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Short Communication

Molecular Study of *Bcl1* (+647 C/G) Glucocorticoid Receptor Polymorphism of Some Iraqi Patients with Type 2 Diabetes Mellitus

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ABSTRACT

BclI is one of the polymorphisms of glucocorticoid receptor (GR) gene that is associated with increased insulin resistance. The aim of this study was to evaluate the prevalence of *Bcl1* (+647 C/G) polymorphism of the glucocorticoid receptor gene in Iraqi patients with type 2 diabetes mellitus (T2DM) and correlate it with levels of some biochemical parameters. In the study, 70 blood samples were collected from type 2 diabetic patients, and 50 blood samples from healthy individual as a control group. Biochemical parameters such as insulin, glutathione, ceruloplasmin and lipid profiles were estimated using enzymatic assays, while functional Bcl1 (+647 C/G) GR polymorphism was genotyped for both groups using a Tetra-amplification refractory mutation system polymerase chain reaction (T-ARMS PCR). The results revealed a significant increase in the levels of insulin, cholesterol and TG in the control compared with treatment groups, while there was no significant variance in the amounts of CP and HDL-C in both groups. Also, the differences in the genotype and allele frequency of *Bcl1* GR polymorphism between both groups were not significant. This observation indicated a negative effect of the mutant G allele of *Bcl1* GR polymorphism on T2DM patients. The finding suggests that GG genotype and allele of *Bcl1* GR polymorphism may act as molecular markers of T2DM in Iraqi patients and have negative effects on insulin and other biochemical parameters.

***Keywords*:** *Bcl1* glucocorticoid receptor, Polymorphism, T-ARMS PCR, Type 2 Diabetes mellitus.

Introduction

The polygenic type 2 diabetes mellitus (T2DM) is a frequent endocrine and metabolic disorder that has long being found to induce cardiovascular disease, retinopathy, and complications of the kidneys and nerves.[1] Glucocorticoid (GC) hormones are released by hypothalamic-pituitary-adrenal (HPA) axis-controlled adrenal cortex, its play a vital role in controlling of the several basic metabolic processes.[2] By binding two receptors, the glucocorticoid receptor (GR) and mineral corticoid receptor, they exercise their function in different target tissues. The effects of glucocorticoids are well known to have a large variability between individuals due to the different sensitivity of the receptor that is mostly genetically determined.[3] GCs are known to be involved in the pathophysiology of diabetes among the different conditions and causes. They catalyze gluconeogenesis by inducing gluconeogenic gene expression in the liver by inhibition glucose absorption in the skeletal muscle and adipocytes which induce insulin resistance. Insulin resistance is therefore likely to be an essential factor leading to diabetes by excess GCs.[4]

In a large variety of biological processes in the body, glucocorticoids play a central role, influencing lipid and gluconeogenesis, anti-inflammatory responses, development and brain activity.[5] High serum levels of endogenous GC can induce insulin resistance, and in patients with diabetes, this may result in glycemic regulation being calculated.

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Cortisol changes blood sugar in peripheral tissues such as fat and skeletal muscle by manipulating glucose transporters. Therefore, glucocorticoid can contribute to increased levels of blood glucose due to insufficient glucose in the peripheral tissue.[6] Concerning homeostasis, immune function, cell growth and differentiation, GCs play an essential role in endocrine regulation. These are used in premature infants to treat hypotension, bronchopulmonary dysplasia (BPD) and sepsis.[7] GR exerts its effects primarily through an intracellular receptor molecule, being a member of the ligand-activated transcription factors superfamily of the nuclear hormone receptor. The GR is a hormone binding domain, a DNA binding domain, and an amino-terminal region of two spliceosomes, GRa and GRb. GRs control the transcriptional activity of up to 5–20 % of the human genome, positively and negatively.[8]

