Determination the Relationship between (*KLF14* rs972283) Genotype and Type 2 Diabetes in a Sample of Iraqi Patients

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Abstract

This study was aimed to detect *KLF14* gene polymorphism in Iraqi type 2 diabetes mellitus patients (T2DM) to found the correlation between the SNP (rs972283) polymorphism in *KLF14* gene and lipid metabolism and impact on the incidence of type 2 diabetes mellitus (T2DM). The result show high Significant difference was observed in FBS level (P<0.001) in patient group (172.9 ± 79.73) and in control (81.16 ± 7.18),). Cholesterol, LDL-Cholesterol and VLDL-Cholesterol mean level value in diabetic patients was significantly higher than those of control group (p<0.0001), serum HDL-Cholesterol mean value was significantly difference (P<0.05) in serum mean level of HDL between T2DM patients and healthy controls. Real time PCR (HRM) RT-PCR were used to detect SNP (rs972283) in *KLR14* gene (G>A) by using specific primers, as a related with SNP (rs972283) G>A in *KLF14* gene, genotypes and alleles frequencies, odds ratios, 95% confidence intervals and *P* values for the *KLF14* gene In this study, statistical analyses of genotypic frequencies for the *KLF14* (rs972283) revealed significant difference between T2DM patients and controls in the examined population. GG genotype was significantly (P = 0.0006) more frequent in the patient group. The observed G/G, A/G, and A/A genotype frequencies were 50%, 42%, and 8%, respectively .The A (wild-type) and G (variant) allele frequencies were 29% and 71% , respectively in patient group while the observed G/G, A/G, and A/A genotype frequencies were 22%, 48%, and 30, respectively and the A (wild-type) , G (variant) allele frequencies were 54% and 46%, respectively in control group (P <0.0003). GG genotype was significantly (P = 0.0006) more frequent in the patient group, **Conclusion:** There was a relationship between polymorphism of *KLF14* gene SNP (rs972283) and the incidence of T2DM in sample of Iraqi patients.

Keywords: *KLF14 rs972283, Genotype, Type 2 Diabetes*

Introduction

Diabetes is a chronic disease that occurs when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces, Hyperglycemia, or increase in blood sugar, is a common effect of uncontrolled diabetes and over time it leads to serious damage to the body system, especially nerves and blood vessel [(1)].

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The global epidemic of diabetes is a major public health problem, and the number of cases has increased four times in the past 30 years [(2)]. Incredibly, 1 in 11 adults suffered diabetes globally. It is estimated that about 463 million adults were living with diabetes mellitus worldwide in 2019, and most of them had type 2 diabetes mellitus (T2DM). Moreover, this number is expected to increase to 642 million by 2040 [(3)]. Considering its high prevalence and rapid increasing speed, there are increasing numbers of investigations focusing on risk factors and susceptibilities for T2DM. However, the underlying etiology of T2DM remains unclear. Therefore, we conducted this meta-analysis to

further demonstrate whether genetic factors play a vital role in the pathogenesis of T2DM [4] or not.

Lines of evidence suggest that genetic polymorphism plays a pivotal role in the pathogenesis of a wide range of human disorders including cancers, diabetes, cardiovascular disorders, kidney diseases, and neurodegenerative diseases [5].

Several polymorphisms in candidate Kruppel-like factors that may influence susceptibility to T2DM. The transcription factor *KLF14,* is a master trans-regulator of multiple genes that are associated with metabolic phenotypes in adipose tissue, *KLF14*, is located on chromosome 7q32.3. Variations in these genes are associated with T2DM [4].

Materials and Methods

This study conducted during the period from 1 November 2020 until the end of March at University of Baghdad / Institute of Genetic Engineering and Biotechnology for post Graduate Studies. The study consisted of 50 patient with type 2 diabetic were selected from those attended Al Shahed mohammed Baqer alhakim Hospital. Their ages ranged between (25-65) years.

