ORIGINAL ARTICLE

FUNCTION ROLE OFIL-6-174 GENE POLYMORPHISMS IN ASSOCIATION WITH IL-6 LEVELS IN TYPE 2 DIABETES MELLITUS

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ABSTRACT

The aim: To distinguish the function role of IL-6 protein levels as well as IL-6 (-174) gene polymorphism in diabetes mellitus patients.

Materials and methods: in present case-control study160 (cases 86, control 74) volunteers were enrolled in this study 2.5 ml were added to EDTA tube for molecular investigation IL6 (-174), and other 2.5 ml use for measurement of fasted glucose by spectrophotometry and insulin levels as well as IL-6 level by ELISA.

Results: Regarding IL-6-174 (*rs*1800795), results revealed significant difference at genotype CC (p- value 0.05 with odds ratio 3.49) and allele frequency C (p-value 0.02 with odds 2.17), also at protein level the IL-6 showed significant difference between IL-6 levels and T2DM especial in insulin resistance (IR) group (p-value 0.03) compared with control one (Cont).

Conclusions: There is potent relation between IL-6 levels with T2DM and IR groups. There is potent relationship between CC variation of IL-6 (-174) and risk of each T2DM and IR condition.

KEY WORDS: type 2 diabetes mellitus, interlukine-6, IL-6 (-174), insulin resistance

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INTRODUCTION

Diabetes mellitus one of the most important diseases worldwide, characterized by increased blood sugar [1]. According to the International Diabetes Federation, in 2020 approximately 463 million patients were registered in world with diabetes mellitus [2]; in report of the WHO (2018) around 1.4 million,of Iraqis, have diabetes [3]. There are many factors that may be considered as risks for developing of T2DM including obesity, hypertension, lifestyle, age, nutrition regimen and physical activity, as well as genetic factor [4, 5]. Accumulated evidences reported on strong correlation between immune factors and hyperglycemia [6, 7]. Particularly, IL-6 at both protein and genetic levels showed significant relation with development of T2DM and special insulin resistance [8]. Elevation of IL-6 level involves in the pathogenicity of T2DM, effecting the insulin signaling pathway, leading to prevent the glucose uptake as well as insulin resistance [9]. On the other hand, IL-6-174 gene polymorphism (rs1800795) effects the rate of IL-6 gene expression and production of IL-6 [10, 11]. The mutant allele of IL-6-174 gene polymorphism is higher in patient with T2DM rather than in control [12].

THE AIM

To distinguish the function role of IL-6 protein levels as well as IL-6 (-174) gene polymorphism in diabetes mellitus patients.

MATERIALS AND METHODS

Present case-control study was conducted in 160 Iraqi participants (30 – 70 years): 86 T2DM patients diagnosed by consultant internists and 74 healthy volunteers in ALEmamain AL-Kadhemain Medical City at Baghdad, during the period December 2020 - March 2021. Patients were divided according to HOMO-IR into following groups: Insulin resistance (24 participants, 9 male and 15 female) and Insulin sensitive (62 participants, 22 male and 40 female). Five ml of venous blood were collected from each subjects, 2.5 ml were added to EDTA tube for measurement (SNP) for IL-6 (-174). Sera were isolated from the remaining 2.5 mL of the blood then stored until performing the biochemical and immunological assay (ELISA). Allele specific PCR was used to determine the gene polymorphism of IL-6 -174 using specific primers design online, as shown in table I.

Biochemical assay (Human, Germany) was used to measure FBS, while ELISA assays (Sunlong, Chinese) were used to measure levels of each of fasted insulin and IL-6 levels.

STATISTICAL ANALYSIS

This case control report was analyzed statistically with SPSS version 24.0 and Microsoft Excel 2010.Using (ANOVA) for Way Analysis of Variance to test the discrepancy between the groups tested. The Pearson test was used to determine the degree of association between the variables under consideration.