The NR3C1 gene produces four types of GR isoforms (GCRa, GCRb, GCRc and GCRd). GR comprises 777 amino acids and the N-terminal AF1 (activation domain 1) which establishes the relevant biological receptor function mechanisms in addition to interactions with transcription elements and coactivator proteins.[9] The presence of point mutations or particular polymorphisms in the GR gene can lead to an impairment of the GR complex or alter the process of transactivation or transrepression.[10] Cytoplasmic GR, a nuclear receptor family member that is transcribed from GR gene (NR3C1), mapped to 5q31 chromosome and consists of 9 exons and 8 introns.[11,12] The GR (NR3C1) gene contains some polymorphisms such as N363S (rs6195), ER22/23EK (known as rs6189 and rs6190), A3669G (rs6198) and *Bcl*I (rs41423247).[13] Numerous studies have revealed that these polymorphisms can be correlated with modified glucocorticoid GC sensitivity resulting in adverse or beneficial effects on metabolic profile.[14] The *BclI* polymorphism consisting of a replacement of C to G is 646 bp downstream from exon 2. It correlated with hyperglycaemia, improved insulin secretion, with sensitivity to

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steroids and abdominal obesity.[15,16] The presence of the minor (G) allele causes a rising sensitivity to GCs, hyperinsulinemia, increased body mass index and high distribution of abdominal fat.[17]
To our knowledge, there has been no any study until now on the relationship between the BclI polymorphism of GR (NR3C1) gene and Type 2 diabetes in Iraqi society. The study investigated the *BclI* polymorphism of GR (NR3C1) gene in Iraqi patients with T2DM, and correlating it with levels of some biochemical parameters.

Materials and Methods

Study group
A total of 70 T2DM patients were recruited for the study and referred to as the experimental group, while 50 apparently healthy (from all type of diseases) individuals, as control group. Both genders (36 males and 34 females) participated and the age range was between 45 to 75 years. The T2DM subjects included for this study were confirmed with Type 2 diabetes by the physicians in Tikrit Central Hospital or Ibn-Sina Center, Tikrit, Iraq.

Blood sample collection
Blood samples were collected with the consent of the participants. An aliquot of 5 ml blood sample was took from the vein and divided into two parts. The first part (2 ml) was collected into an EDTA tube and kept at -20 °C for molecular analysis, while serum that was used for biochemical tests was recovered from the second blood part (3 ml).

Biochemical analyses
Lipid profile (TC, HDL-C, TG) levels were measured by enzymatic method,[18a,b] while (LDL-C and VLDL-C were estimated by Friedewald formula.[19] The levels of GSH,[20] insulin,[21] and ceruloplasmin,[22] were assayed for both the experimental and control groups accordingly.

Molecular analysis
Genomic DNA was extracted from EDTA anticoagulated whole blood sample from both the experimental and the control groups according to the procedure described by (Ali *et al*. 2008).[23] Agarose gel electrophoresis at 1 % was used to evaluate the integrity of genomic DNA and the concentration was measured by Nanodrop (Thermo Scientific, Germany). To determining *Bcl1* (+647 C/G) genotype, T-ARMS PCR was used.[24] The sequences of the primers used included: a reverse primer specific for the wild-type allele (5'-CAA TTC CTC TCT TAA AGA GAT TG-3') and a forward primer specific for the mutant allele (5'-GAC AAG TTA TGT CTG CTG ATG-3'); and an outer forward primer (5'-AGA GCC CTA TTC TTC AAA CTG-3') and reverse primer (5'-GAG AAA TTC ACC CCT ACC AAC-3'). PCR amplification was achieved in a total reaction volume of 20 µL containing 10 µL of 2X GoTaq green master mix (Promega Company, USA), 4 µL of genomic DNA, 1 µL of each forward and reverse primer and 4 µL of DNase/RNase free water. The PCR program involved: initialization at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 sec, annealing at 51 °C for 30 sec, extension at 72 °C for 1.5 min and a final extension at 72 °C for 10 min and for 4 C for 2-5 min. At the end of the amplification, the PCR products were fractionated on 2 % agarose gel electrophoresis with the inclusion of 100 bp DNA ladder (Biolabs-England). Then, the gel was stained with Redsafe, destained and visualized on Gel Documentation unit (Cleaver scientific).