Fifty healthy controls with normal fasting blood glucose (80–110 mg/dl). And age range between (25-65) years. They were randomly selected from the people who attend the clinics for checkup also from relatives and colleagues. Questionnaire that includes information about age, sex, family history, BMI for all subjects had measured. Both groups were classified according to BMI, age, gender and family history**.**

Samples collection

Amount of five ml of venous blood was withdrawn from each subject under aseptic conditions. Two ml of blood was placed in EDTA tube (1.5 mg/ml) and kept at -20 Cº to be used in molecular study. The remaining 3 ml venous blood was placed into clot activator and gel serum separation tubes (5 ml) and left to stand at room temperature (18-22°C). Then, the serum separated by centrifugation at 3000 rpm for 15 minutes. Later, it was divided into three aliquots in microcentrifuge tubes for biochemical test.

Genomic DNA extraction:

Genomic DNA was automatedly extracted from the whole blood samples of all subjects by using Blood DNA Extraction Kit 200 (MagPurix/Taiwan).

The MagPurix technology is a state of the art platform that uses magnetic beads to extract nucleic acids from samples. The platform commits a truly walk-away automation in nucleic acid purification from samples to results. The purification process contains steps of lysis, binding, washing and elution. After genomic DNA was extracted, agarose gel electrophoresis was adopted to confirm the presence and integrity of the extracted DNA.

Fasting blood sugar FBS, Total cholesterol TC, high density lipids HDL, low density lipids LDL and Triglycride were measured using kits supplied by (Spainreact, Spain), while glycosylated hemoglobin HbA1C measured using kit supplied by (Nycocard ,Norway).

Genotyping:

Genotyping was carried out For SNP rs972283of *KLF14* gene polymorphism analysis, DNA was amplified using the forward primer 5'-GCTATTGAACCATCATTGT -3' and Reverse primer 5'-AGCAAATACAGTTTAGTAATATG -3'. The qRT-PCR-HRMwas performed in a 20 µl total volume, Primer forward 0.75 µl, Primer reverse 0.75 µl , DNA Template 3.5 µl, PCR Re Mix (Ready to use) EVA Green 10 µl and D.W. 5 µl. A total of 40 PCR cycles with denaturation at 95 ºC for 15 sec., annealing for 40 Sec at 60 ºC and extension at 72 ºC for 20 Sec.

Result and Discussion

All serum lipid and lipoproteins were significantly higher in diabetic patients compared to healthy control group. In Figure (1), T. Cholesterol mean level value in diabetic patients was significantly ($p<0.0001$) higher

than control group, this increase may be due to an increasing in the plasma concentration of VLDL and LDL, which may be due to the increase in hepatic production of VLDL or decrease in the removal of VLDL and LDL from the circulation. (Ganong, 2003).