RESULTS

Level of Fasted blood sugar has shown the significant difference between each of IS (135.58 ± 49.82, P value ≤0.001) and IR (192.31 ± 64.31, P value ≤0.001) groups compared to cont. (90.40 ± 19.74) group. Also we detected significant difference between IS (P value ≤0.001) compared to IR group, as shown in table II. Data related to the level of insulin, showed non-significant and significant difference in IS (3.87 ± 2.67, P value 0.31) and IR (8.58 ± 2.38, P value ≤0.001) groups respectively compared to cont. (4.32 ± 2.64) group, while there was significant difference between IS (P value ≤0.001) compared IR groups, as shown in table III. Results related to the level of HOMO-IR, showed non-significant and significant difference in IS (1.13 ± 0.68, P value 0.12) and IR (3.90 ± 1.39, P value ≤0.001) groups respectively compared to cont. (0.92 ± 0.58) group, while there was a significant difference between IS (P value ≤0.001) compared to IR group, as shown in table IV.

Level of IL-6 showed non-significant and significant difference in IS (20.25 ± 0.87, P value 0.39) and IR (25.17 ± 1.87, P value 0.03) groups respectively compared to cont. (21.34 ± 0.83) group, with significant difference between IR (P value 0.01) and IS groups, as shown table V.

VARIATION OF IL-6 (RS 1800795)

Gel electrophoresis of ARMS-PCR production was done to determine the variations in the studied SNPs that had three genotypes in IL-6: heterozygous genotype (GC), homozygous genotype (CC) and Wild genotype (GG), shown in figure 1.

ASSOCIATION OF (RS1800795) WITH STUDY GROUP

Interleukin-6 SNP rs1800795 which had G>C Allele variations in control group made 33 (45%) Wild genotype (GG), 24 (32%) of GC and 17 (23%) of CC variation, with DM2 patient variation equaling 27 (31%) of GG, 41 (48%) of GC and 18 (21%) of CC. We used Logistic regression test for association of variation with incidence of DM2, which revealed insignificant association with each of variation (p value > 0.05). Furthermore (G and C) allelic distribution did not show significant differences (p value > 0.05) between control group and DM2 patients as shown in table VI.

ASSOCIATION OF (RS1800795) WITH CONTROL AND IR DM2

The studied of SNP rs1800795 of IL-6 had G>C Allele. The heterozygous genotype frequency (GC) was found in 10 (42%) DM2 patients and control group 24 (32%), whereas wild genotype (GG) was present in (21%) 5 patients with DM2 and 33 (45%) among healthy control, while homozygous genotype frequency (AA) was in 9 (38%) of DM2 patients and 17 (23%) of healthy control group. As shown by the outcomes, there was a positively significant difference (P value 0.05, odds 3.49) between DM2 patients for genotype (CC) compared to GG control group and allele frequencies showed statistically significant difference between DM2 patient and healthy control group (P value = 0.02, odds 2.17), as shown in Table VII.

Table I. The sequencing of primer

Primer	Sequence (5'-3')	Product size
Wild type Forward	CCCTAGTTGTGTCTTGGG	281bp
Mutant Forward	CCCTAGTTGTGTCTTGGC	
Common Reverse	GCACTTACTTGTGGAGAAGG	

Table II. the levels of FBS and insulin as well as HOMO-IR in studied groups

FBS		P value (sg≤0.05)	
Groups	M±SD	Sensitive	Resistance
Control	90.40 ± 19.74	0.00	0.00
Sensitive	135.58 ± 49.82		0.00
Resistance	192.31 ± 64.31		

Table III. the levels of insulin in studied groups

INSULIN		P value (sg≤0.05)	
Groups	M±SD	Sensitive	Resistance
Control	4.32 ± 2.64	0.31	0.00
Sensitive	3.87 ± 2.67		0.00
Resistance	8.58 ± 2.38		