Statistical analysis
Statistical analysis of data obtained were carried out with SPSS (version 20 PC program). Pearson's chi-square test was used to determine frequency of alleles, Hardy–Weinberg equilibrium, genotypes, odds ratios (OR) and Confidence Intervals (CI). Student T-test and one-way ANOVA were used to compare the Mean ± Standard Deviation (SD) of biochemical parameters between the experimental and control groups as well as among the genotypes of Bcl1 (+647 C/G) polymorphism. $P \leq 0.05$ was considered significant and $p \leq 0.01$ was highly significant.

Results and Discussion

Comparison of lipid profiles between T2DM patients and healthy control group
Diabetes mellitus has the features of chronic disorder resulting from absolute insulin secretion or a deficiency of its function.[25] In the study, 120 Iraqi subjects were recruited; 70 patients with T2DM were regarded as the treatment group, while 50 healthy individuals as the control group. Table 1 presents the results obtained for biochemical analyses, which indicated that the levels of insulin, cholesterol GSH, TG, LDL-C and VLDL-C were significantly higher in the control group compared to the experimental group. Meanwhile, there was no significant difference in the levels of CP and HDL-C in both groups. Also, table 1 shown that there was a significant increase ($p \leq 0.01$) in insulin level in the T2DM patients (3.52 ± 19.79 µIU/ml) compared with the control (0.51 ± 10.48 µIU/ml). Oxidative stress results from an increase in the formation of reactive oxygen species (ROS), besides a decrease in the activity of antioxidant defense system such as antioxidant enzymes.[26] In T2DM, hyperglycemia and hyperinsulinemia might increase oxidative stress.[27] The results from Table 1 revealed a high significant reduction ($p \leq 0.05$) in GHS activity in T2DM group (4.27 ± 1.43 µmol/L) compared with the control (12.02 ± 4.38 µmol/L). In contrast, a non-significant increase was observed in CP activity in the T2DM patients (0.68 ± 0.03gm/L) compared with the control (0.64 ± 0.04 gm/L). This observation is in agreement with other studies which reported that CP mimics transferrin formation that inhibits the formation of ROS from lipid peroxidation.[28,29] More so, serum lipids (TC, TG, LDL-C, and VLDL-C) increased significantly ($p \leq 0.05$), except HDL-C which showed a non-significant reduction ($P \leq 0.05$) in T2DM patients compared with the healthy control. These observations indicated that in T2DM, deficiency in the amount of insulin affects lipid metabolism.[30]

Frequencies of genotypes and alleles
The variety in individual responds to both exogenous and endogenous glucocorticoids is regulated by GR gene polymorphisms.[31] Many of GR gene polymorphisms have been affect the receptor function and correlated with many diseases.[32] The results of the Tetra-ARMS-PCR analysis of Bcl1 (+647 C/G) polymorphism of the 70 T2DM patients and 50 control revealed three genotypes: homozygous wildtype (CC), heterozygous mutant (CG) and homozygous mutant (GG) as shown in Fig. 1. The genotype of *BclI* (+647 C/G) and the allelic frequencies were illustrated in Table 2. Genotype distribution was conducted using the Hardy-Weinberg equilibrium.

Table 1: Comparison between antioxidant and lipid profiles of T2DM patients and healthy control group

Parameter	Study population		p value
	Patient (N=70)	Control (N=30)	
Insulin (µIU/ml)	10.48 ± 0.51	19.79 ± 3.52	≤ 0.05*
GSH (µmol/L)	12.02 ± 4.38	4.27 ± 1.43	≤ 0.05*
CP (gm/L)	0.64 ± 0.04	0.68 ± 0.03	N.S
Cholesterol (mg/dl)	153.17 ± 3.03	206.02 ± 5.65	≤ 0.05*
TG (mg/dl)	151.69 ± 3.49	262.11 ± 14.30	≤ 0.05*
HDL-C (mg/dl)	56.15 ± 1.74	53.76 ± 1.37	NS
LDL-C (mg/dl)	61.26 ± 2.36	100.03 ± 6.21	≤ 0.05*
VLDL-C (mg/dl)	32.03 ±1.02	44.31 ± 2.10	≤ 0.05*

*: Significant at $p \leq 0.05$; NS: Non-significant.