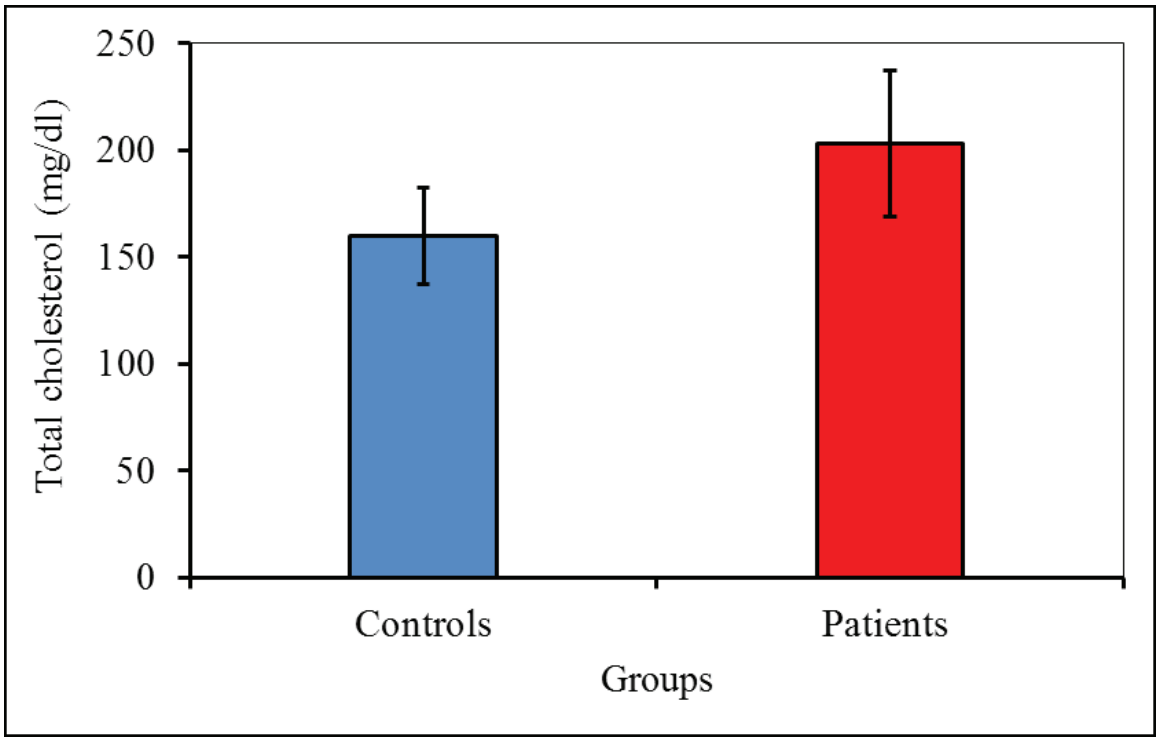

Figure (1): Comparison between control and patients In T. cholesterol

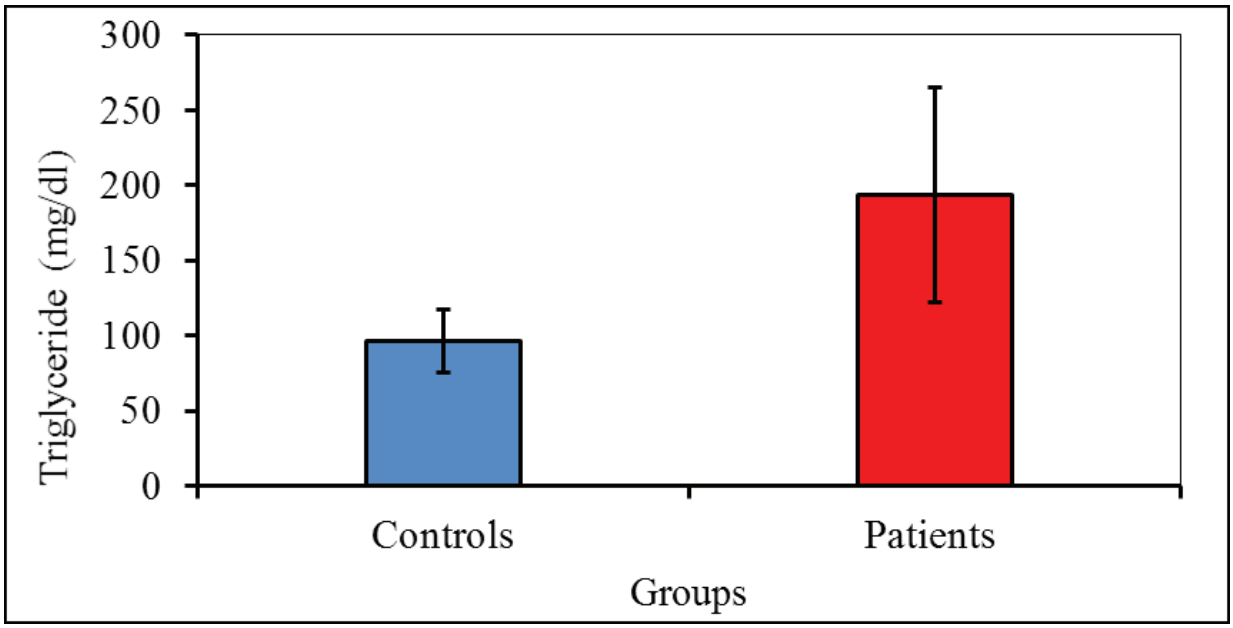

Figure (2): Comparison between control and patients in Triglyceride.