Table IV. the levels of HOMO-IR in studied groups

HOMO IR		P value (sg≤0.05)	
Groups	M±SD	Sensitive	Resistance
Control	0.92 ± 0.58	0.12	0.00
Sensitive	1.13 ± 0.68		0.00
Resistance	3.90 ± 1.39		

Table V. value of IL-6 in studied groups

IL-6		P value (sg≤0.05)	
Groups	M±SD	Sensitive	Resistance
Control	21.34 ± 0.83	0.39	0.03
Sensitive	20.25 ± 0.87		0.01
Resistance	25.17 ± 1.87		

DISCUSSION

There are many clinical studies referring presence of strong correlation between development of T2DM with many proinflammatory factor such as (IL-6, IL-1β and TNF-α) [13-15]. IL-6 is major responsible for the insulin resistance by effecting the internal signal of cells to uptake the glucose [9]. The result of our study showed that levels of fasted blood sugar in IR patients is higher than level in IS patients with significant value and both of them are significantly different, compared to control group. Elevation of FBS may be resulted by increasing of IL-6

Table VI. Distribution of Genotypes and Alleles of IL-6 gene polymorphism (Rs1800795) in study groups

		Rs1800795 Genotype Frequency (%)			
IL-6 Variations	Control (74)	DM2 (86)	P value	Odds ratio	C.I (95%)
GG	33 (45%)	27 (31%)	0.38	1	
GC	24 (32%)	41 (48%)	0.41	1.35	0.662-2.769
CC	17 (23%)	18 (21%)	0.17	1.76	0.781-3.967
		Allele frequency (%)			
IL-6 alleles	Control (148)	DM2 (172)	P value	Odds ratio	C.I (95%)
G	90 (61%)	95 (55%)	0.31	1.26	0.81-1.97
C	58 (39%)	77 (45%)			

Table VII. Distribution of Genotypes and Alleles of IL-6 gene polymorphism (Rs1800795) between control and Insulin resistance

		Rs1800795 Genotype Frequency (%)			
IL-6 Variations	Control (74)	IR DM2 (24)	P value	Odds ratio	C.I (95%)
GG	33 (45%)	5 (21%)	0.12	1	
GC	24 (32%)	10 (42%)	0.09	2.75	0.832-9.088
CC	17 (23%)	9 (38%)	0.05	3.49	1.011-12.074
		Allele frequency (%)			
IL-6 alleles	Control (148)	IR DM2 (48)	P value	Odds ratio	C.I (95%)
G	90 (61%)	20 (42%)	0.02	2.17	1.12-4.21
C	58 (39%)	28 (58%)			