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There was no significant difference (p = 0.845) between the frequencies of the genotypes and alleles of *BclI* (+647 C/G) polymorphism in the treatment compared with control groups. There was a high frequency of CC genotype (61.4%) compared with GG genotype (6.6%), while the CG genotype (33%) remained intermediate in the treatment group. Also, the results revealed that the frequency of C allele was 77.9 %, while that of the G allele was 22.1% in the T2DM patients.

There was no significant difference (p = 0.9406) in the allelic frequencies in both the treatment and control groups. T2DM patients had high CG genotype (0.88 OR and 95 % CI 0.35 – 2.16) but OR for GG allele (1.67) with (95 % CI 0.17 – 16.04). As a result of this observation, GG genotype may be considered as a risk factor, while CG genotype and G allele seemed to be a protective factor for T2DM in the Iraqi population examined. The results also indicated that the number of patients who had the polymorphism CC was 33 (61%) in comparison with that of healthy individuals which was 18 (60 %). Patients who have the polymorphism CG were 23 (33%) in comparison with the control group which was 11 (37%). Meanwhile, the mutant polymorphism GG appeared in 4 (6.6%) cases in T2DM patients, while it appeared in only 1 case (3.3%) in the control group.

The frequencies of *BclI* polymorphism demonstrated that CC and GG genotypes (61.4 and 6.6%, respectively) in the T2DM patients were non-significantly higher than the control groups (60 and 3.3%, respectively). Furthermore, the CG genotype was non-significantly lower (33%) in the T2DM patients compared with the healthy control (36.7%). This observation indicated that these genotypes have no association with the genetic predisposition of T2DM. The C allele frequency was non-significantly lower (77.9%) in the T2DM patients compared with the healthy control (78.3%), while the G allelic frequency showed a non-significant increase in the treatment group (22.1%) compared with the control group (21.7%). This outcome is in accordance with Geelen et al. who reported that homozygous carriers of the G allele had an effect on insulin resistance.[33]

Effect of BclI glucocorticoid receptor gene polymorphism on biochemical parameters

The outcomes of the effect of *BclI* GR gene polymorphism on biochemical parameters in the T2DM patients are presented in Table 3. There were significantly ($p \leq 0.01$) high amounts of insulin (27.525 ± 5.001 µIU/mL), cholesterol (226.5 ± 10.408 mg/dl) and LDL-C (108.41 ± 13.547 mg/dL) in the GG genotype of T2DM patients compared with other genotypes. Also, GSH level (10.623 ± 1.337 µmol/L) of patients with CC genotype was significantly ($p \leq 0.01$) higher than the other genotypes. There was no significant ($p \leq 0.01$) difference in the levels of CP and TG among all the three genotypes.

Statistical relationship between CC, CG and GG genotypes in relation to biochemical parameters in our study revealed a significant difference at $p \leq 0.01$. High homozygous G allele carriers had elevated and more insulin resistance (27.525 ± 5.001 µIU/ml) in relation to TC (226.5 ± 10.408 mg/dL). Although the elevation of HDL-C (56.35 ± 3.777 mg/dl), LDL-C (108.41 ± 13.547 mg/dl) and VLDL-C (45.865 ± 3.936 mg/dl) was comparable to other groups of CC and CG carriers. These observations are in agreement with other studies that G allele carriers had significantly higher blood glucose or insulin resistance.[33,34]