In Figure (2) mean value of triglycerides in diabetic patients was significantly (P>0.05) increased compared to mean of control group. The elevated triglyceride levels can arise from two abnormalities, The impaired lipolysis of triglycerides and over production of VLDL and patients with type 2 diabetes have an over production of triglyceride-rich VLDL level, which is a result of high free fatty acid levels, hyperglycemia, obesity, and

insulin resistance [7].

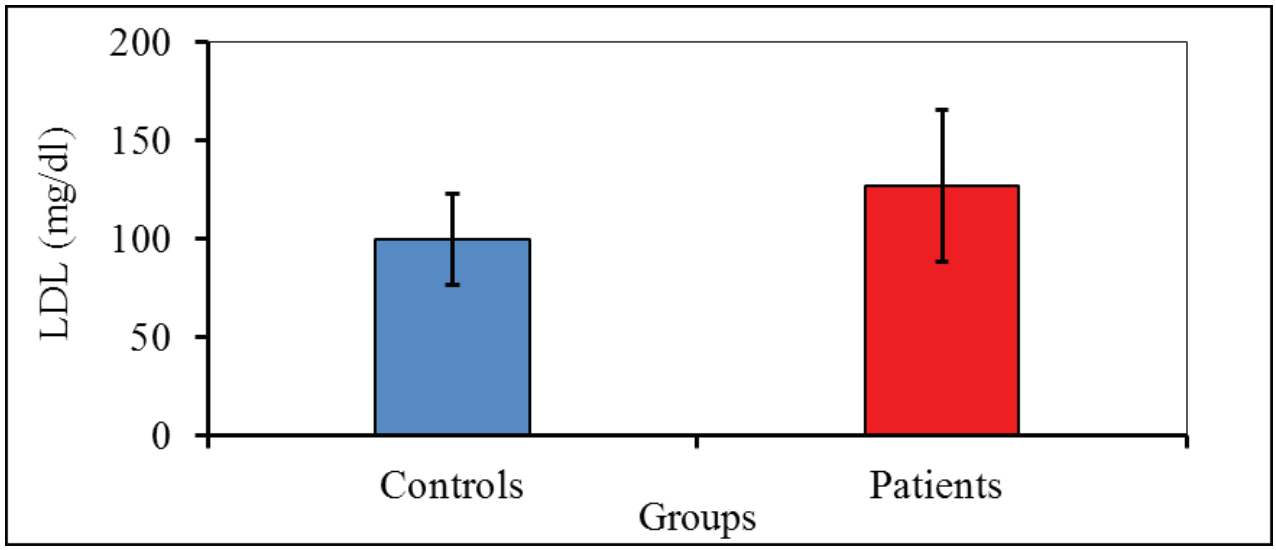

Figure (3): Comparison between control and patients in LDL

In Figure (3) LDL-Cholesterol mean value in diabetic patients was statistically significant ((p<0.0001) higher than the mean value of control group**.** The increased level of LDL in diabetic patients is due to insulin increases the number of LDL receptor, so chronic insulin deficiency might be associated with a diminished level of LDL receptor. This causes the increase in LDL particles and results in the increase in LDL-cholesterol value in diabetes mellitus [8].

