension (OR0.7813 (0.2024-3.0161), slightly differences appeared in AG and GG between groups. The impact of genotyping in glycemic parameters is non-significant between genotyping within the group except in HbA1C (P 0.007) in the T2D group. The present study concluded that the XRCC1 (Arg399Gln) did not associate with hypertension in T2D patients. Also this polymorphism had no association with the studied biomarkers (diabetic-related parameters, blood pressure parameters, age, and BMI).

Keywords: Diabetes Mellitus Type 2, Genotyping, Hypertension, X-Ray Repair Cross-Complementing Group 1.

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INTRODUCTION

Diabetes Mellitus (DM) is a chronic endocrinal disease that impacts most of the world population and becomes epidemic in some countries.[1] It is characterized by impaired metabolism of sugar and lipids as a results of defects in the secretion or function of insulin.[2]

Type 2 diabetes mellitus (T2D) accounts for over 90% of DM cases developing due to an interaction among genetic, epigenetic, behavioral, and environmental factors.[3] It is linked with an increased risk of early mortality and morbidity due to cardiovascular diseases as hypertension, stroke, and end-stage renal disease.[4] Diabetics often develop hypertension. The community-based surveys found that only half of patients with T2D had normal systolic and diastolic blood pressures, reflecting a great overlap in the mechanisms and causes of these diseases.[5,6]

In general, the hypertension definition in T2D is similar to the general population, and the threshold for therapy is persistent blood pressure values ≥l40/90 mmHg.[7] The coexistence of hypertension and diabetes in many patients is not coincidental; it involves intense interactions among genetic predisposition, environmental and biological factors.[8,9] Hyperglycemia, smoking, and alcohol consumption may boost the production of reactive oxygen species (ROS) that engaged in T2D and its complications.[10,11] It has been reported that ROS associated with metabolic disorders related to diabetes like hypertension.[12] The overproduction of ROS leads to damage to the biological macromolecules, like DNA, the damaged DNA may occur due to several endogenous and exogenous factors involving telomere erosion, genotoxic stress, and metabolic stress.[13]

The DNA repair genes have a significant role in sustaining the genomic integrity by repairing the damaged DNA through several mechanisms in close nucleotide excision repair, base excision repair (BER), and mismatch repair.[14] Genetic variations, including single nucleotide polymorphism in DNA repair genes could hamper the repair activity, affecting the susceptibility to T2D and its vascular complications.[15]

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X-ray repair cross-complementing group 1 (XRCC1) is a marked DNA repair gene in BER pathway.[16] It is positioned on the chromosome 19, at 19q13.2, and encodes XRCC1 protein, a pivotal enzyme that plays an important role in coordinating the series stages of the BER pathway due to its interactions with several proteins.[13,17] The XRCC1 gene has an important role either directly through repair the single-strand breaks or indirectly through the BER. Lack the activity of XRCC1 gene resulted in genetic instability, together with growing the frequency of involuntary and/or catalyzed chromosome deletions and translocations.[18,19]

About fifty polymorphic regions had been specified for XRCC1 gene, but the most frequent deliberated genetic variant is Arg399Gln, which involves transition of G/A, which leads to the substitution of Arg to Gln at codon 399.[20,21] It has been revealed that this genetic variant at XRCC1 gene was associated with reducing the XRCC1 enzyme activity.[22]

Several studies have dealt with XRCC1 Arg399Gln polymorphism as risk factor for numerous types of cancer in assorted populations.[19,23] However, the research concerning the relation of this polymorphism with the tendency to T2D and its complications was relatively scarce. So, the current study aimed to determine whether the XRCC1Arg399Gln polymorphic variant is related with susceptibility to hypertension in Iraqi patients with T2D.

METHODOLOGY

Sample Collection and Study Sitting

Forty T2D patients were enrolled in the present study, blood samples were collected from all patients with and without hypertension by written consents. Glycemic biomarkers included fasting blood glucose (FBG), glycated hemoglobin (HbA1C), insulin, insulin resistance (IR), and insulin sensitivity (IS) were detected in addition to age, body mass index (BMI), and blood pressure parameters (systolic and diastolic blood pressure).

Biomarkers Analysis

The Assessment of FBG

The enzymatic colorimetric method has been utilized to assess the levels of FBG (mg/dL) used the Linear kit, Spain.[24]

The Assessment of HbA1C

The levels of HbA1C was estimated by an automated Epithod®6161 Analyzer (DxGen /Korea) based on the principle of the boronate affinity.[25]

The Assessment of Insulin Level

Insulin hormone has been measured using (CALBIOTECK// USA) ELISA kit based on the standard sandwich enzyme-linked immune-sorbent technique.[26]

The Calculation of IR and IS

Values of IR has been calculated by the mean of determining the homeostasis model estimation of IR (HOMA-IR) and assessed using the equation ahead:[27]

$$IR = \frac{(\text{Fasting insulin level} \times \text{Fasting glucose level})}{405}$$

The quantitative insulin sensitivity check index (QUICKI) is derived employing inverse of the summation of logarithms of fasting serum insulin and fasting glucose level,[28] as mentioned in the equation below:

$$IS = \frac{1}{(\log \text{fasting insulin level } \mu IU/mL) + \log (\text{fasting glucose level mg/dL})}$$

The Calculation of BMI

The values BMI has been calculated using the BMI formula that mentioned below:[29]

$$BMI = \text{weight (kg)} / \text{height}^2 \text{ (m)}^2$$

The Measurement of Blood Pressure Parameters

Mercury sphygmomanometer has been employed to measure arterial blood pressure of participant patients for two additional times, then recorded the reading rates.

