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Associations of *KCNQ1* (rs2237892) Polymorphisms with the Risk of Type 2 Diabetes Mellitus in a Sample of Iraqi Patients

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Abstract:

This study was aimed to detect *KCNQ1* gene polymorphism in Iraqi type 2 diabetes mellitus patients (T2DM) to found the correlation between the SNP (rs2237892) polymorphism in *KCNQ1* 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 (rs2237892) in *KCNQ1* gene (T>C) by using specific primers, as a related with SNP (rs2237892) T>C in *KCNQ1* gene, genotypes and alleles frequencies, odds ratios, 95% confidence intervals and P values for the *KCNQ1* gene In this study, statistical analyses of genotypic frequencies for the *KCNQ1* (rs2237892) revealed significant ($P<0.001$) difference between T2DM patients and controls in the examined population.

. The observed C/C, C/T, and T/T genotype frequencies were 96%, 4%, and 0%, respectively table (4-5). The C (wild-type) and T (variant) allele frequencies were 98% and 2% , respectively in patient group while the observed C/C, C/T, and T/T genotype frequencies were 72%, 28%, and 0, respectively and the C (wild-type) , T (variant) allele frequencies were 86% and 14%, respectively in control group ($P <0.01$). The *KCNQ1* CC genotype were significantly ($P <0.001$) more frequent in the patient group**. Conclusion:** There was a relationship between polymorphism of *KCNQ1* gene SNP (rs2237892) and the incidence of T2DM in sample of Iraqi patients.

Keywords: KCNQ1, rs2237892, Polymorphisms, Type 2 Diabetes Mellitus

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 (Witka *et al.,* 2019).

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 (Zheng and Hu 2018). 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 (Ogurtsova *et al*.2017). 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 (Yu, 2020) 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 (Malazy *et al.*, 2017).

Several polymorphisms in candidate as KQT-like subfamily Q member1 (KCNQ1) that may influence susceptibility to T2DM. KQT-like subfamily Q member1 (KCNQ1) is a family of voltage-gated potassium channels, and the *KCNQ1* gene located on 11p15.5 encoded the protein Variations in these genes are associated with T2DM (Yu, 2020)

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 shahid mohammed baqir 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 C° 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'- AGCTGTCACAGGACTTTG-3' and Reverse primer 5'-TAAGGCATCTGGTGGAGA-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 ^{o}C for 15 sec., annealing for 40 Sec at 60 ^{o}C and extension at 72 ^{o}C 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).