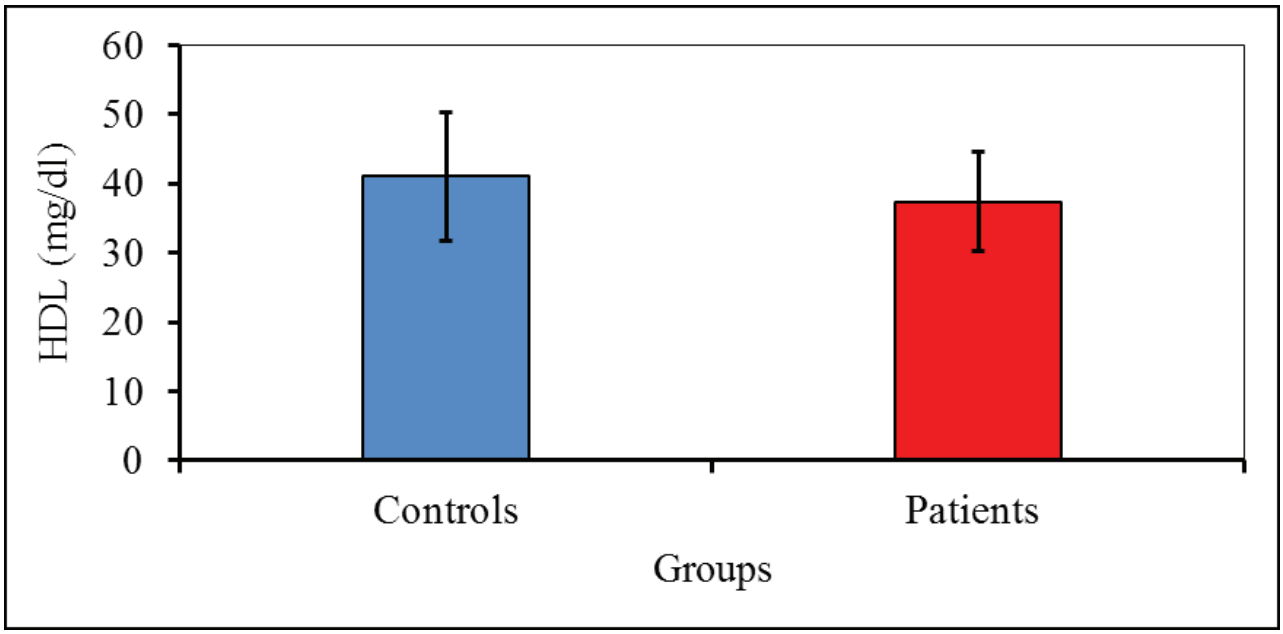

Figure (4): compare between control and patients In HDL.

In Figure (4) Serum HDL-Cholesterol mean value was significantly (p= 0.0036). Difference in serum mean level of HDL between T2DM patients and healthy controls**.**

