RESEARCH ARTICLE

Evaluation of serum IL 18 / IL 18 binding protein ratio and their relation with IL-18 gene polymorphisms in sample of Iraqi type 2 diabetes mellitus patients. A case control study

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Abstract

Objective: To evaluate the ratio of interleukin18 and interleukin18 binding protein in type 2 diabetes mellitus patients, and its relation with the presence of interleukin18-607C/A and interleukin18-137G/C gene polymorphisms and the type of complications.

Method: The case-control study was conducted at the endocrinology clinic of the Madinat Al-Imamin Al-Kazemin Teaching Hospital, Iraq, from September 2020 to July 2021, and comprised diagnosed patients of type 2 diabetes mellitus, and healthy controls matched for age and gender. Blood samples were obtained that were processed for serum separation. Serum interleukin18 and interleukin18 binding protein levels were assessed, and part of the sample was used for deoxyribonucleic acid extraction using tetra-primer amplification refractory mutation system polymerase chain reaction with four specific primers for interleukin18-607C/A and interleukin-18-137G/C gene polymorphisms in both the groups. Data was analysed using SPSS 20.

Results: Of the 168 subjects, 86(51.2%) were patients; 67(77.9%) females and 19(22.1%) males with mean age 52±8.97 years. There were 82(48.8%) controls; 56(68.3%) females and 26(31.7%) males with mean age 48±9.44 years ($p>0.05$). Higher serum level of interleukin18 and lower level of interleukin18 binding protein were seen in type 2 diabetes mellitus group compared to the controls ($p<0.001$). Interleukin18-137G/C and interleukin18-607C/A mutant alleles had odd ratios 3.52 (confidence interval: 1.91- 6.63) and 3.25 (confidence interval: 1.77-5.83), respectively, as risk factors for the occurrence of type 2 diabetes mellitus. There was an association of interleukin18-137G/C homozygous mutant genotype with the occurrence of retinopathy among type 2 diabetes mellitus patients ($p=0.046$), but risk allele C was not associated with retinopathy ($p>0.05$).

Conclusions: The presence of interleukin18-137G/C and interleukin18-607C/A gene polymorphism might be considered a risk factor for type 2 diabetes mellitus.

Key Words: Alleles, Interleukin, Diabetes, Genotype, Polymerase, DNA, Retinal, Mutation

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Introduction

Type 2 diabetes mellitus (T2DM) is a chronic multisystem illness defined by abnormalities of carbohydrate and lipid metabolism, leading to macro- and micro-vascular issues and microangiopathy[1]. These are related with low-grade chronic inflammation at the cellular level due to hyperglycaemia, which induces the formation of harmful free radicals and oxidative stress, which, in turn, increases the expression of pro-inflammatory cytokines[2]. One of these cytokine is interferon gamma inducer factor (IGIF), or interleukin18 (IL18)[3], IL18 is an immune cytokine belonging to IL1 super family group, which has an important role to regulate both innate and acquired immune responses. IL18 is expressed in many cell types, including macrophages, dendritic cells, Kupffer cells, keratinocytes, osteoblasts, intestinal epithelial cells and microglial cells[4]

IL18 induction to inflammation begins at the cellular level. Until it induces complications[5], the level of IL18 excretion can be influenced by the presence of specific polymorphism of IL18 gene, and also the regulation of the free circulating IL18 by the presence of IL18 binding protein (IL18BP), which is a natural inhibitory control, preventing IL18 from binding to its cellular receptor[6].

Some polymorphisms in the IL18 gene have been associated with IL18 production by monocytes or with IL18 circulating levels[7]. Two single nucleotide polymorphisms (SNPs) have functional relevance; a change from cytosine (C) to adenine (A) at position 607 (rs1946518) and a substitution at position 137 from guanine (G) to C (rs187238). It has been reported that the promoter 607 and 137 polymorphisms of IL18 influence the level of cytokine IL18 expression. Individuals

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homozygous for the 607 C and 137 G express higher levels of IL18[8].

The current study was planned to investigate the serum level of IL18 and IL18BP in T2DM patients, and relation of the two with IL18 gene polymorphisms along with its complications.