The PCR and Amplification Conditions

The XRCC1 (Arg399Gln) were detection belong to the method mention by (30) using the oligosF1, TCC, CTG,CGC, CGC, TGC, AGT, TTC, T; R1 TGG, CGT, GTG, AGG, CCT, TAC, CTC, C; F2 TCG, GCG, GCT, GCC, CTC CCA; and R2, AGC, CCT, CTG, TGA, CCT, CCC,AGG, C. via annealing Tm 59 ºC, the products consist 447bp G allele (399Arg), 222 of A allele (399 Gln) and 630 bp common band.

Electrophoresis was used to visualize genotyping (70 V, 1% agaros, 20 MA for 45 minutes) and SPSS version 23 to analyze data, at $p < 0.05$.

RESULTS

The present study deal with the genotyping of an important DNA repair genes (XRCC1) in hypertension patients with T2D. Results show a non-significant ($p > 0.05$) association between AG, GG with hypertension in T2D patients (OR 0.783, P 0.720), AA did not observe in booth groups Table 1.

The impact of XRCC1 genotyping (Figure 1) in the studied biomarkers (diabetic-related parameters, blood pressure parameters, age, and BMI) is illustrated in Table 2. The impact of genotyping in glycemic parameters showed non-significant ($P > 0.05$) differences between genotyping within the group except for HbA1C, which showed a significant ($p \leq 0.05$) increased in T2D group, who have AG genotype. While the genotyping was a non-significant ($p > 0.05$) effect on the remaining biomarkers.

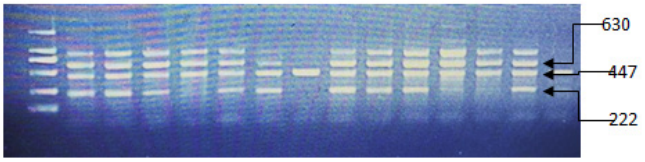

Figure 1: The genotyping of XRCC1 in study the genotyping of XRCC1 in study groups (70 V, 20mA, 1% agaros, 45 min with ethidum brommid staining)

Table 1: Genotyping distribution of XRCC1 gene in T2D andT2D with hypertension.

Genotyping	*T2D*	*T2D with hypertension*	*Odd ratio (CI%)*	*Sig*
AG	15	12	0.7813 (0.2024 - 3.0161	0.7202
GG	8	5		
AA	0	0		

Table 2: Effect of XRCC1 genotyping in study parameters

	T2D			*T2D with hypertension*		
Biomarker	*AG*	*GG*	*sig*	*AG*	*GG*	*sig*
FBG	257.53 ± 30.09	163.87 ± 30.08	0.059	209.83 ± 26.04	254.60 ± 73.40	0.475
HbA1C	9.64 ± 0.451	7.54 ± 0.46	0.007	8.37 ± 0.518	7.88 ± 0.79	0.614
Insulin	3.69 ± 0.68	5.83 ± 0.91	0.077	3.45 ± 0.900	3.31 ± 1.28	0.929
IR	2.19 ± 0.43	1.91 ± 0.26	0.651	1.64 ± 0.448	1.32 ± 0.40	0.677
IS	0.36 ± 0.017	0.35 ± 0.016	0.847	0.39 ± 0.022	0.39 ± 0.03	0.928
Systolic	12.26 ± 0.20	12.00 ± 0.00	0.361	14.66 ± 0.41	15.00 ± 0.54	0.657
Diastolic	7.93 ± 0.066	7.75 ± 0.163	0.232	9.12 ± 0.23	9.10 ± 0.24	0.950
Age	45.06 ± 3.161	48.62 ± 4.04	0.505	54.83 ± 2.51	47.40 ± 5.57	0.177
BMI	30.34 ± 1.46	29.57 ± 2.34	0.773	30.30 ± 1.32	28.32 ± 2.09	0.431

DISCUSSION

Given that an ineffective DNA repair system may be engaged in the development of diabetic complications, the current study goal was to investigate the distribution of polymorphism of genotypes and alleles frequencies of XRCC1 gene in diabetic patients without hypertension compared to hypertensive diabetic individuals. Based on this goal, this study determined the association between studied biomarkers (diabetic-related parameters, blood pressure parameters, age, and BMI) and altered activity of the XRCC1 gene due to Arg399Gln polymorphism. Comparison of allele frequency of XRCC1 gene between the two studied groups (T2D patients with/without hypertension) showed statistically non-significant differences. Except for HbA1C (P 0.007) in T2D group, the genotyping of XRCC1 gene had a non-significant association with all studied biomarkers in both group (T2D patients with/without hypertension). These returns suggest that there is no relation between state of hyperglycemic and XRCC1 Arg399Gln polymorphism.

The role of XRCC1 Arg399Gln polymorphism was recorded to be related to the increased risk of nephropathy and neuropathy in diabetic patients.[21,31] It is worth noting that none of the previous researches has assessed the impact of this polymorphism in growing the risk for T2D and hypertension with each other. The current study's findings agree with 32 who did not find any relationship between XRCC1 Arg399Gln polymorphism and T2D. The current study also agrees with the previous research results,[33] who reported a non-significant association between this polymorphism and the severity of diabetic nephropathy. On the other hand, the current study contrasts with a previous investigation on the effect of other DNA repair gene polymorphisms on diabetic patients with hypertension. It indicated that the polymorphisms in RAD18 and XPD genes increase the risk for T2D and hypertension.[34]

In conclusion, further research should be carried out with a larger size of samples to clarify the role of XRCC1 Arg399Gln gene polymorphism and the development of hypertension in patients with T2D in the Iraqi population. Furthermore, evaluating the expressions of important DNA repair genes and studying the impact of epigenetic changes may be valuable to illuminate the correlation between diabetes and hypertension and the mechanisms underlying boosted DNA damage and inactive repair in T2D.

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