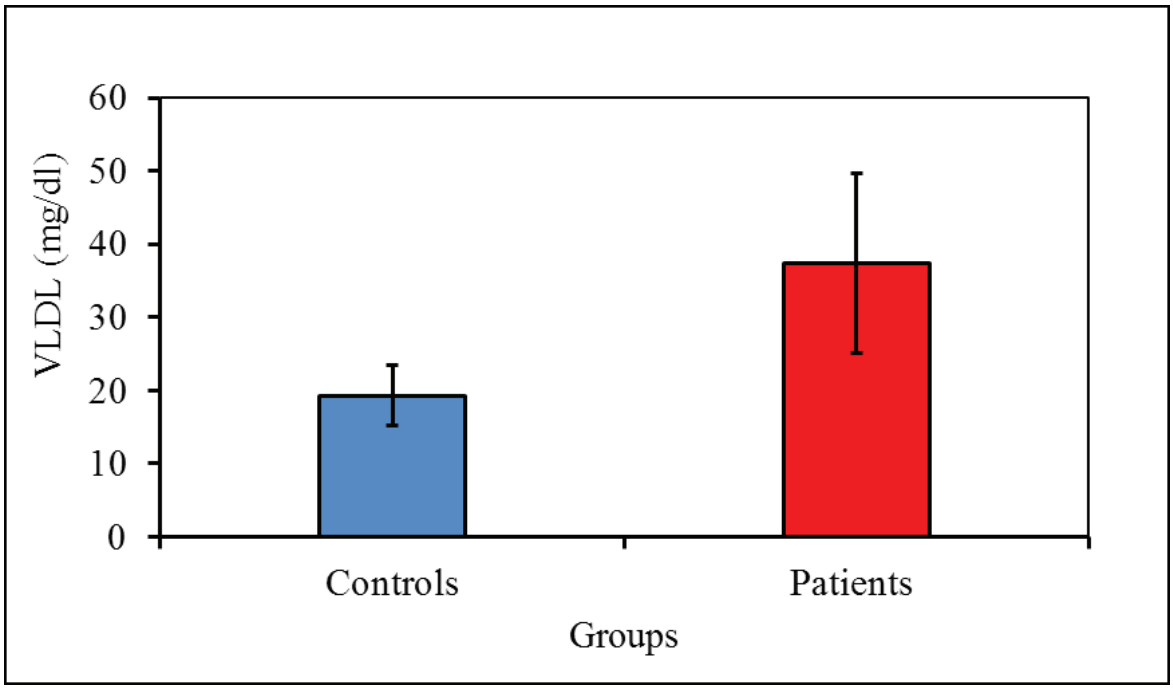

Figure (5): Comparison between control and patients in VLDL.

In Figure (5) VLDL-Cholesterol mean value in diabetic patients was significantly (P=0.00385) increased compared to mean of control group Higher level of VLDL was the consequence of insulin resistance in which the skeletal-muscle system stimulates the conversion of energy from consumed carbohydrate to raise liver triglyceride synthesis. As a result, it will produce atherogenic TGs-rich lipoprotein units, like VLDL [9].

Quantitative Real-Time Polymerase Chain Reaction - High Resolution Melting

Real-time PCR (RT-PCR) is also called quantitative PCR or qPCR. The key feature in RT-PCR is that amplification of DNA detected in real- time as PCR is in progress by the use of fluorescent reporter. The fluorescent reporter signal strength is directly proportional to the number of amplified DNA molecules [10].

The current study uses qPCR-HRM assay to determine the SNP rs972283 of the *KLF14* gene **(G>A)**, in T2DM in Iraqi patients, by using specific designed primer and positive, negative control which ensure a high degree of specificity.

Distribution genotype and allele frequency of *KLF14* (rs972283 G>A) polymorphism in patients and controls.

KLF14 is a master trans-regulator of multiple genes that are associated with metabolic phenotypes in adipose tissue. Table (1)illustrates genotypes and alleles frequencies, odds ratios, 95% confidence intervals and *P* values for the *KLF14* gene In this study, statistical analyses of genotypic frequencies for the *KLF14* (rs972283) revealed significant difference between T2DM patients and controls in the examined population. GG genotype was significantly (P = 0.0006) more frequent in the patient group.

The observed G/G, A/G, and A/A genotype frequencies were 50%, 42%, and 8%, respectively table (1). The A (wild-type) and G (variant) allele frequencies were 29% and 71% , respectively in patient group while the observed G/G, A/G, and A/A genotype frequencies were 22%, 48%, and 30, respectively and the A (wild-type) , G (variant) allele frequencies were 54% and 46%, respectively in control group (P <0.0003).

Table (1): Genotypes and alleles frequencies of *KLF14* (rs972283 G>A) genes polymorphism in diabetic and control subjects.

Genotype	Patients N=50		Control N=50		Odds ratio (95% CI of OR)	Chi-Square	P value
	No.	%	No.	%			
GG	25	50%	11	22%	8.52(2.30-31.63)	11.68	P = 0.0006
AG	21	42%	24	48%	3.28 (0.94-11.44)	3.68	P = 0.05
AA	4	8%	15	30%	1		
Allele Frequency							
G	71 (71%)		46 (46%)		0.35 (0.19-0.62)	12.87	P = 0.0003
A	29 (29%)		54 (54%)				

Conclusion

The present case-control study focused on the contribution of *KLF14* (rs972283 G>A) polymorphisms to the risk of having T2DM in Iraqi Population. The study also examined the relation between those polymorphisms and BMI and various biochemical parameters in the study sample. The results of the study can be summarized as *KLF14* (rs972283 G>A) polymorphism revealed a significant difference between T2DM patients and controls; GG genotype was significantly more frequent in the patient group. The highly levels of Total Cholesterol, triglycerides, LDL and VLDL may associated with T2DM in people with risk variants in *KLF14* gene.

Conflict of Interest: None

Funding: Self

Ethical Clearance: Not required

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