Materials and Methods

The case-control study at the endocrinology clinic of the Madinat Al-Imamin Al-Kazemin Teaching Hospital, from September 2020 to July 2021. The study was approved according to Council Decision No:202011151 in 2/9/2020 from AL_Nahrain University – College of Medicine. Using consecutive non-probability sampling technique, patients of either gender aged 20-65 years previously diagnosed with T2DM and attending the endocrinology clinic were included. The T2DM diagnosis was based on the Standards of Medical Care in Diabetes 2020 guidelines.[9] Healthy controls matched for age and gender were enrolled from a blood donation centre. A verbal consent was obtained from all the participants. Exclusion criteria entailed patients with type 1 diabetes mellitus, those taking immunosuppressive drugs, and those diagnosed with any malignancy.

Age, disease duration, smoking status, T2DM complications as well as the latest fasting blood glucose (FBS) and glycated haemoglobin (HbA1C) levels were recorded from the institutional patient registry.

Enzyme-linked immunosorbent assay (ELISA) was used to measure the serum level of free circulating IL18 (Catalogue No. abx250150, Abbexa, United Kingdom) and IL18BP (Catalogue No. abx351837, Abbexa, UK) as per the manufacturer's instructions.

After deoxyribonucleic acid (DNA) extraction using a commercial kit (Blood Genomic DNA Kit, Catalogue No. abx098078, Abbexa, UK), a tetra-primer amplification refractory mutation system (TARMS-PCR) technique was used screening IL18 SNPs 607 C/A(10) and 137 G/C[11]. The agarose gel with 2% concentration were used to analyse electrophoresis of DNA fragments under ultraviolet (UV) light, comparing them with a 100bp DNA ladder.

Data was analysed using SPSS 20. Continuous variables were subjected to normality test. Data with normal distribution was subjected to t-test for comparison of mean values. For non-normally distributed data, either Mann-Whitney U-test or Kruskal Wallis test was used for comparison between median values. These variables were expressed as either mean ± standard deviation (SD) or median (interquartile range [IQR]). Categorical variables were expressed as frequencies and percentages, and analysed with chi-square, or fisher exact tests. Odds ratio (ORs) and corresponding 95% confidence intervals (CIs) were calculated to assess the association between different SNPs of IL18 risk and T2DM. Two-sided p≤0.05 was accepted as the level of significance.

Results

Of the 168 subjects, 86(51.2%) were patients; 67(77.9%) females and 19(22.1%) males with mean age 52±8.97 years. There were 82(48.8%) controls; 56(68.3%) females and 26(31.7%) males with mean age 48±9.44 years (p>0.05). Regarding complications, there were 44 (51.16%) with retinopathy, 27 (31.4%) with nephropathy, 17 (19.77%) with neuropathy, 12 (13.95%) with heart disease, 9 (10.47%) with peripheral vascular disease and only 2 (2.33%) with stroke. Differences related to serum CRP, FBS, HbA1c, IL18 and IL18BP were significantly different between the groups (Table 1).

Regarding 137 G/C, the mutant homozygous genotype

Table-1: Demographic, clinical and laboratory data.

	Study Groups		
	T2DM	Control	P value
Age			
Mean±SD	52±8.97	48.48±9.44	0.328NS
Gender			
Female	67 (77.9%)	56 (68.3%)	0.349NS
Male	19 (22.1%)	26 (31.7%)	
Complication			
Retinopathy	44 (51.16%)		
Nephropathy	27 (31.40%)		
Neuropathy	17 (19.77%)		
Stroke	2 (2.33%)		
Heart Disease	12 (13.95%)		
Peripheral Vascular disease	9 (10.47%)		
CRP			
Positive	32 (37.2%)	0 (0.0%)	<0.001**
Negative	54 (62.8%)	82 (100.0%)	
FBG			
Median (5-95%CI)	205.50 (95-423)	89.5 (74-110)	<0.001**
HbA1C			
Median (5-95%CI)	7.6 (5.8-12)	5.3 (5-5.7)	<0.001**
Serum IL-18 (pg/ml)			
Median (5-95%CI)	283.49 (137.93-740.58)	73.96 (35.04-136.29)	<0.001**
Serum IL-18BP (ng/ml)			
Median (5-95%CI)	1.34 (0.53-5.83)	2.07 (0.82-7.47)	0.003*
IL18/IL18BP			
Median (5-95%CI)	0.22 (0.07-0.52)	0.03 (0.01-0.12)	<0.001**