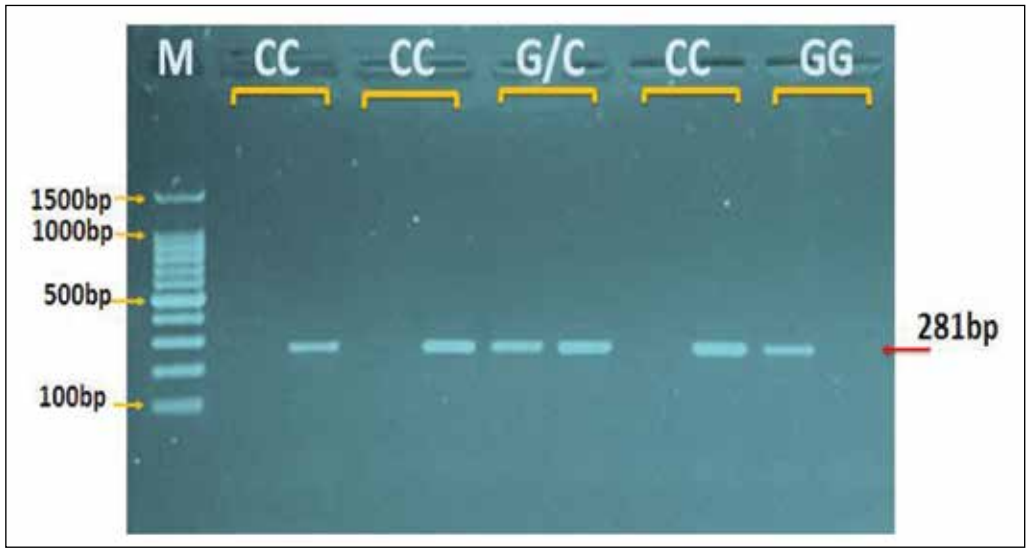

Fig. 1. Agarose gel electrophoresis image showed the ARMS-PCR product analysis of rs1800795 (C/G) gene polymorphism. Where M: marker (1500-100bp). The presence of A or G allele was observed at 281bp product size. The (GG) wild type homozygote was present in G allele only, the (CC) mutant type homozygote showed in C allele only, whereas the (G/C) heterozygote showed in both G and C allele.

level that we detected in our study. The result of our study agrees with another clinical study, conducted by Park et al; (2015) that showed significant increasing of FBS in IS and IR groups [16]. Fryk et al; (2021) also detected it in patients with insulin resistance compared with control group with normal glucose level [17]. The results of our studying of related insulin levels showed non-significant and significant differences in each of IS and IR patients respectively compared with control group. There was also a significant difference between IS patients compared to IR groups. Accumulated studies conducted by many research teams support the result of our study, regarding the level of insulin in each of studied group [18-20]. Present study revealed the elevation of HOMO-IR in IR group compared to IS group, as well as its increased level in IR compared to control group. This significant elevation in IR comes from increasing of glucose and insulin levels due to defect in insulin signaling pathway. This fact is proved by accumulated studies, supported by results of our study [21-23]. In present study the Level of IL-6 showed non-significant and significant difference in IS and IR groups respectively compared to control group, with parallel significant difference between IR and IS groups. This increasing of IL-6 level in association with increasing of FBS may refer to its effects on blood sugar metabolism leading to IR condition due to affected of internal signaling responsible for cell

glucose uptake. Furthermore, non-elevation of IL-6 levels in IS group in association with non-elevated of FBS may agree the results mentioned above. This result consent with the findings of Bashir et al; (2020) who stated a statistical difference between the levels of IL-6 in each of IS patients and IR patients [23].

Also, Ayelign et al; (2021) have reported the association between IL-6 level as pro-inflammatory cytokine and T2DM; they mentioned that chronic elevation of IL-6 at low-grade leads to rising of glucose levels, then to predisposition of T2DM [24]. Regarding genetic investigation of IL-6-174 (*rs*1800795) SNP reveled non-significant association of each variation with control group and T2DM. However, detected association between IR compared control at CC variation (mutant allele) means the patient-carrier of CC genotype has high risk for development of IR condition, compared to patient carrier GG genotype. Furthermore, the allelic C -carriers have risk higher by 2.04 times to development of IR condition than those carrying allelic G. These results agree with Todendi et al; (2015) study that showed non-associated IL-6 gene variation and level of IL-6 [8]. While Frota, et al; (2021) reported potent effect of polymorphism IL-6-174 on this gene expression [25]. IL-6 SNP (*rs1800795)* is associated with diseases such as T2DM and insulin resistance [26, 27]. Whereas other study conducted by Tabassum et al; (2012) showed not associated IL-6-174 SNP (*rs1800795),* obese and T2DM [28]. A study was conducted in London in 571 patients. 76 % of them were patient-carriers of CC genotype with metabolic syndrome, compared to patients who had GG genotype 56%. The C allele has association with metabolic syndrome compared to patient without this syndrome [29]. According to a study performed by Qi et al; (2006) IL-6-174 G/C polymorphism is not associated with the risk of T2DM development [30]. Other study by Dhamodharan et al; (2015) showed that IL-6-174 GC and CC variation conferred protection against of T2DM, and also reported the C allele of CC and GC variation has significant protection against T2DM [31].