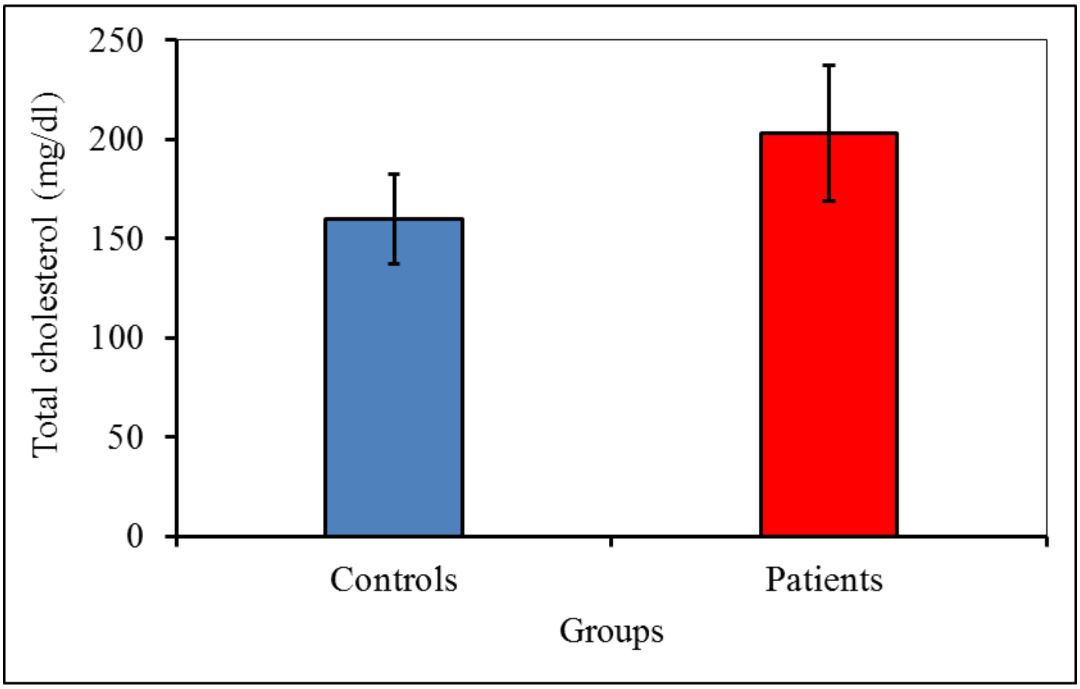

Figure (1): Comparison between control and patients In T. cholesterol

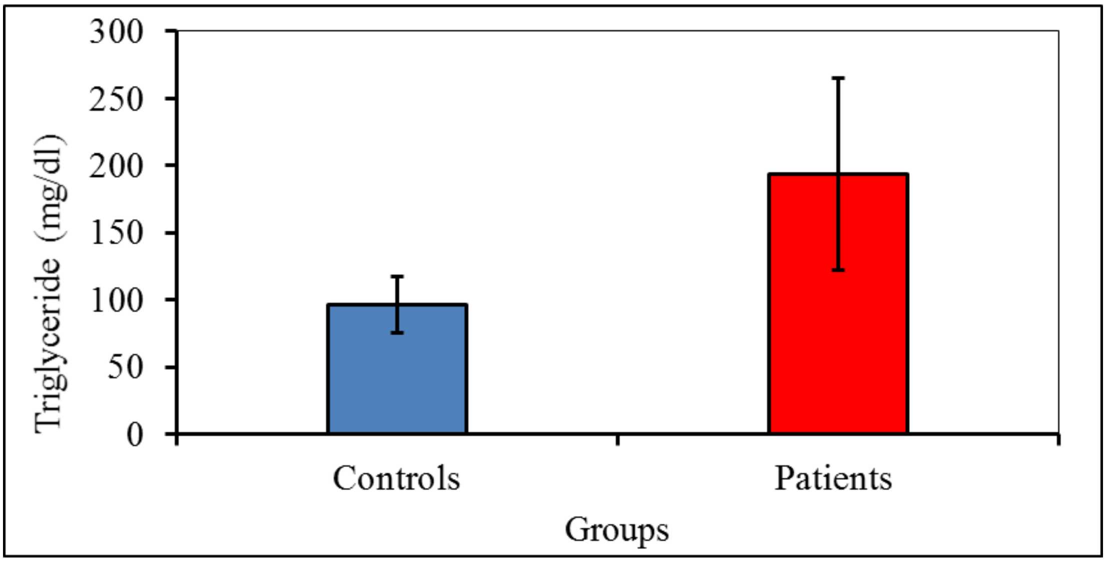

Figure (2): Comparison between control and patients in Triglyceride.

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 (Daniel, 2011).

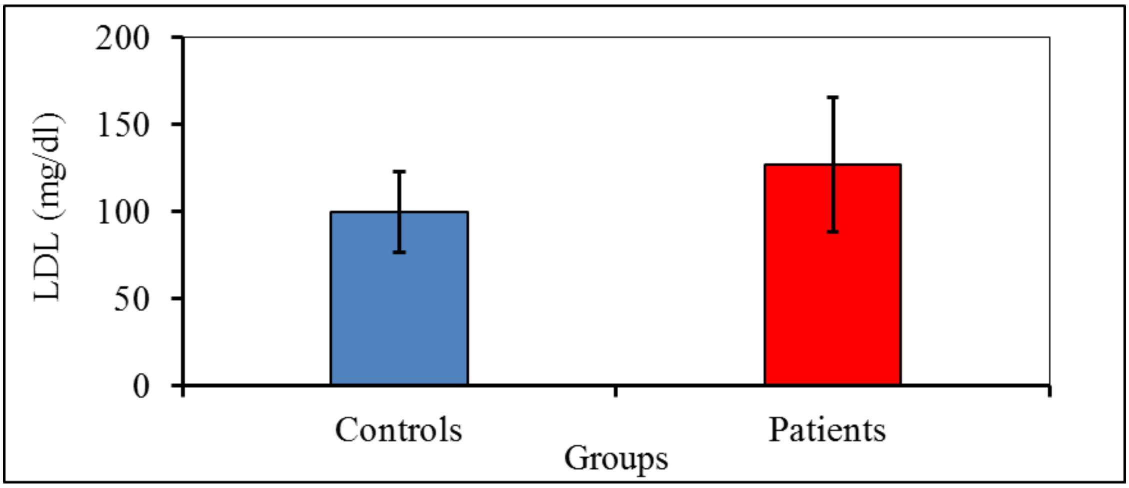

Figure (3): Comparison between control and patients in LDL

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 (Suryawanshi *et al*., 2006).