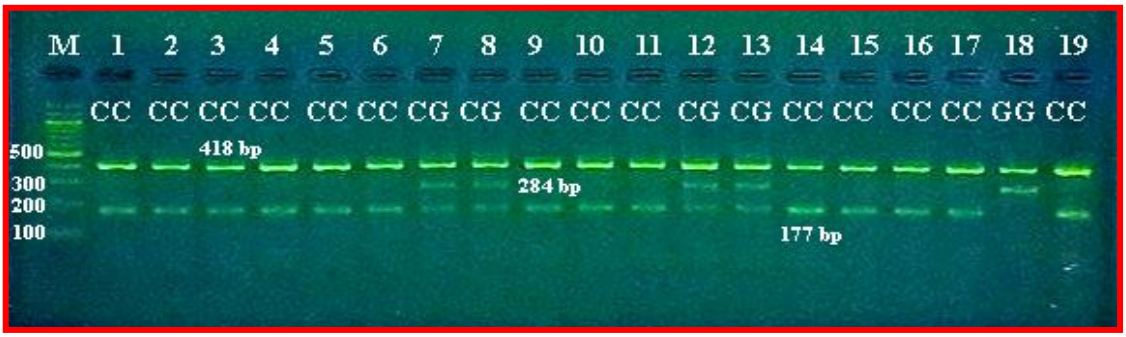

Figure 1: 2 % Agarose gel electrophoresis of Tetra-ARMS-PCR products of the BclI GR gene polymorphism.
Lane M: 100 bp DNA ladder; Lanes 1-6: CC wildtype homozygote (418 & 177 bp); Lanes 7 & 8: CG heterozygote (418, 284 & 177 bp); Lane 18: GG mutant homozygote (418 & 284 bp).

Table 2: Distribution of genotypic and allelic frequencies of *BclI* glucocorticoid receptor gene polymorphism of T2DM patients and healthy control group

	Patients (N = 70)		**Control (N = 30)**				
Genotype	**No.**	**Freq (%)**	**No.**	***Freq (%)**	***P* value**	**OR**	**95 % CI**
CC	43	61.4	18	60		1 Ref.	-
CG	23	33	11	36.7	0.845	0.88	0.35 – 2.16
GG	4	6.6	1	3.3		1.67	0.17 – 16.04
Alleles	No.	Freq (%)	No.	Freq (%)	*P* value	OR	95 % CI
C	109	77.9	47	78.3	0.940	1 Ref.	-
G	31	22.1	13	21.7		1.028	0.494 to 2.138

*: Frequency

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Table 3: Relationship between biochemical parameters and insulin levels in with genotypes of *BclI* glucocorticoid receptor gene polymorphism

Parameter	Genotype			*P* value
	CC (N = 43)	CG (N = 23)	GG (N = 4)	
Insulin (µIU/mL)	16.339 ± 5.609	11.787 ± 4.667	27.525 ± 5.001	0.0001**
GSH (µmol/L)	10.623 ± 1.337	8.769 ± 2.078	9.36 ± 0.804	0.0001**
CP (gm/L)	0.642 ± 0.187	0.753 ± 0.225	0.697 ± 0.180	0.107
Cholesterol (mg/dl)	198.997 ± 18.483	216.104 ± 13.132	226.5 ± 10.408	0.01*
TG (mg/dl)	260.258 ± 11.175	259.956 ± 12.496	244.575 ± 2.508	0.759
HDL-C (mg/dl)	52.934 ± 9.105	53.4 ± 9.611	56.35 ± 3.777	0.771
LDL-C (mg/dl)	97.754 ± 9.368	97.683 ± 9.495	108.41 ± 13.547	0.773
VLDL-C (mg/dl)	43.218 ± 4.616	45.638 ± 2.186	45.865 ± 3.936	0.770

$P \geq 0.05$: Non-significant; *: Significant at $p \leq 0.05$; **: Highly significant at $p \leq 0.01$

Conclusion

The results indicated that *BclI* (+647 C/G) polymorphism was in association with Iraqi patients with T2DM. The GG genotype and G allele could be considered as markers of genetic predisposition to lower levels of GSH and CP; and higher levels of insulin, TC, HDL-C and VLDL-C in Iraqi population, thereby leading to increased susceptibility to T2DM complication.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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