CONCLUSIONS

There is potent relation between IL-6 levels with abnormality of blood sugar metabolism in T2DM, this effect was highly obvious with protein levels in IR groups of IL-6 (-174) variations. A potent relation between CC variation of IL-6 (-174) and risk of each of T2DM and IR condition in T2DM, is supported by the risk of C allele with incidence in both of T2DM and IR condition of T2DM.

REFERENCES

1. Nandan S., Rao M.M., Obulesu G. The study of diabetic complications in patients of newly diagnosed type 2 diabetes mellitus. IAIM. 2017; 4(1): 38-43.
2. Federation I.D. IDF DIABETES ATLAS 9th 2019. International Diabetes Federation, Brussels, Belgium. 2020, 65p.
3. Mohammed A. et al. Iraqi experts' consensus on the management of type 2 diabetes/prediabetic in adults. Clinical Medicine Insights: Endocrinology and Diabetes. 2020; 13: 1179551420942232.
4. Wu Y., Ding Y., Tanaka Y., Zhang W. Risk factors contributing to type 2 diabetes and recent advances in the treatment and prevent International journal of medical sciences. 2014; 11 (11): 1185.
5. American Diabetes Association. Lifestyle Management. Diabetes Care. 2018; 40(1): 33–43.
6. Nariman M. et al. Association of circulating CTRP9 with soluble adhesion molecules and inflammatory markers in patients with type 2 diabetes mellitus and coronary artery disease. PloS one, 2018; 13(1): e0192159.
7. Afiat B. et al. Type 2 diabetes and its impact on the immune system. Current diabetes reviews. 2020; 16(5): 442.
8. Pâmela T.F. et al. Association of IL-6 and CRP gene polymorphisms with obesity and metabolic disorders in children and adolescents. Anais da Academia Brasileira de Ciências. 2015; 87(2): 915-924.
9. Akbari M., Hassan-Zadeh V. IL-6 signalling pathways and the development of type 2 diabetes. Inflammopharmacology. 2018; 26(3): 685-698. doi: 10.1007/s10787-018-0458-0.
10. Daniel F. et al. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. The Journal of clinical investigation. 1998; 102(7): 1369-1376.
11. Olivieri F., Bonafè M., Giovagnetti S. et al. In vitro IL-6 production by EBV-immortalized B lymphocytes from young and elderly people genotyped for -174 C/G polymorphism in IL-6 gene: a model to study the genetic basis of inflamm-aging. Mech Ageing Dev. 2003;124(4):549-53. doi: 10.1016/s0047-6374(03)00035-6.
12. Haghnazari L., Sabzi R. Relationship between TP53 and interleukin-6 gene variants and the risk of types 1 and 2 diabetes mellitus development in the Kermanshah province. J Med Life. 2021; 14(1):37-44. doi: 10.25122/jml-2019-0150.
13. Chen L. et al. Mechanisms linking inflammation to insulin resistance. International journal of endocrinology. 2015,454p.
14. Asmat U., Abad K., Ismail K. Diabetes mellitus and oxidative stress-A concise review. Saudi pharmaceutical journal, 2016; 24(5): 547-553.
15. Akash M.S.H., Rehman K., Chen S. Role of inflammatory mechanisms in pathogenesis of type 2 diabetes mellitus. Journal of cellular biochemistry. 2013; 114(3) : 525-531.
16. Emanuel F. et al. Hyperinsulinemia and insulin resistance in the obese may develop as part of a homeostatic response to elevated free fatty acids: A mechanistic case-control and a population-based cohort study. EBio Medicine. 2021; 65: 103264.
17. Misaki Y., Miyauchi R., Mochizuki K. et al. Plasma interleukin-1β concentrations are closely associated with fasting blood glucose levels in healthy and preclinical middle-aged no overweight and overweight Japanese men. Metabolism. 2010; 59(10): 1465-1471.
18. González-Zavala M.A., Velasco-Morales A., Terrazas-Flores J.J. et al. Levels of insulin and HOMA-IR in adolescents in Saltillo, Coahuila, Mexico. Medicina Universitaria. 2015; 17(67): 80–87. doi:10.1016/j.rmu.2015.02.004.
19. de Fátima Haueisen Sander Diniz M., Beleigoli A.M.R., Schmidt M.I. et al. Homeostasis model assessment of insulin resistance (HOMA-IR) and metabolic syndrome at baseline of a multicentric Brazilian cohort: ELSA-Brasil study. Cad Saude Publica. 2020; 36(8):e00072120. doi: 10.1590/0102-311X00072120.
20. Nadeem A., Mumtaz S., Naveed A.K. et al. Association of IL-6 C-174G (rs 1800795) single nucleotide polymorphism with type 2 diabetes mellitus in Pakistani population. J Pak Med Assoc. 2017; 67(428): 433.