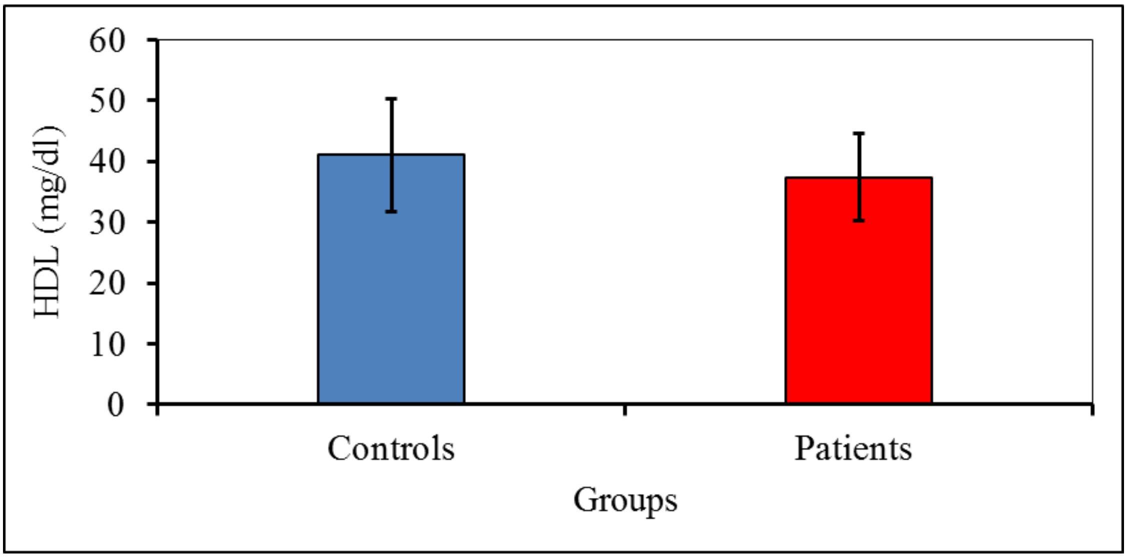

Figure (4): compare between control and patients In HDL**.**

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**.**

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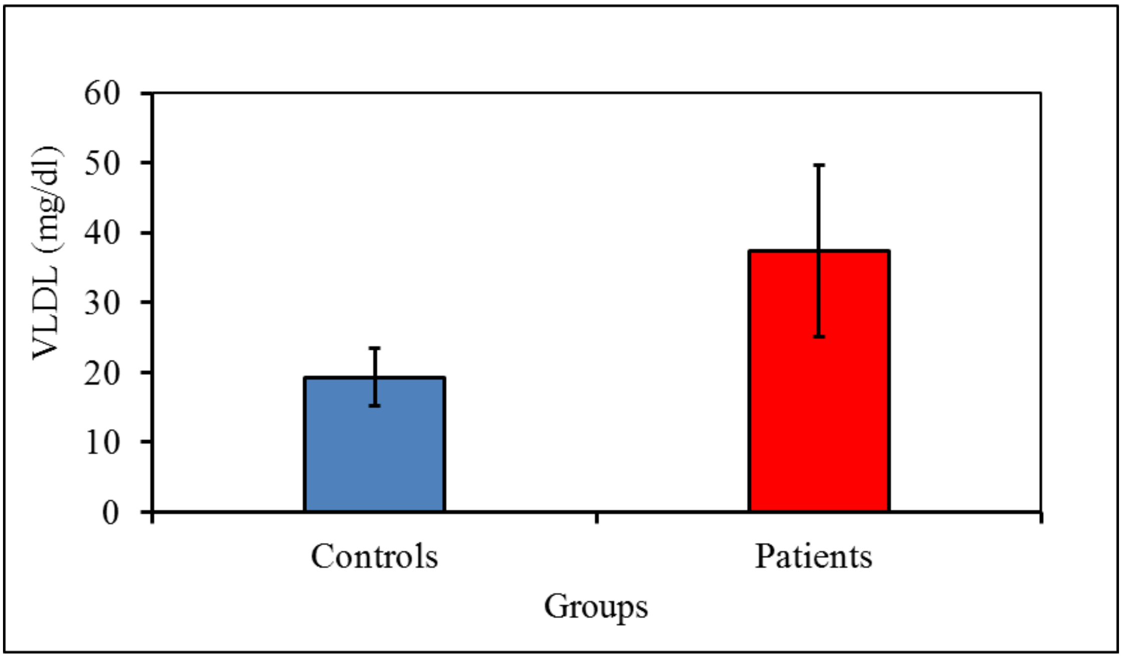

Figure (5): Comparison between control and patients in VLDL.

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 (Petersen *et al*., 2007).

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 (Maddocks and Jenkins, 2017).

The current study uses qPCR-HRM assay to determine the SNP rs2237892 of the *KCNQ1* (T>C), in T2DM in Iraqi patients, by using specific designed primer and positive, negative control which ensure a high degree of specificity.

Positive, negative and hetero controls are included in each qPCR reactions. HRM melting profile result of rs2237892 as shown in figures (6).

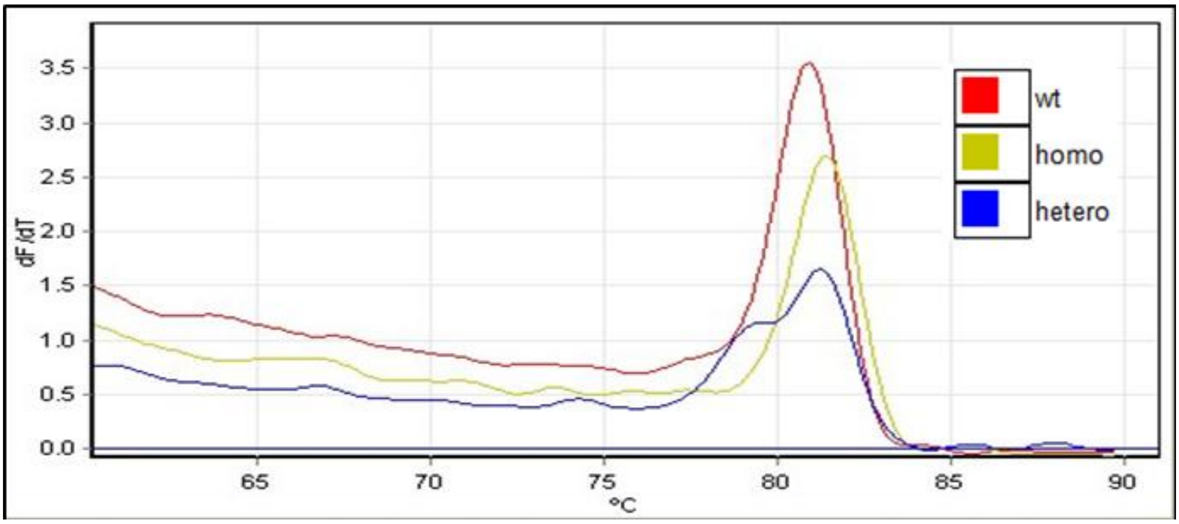

Figure (6): HRM melting profile result of the *KCNQ1* (rs2237892) gene, positive, negative and hetero controls were included in each qPCR reactions.

Distribution genotype and allele frequency of *KCNQ1* (rs2237892 T>C) polymorphism in patients and controls.

KCNQ1 gene is one of the members of voltage-gated potassium channel family. It is located at the 11*th* chromosome 11p l5.5, about 404 kb, and is composed of 17 exons. The exons are in the size range of 47–1122 bp

Table (4-5) illustrates genotypes and alleles frequencies, odds ratios, 95% confidence intervals and P values for the *KCNQ1* gene. In this study, statistical analyses of genotypic frequencies for the *KCNQ1* (rs2237892) revealed significant (P<0.001) difference between T2DM patients and controls in the examined population.

The observed C/C, C/T, and T/T genotype frequencies were 96%, 4%, and 0%, respectively table (4-5). The C (wild-type) and T (variant) allele frequencies were 98% and 2% , respectively in patient group while the observed C/C, C/T, and T/T genotype frequencies were 72%, 28%, and 0, respectively and the C (wild-type) , T (variant) allele frequencies were 86% and 14%, respectively in control group (P <0.01). The *KCNQ1* CC genotype were significantly (P <0.001) more frequent in the patient group**.**

Table (4-5): Genotypes and alleles frequencies of *KCNQ1* (rs2237892 T>C) genes polymorphism in diabetic and control subjects.