21. Fandong M., Qiangwei S., Dongmei Z. et al. Inhibition of Aurora-A improves insulin resistance by ameliorating islet inflammation and controlling interleukin-6 in a diabetic mouse model. Adipocyte. 2020; 9(1):609-619. doi:10.1080/21623945.2020.1829851.
22. Bashir H., Ahmad Bhat S., Majid S. et al. Role of inflammatory mediators (TNF-α, IL-6, CRP), biochemical and hematological parameters in type 2 diabetes mellitus patients of Kashmir, India. Med J Islam Repub Iran. 2020; 34(5). doi:10.34171/mjiri.34.5.
23. Fadaei R., Bagheri N., Heidarian E. et al. Serum levels of IL-32 in patients with type 2 diabetes mellitus and its relationship with TNF-α and IL-6. Cytokine. 2020; 125:154832. doi: 10.1016/j.cyto.2019.154832.
24. Böni-Schnetzler M., Häuselmann S.P., Dalmas E. et al. B-cell-specific deletion of the IL-1 receptor antagonist impairs β cell proliferation and insulin secretion. Cell reports, 2018; 22(7): 1774-1786.
25. Corpeleijn E. et al. Obesity-related polymorphisms and their associations with the ability to regulate fat oxidation in obese Europens: the NUGENOB study. Obesity (Silver Spring). 2010; 18(7): 1369-1377.
26. Mendoza-Carrera F., Ramírez-López G., Ayala-Martínez N.A. et al. Influence of CRP, IL6 and TNFA gene polymorphisms on circulating levels of C-reactive protein in Mexican adolescents. Arch Med Res. 2010; 41(6): 472-477.
27. Tabassum R., Mahendran Y., Dwivedi O.P. et al. Variants of IL6, LEPR, and PBEF1 Are Associated With Obesity in Indian Children. Diabetes. 2012; 61(3): 626-631.
28. Hamid Y.H., Rose C.S., Urhammer S.A. et al. Variations of the interleukin-6 promoter are associated with features of the metabolic syndrome in Caucasian Danes. Diabetologia. 2005; 48(2): 251-260.
29. Qi L., van Dam R.M., Meigs J.B. et al. Genetic variation in IL6 gene and type 2 diabetes: tagging-SNP haplotype analysis in large-scale case-control study and metanalysis. Hum Mol Genet. 2006; 15(11): 1914.
30. Dhamodharan U., Viswanathan V., Krishnamoorthy E. et al. Genetic association of IL-6, TNF-α and SDF-1 polymorphisms with serum cytokine levels in diabetic foot ulcer. Gene. 2015; 565(1): 62-67.
31. Nils W. et al. Is there a role for locally produced interleukin-1 in the deleterious effects of high glucose or the type 2 diabetes milieu to human pancreatic islets? Diabetes. 2005; 54(11): 3238-3244.

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