Genotype	Patients N=50		Control N=50		Odds ratio (95% CI of OR)	Chi-Sque	P value
	No.	%	No.	%			
CC	48	96%	36	72%	9.33 (1.99- 43.68)	10.71	P<0.001
CT	2	4%	14	28%	0.1071 (0.02-0.50)	10.71	P<0.001
TT	0	0%	0	0%			
Allele Frequency							
C	98 (98%)		86 (86%)		7.97 (1.76-36.09)	9.78	P<0.01
T	2 (2%)		14 (14%)				

Comparison of *KCNQ1* rs2237892 (CT) SNP genotypes and alleles frequencies between control and patients' groups is shown genotype CC is more frequent in patients group than in control group, whereas, genotypes CT were less frequent in patients group than in control group. Hence, genotype CT was protective factor against the disease with and odds ratio (OR) of 0.1071. Analysis of alleles has shown that allele C is a risk factor for the disease and allele T is a protective factor against the disease in a highly significant manner ($p < 0.001$). The risk in association with allele C was 7.97 in terms of OR indicating that individuals having allele C are more times liable to have the disease in comparison with individuals having allele T.

Moreover, the TT homozygote genotype was not encountered in any Investigated subjects. The explanation for the absence of this genotype is mainly due to the uncommon occurrence of the T-allele.

There is biological evidence supporting the hypothesis that *KCNQ1* might play a role in the susceptibility of T2DM. *KCNQ1*, encoding the alpha subunit of the IKsK$^+$ channel, is mainly expressed in the tissues or cells of the heart, as well as in pancreas islets, which plays an important role in the regulation of insulin secretion .The variants of *KCNQ1* are associated with impaired fasting glucose, beta-cell function, and impaired metabolic traits.(Qi *et al.*2009)

Conclusion

The present case-control study focused on the contribution of *KCNQ1* (rs2237892 T>C) 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 *KCNQ1* (rs2237892 T>C) polymorphism showed a significant difference between T2DM patients and controls. TT homozygote genotype was not encountered in any of investigated subjects.. The highly levels of Total Cholesterol, triglycerides, LDL and VLDL may associated with T2DM in people with risk variants in *KCNQ1*gene.

Reference

1. Abdelseed , N. A. H. . (2021). Glycemic Control and Knowledge among Children and Adolescents with Type 1 Diabetes Mellitus: A Cross-sectional Study . Journal of Scientific Research in Medical and Biological Sciences, 2(1), 1-9. https://doi.org/10.47631/jsrmbs.v2i1.149
2. Witka, B. Z., Oktaviani, D. J., Marcellino, M., Barliana, M. I., & Abdulah, R. (2019). Type 2 diabetes-associated genetic polymorphisms as potential disease predictors. *Diabetes, metabolic syndrome and obesity: targets and therapy*, *12*, 2689.
3. Zheng, Y., Ley, S. H., & Hu, F. B. (2018). Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nature Reviews Endocrinology*, *14*(2), 88.
4. Ogurtsova, K.; da Rocha Fernandes, J.; Huang, Y.; Linnenkamp, U.; Guariguata, L.; Cho, N. *et al.* (2017). IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. *Diabetes research and clinical practice*, 128: 40-50.

5. Yu, X.; Liao, M.; Zeng, Y.; Gao, X.; Liu, Y.; Sun, W., et al. (2020). Associations of KCNQ1 Polymorphisms with the Risk of Type 2 Diabetes Mellitus: An Updated Meta-Analysis with Trial Sequential Analysis. *Journal of diabetes research*, 2020

6. Malazy, T.; Khodaeian, M.; Bitarafan, F.; Larijani, B. and Amoli, M. (2017). Polymorphisms of Antioxidant Genes as a Target for Diabetes Management. *International Journal of Molecular and Cellular Medicine*, 6(3): 135-147.

7. Ganong, W. (2003). Review of Medical Physiology 2 1 .Endocrine Function of pancreas & Regulation of Carbohydrate Metabolism.324-33.

8. Daniel, M. (2011). Lipid Management in Patients with Type 2 Diabetes. American Drug and Hea. Benefits. 4(5): 312–322.

9. Petersen, K; Dufour, S.; Savage, D.; Bilz, S.; Solomon, G.; Yonemitsu, S.,et al.(2007). The role of skeletal muscle insulin resistance in the pathogenesis of the metabolic syndrome. Proceedings of the National Academy of Sciences, 104:12587– 12594 .

10. Maddocks, S. and Jenkins, R.(2017). Understanding PCR, Chapter 4 - Quantitative PCR: Things to Consider. Science Direct; 45-52.

11. Qi, Q., Li, H., Loos, R. J., Liu, C., Wu, Y., Hu, F. B., ... & Lin, X. (2009). Common variants in KCNQ1 are associated with type 2 diabetes and impaired fasting glucose in a Chinese Han population. Human molecular genetics, 18(18), 3508-3515.