*: statistically significant difference (p≤0.05), **: high statistically significant difference (p≤0.001)

T2DM: Type 2 diabetes mellitus, SD: Standard deviation, CRP: C-reactive protein, HbA1c: Glycated haemoglobin, FBG: Fasting Blood Glucose, IL18: Interleukin18, IL18BP: Interleukin18 binding protein, CI: Confidence interval

Table-2: Descriptive frequency and risk estimation of -137G/C and -607C/A of IL18 gene polymorphisms in the study groups.

	Study Groups			Odds ratio
	T2DM	Control	P value	(95% CI)
-137 G/C				
CC	9 (10.5%)	3 (3.7%)	<0.001**	
GC	27 (31.4%)	9 (11.0%)		
GG	50 (58.1%)	70 (85.4%)		
Mutant allele (C)	45 (26.2%)	15 (9.1%)	<0.001**	3.52 (1.91- 6.63)
Wild allele (G)	127 (73.8%)	149 (90.9%)		
-607 C/A				
AA	14 (16.3%)	5 (6.1%)	<0.001**	
CA	19 (22.1%)	7 (8.5%)		
CC	53 (61.6%)	70 (85.4%)		
Mutant allele A	47 (27.3%)	17 (10.4%)	<0.001**	
Wild allele C	125 (72.7%)	147 (89.6%)		3.25 (1.77-5.83)

A: Adenine, T: Thymine, G: Guanine, C: Cytosine, T2DM: Type 2 diabetes mellitus, IL18: Interleukin18, CI: Confidence interval.

Table-3: Association of -137G/C and -607C/A IL18 gene polymorphism with IL18 and IL18/IL-18BP ratio serum levels.

	Serum IL-18 (pg/ml)	P value	Serum IL-18/ IL-18BP	P value
-137				
CC	310.50 (138.37-845)	0.038*	0.21 (0.28-0.04)	
GC	233.94 (136.62-633.86)		0.20 (0.43-0.07)	0.082^{NS}
GG	207.27 (137.93-419.27)		0.23 (0.60-0.09)	
Mutant allele (C)	306.97 (138.37-881.46)	0.009*	0.21 (0.42-0.04)	0.090^{NS}
Wild allele (G)	225.3 (137.93-415.9)		0.23 (0.55-0.08)	
-607				
AA	347.24 (136.62-982.61)	0.007*	0.21 (0.52-0.01)	
CA	290.08 (154.51-579.91)		0.25 (0.86-0.04)	0.365^{NS}
CC	282.61 (137.93-819.27)		0.23 (0.55-0.08)	
Mutant allele A	322.3 (149.04-861.46)	<0.001**	0.22 (0.52-0.04)	0.973^{NS}
Wild allele C	232.61 (137.93-519.27)		0.23 (0.55-0.07)	

A: Adenine, T: Thymine, G: Guanine, C: Cytosine, IL18: Interleukin18, IL18BP: Interleukin18 binding protein.

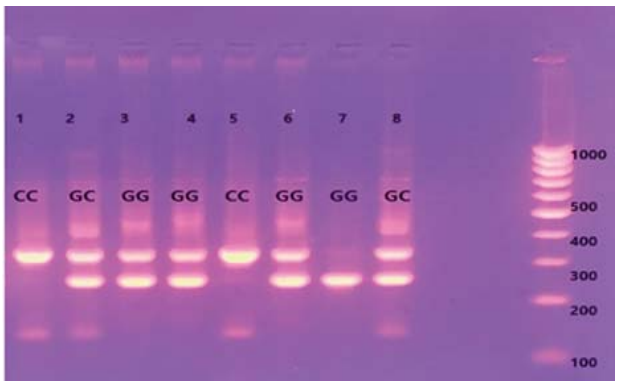

Figure-1: Gel electrophoresis of polymerase chain reaction (PCR) product of -1137G/C genotyping. Lane 3 represents homozygous wild, lane 2 represents heterozygous, and lane 1 represents homozygous mutant genotype. Product size was 342 base pair (bp). For G allele, the product size was 257bp, and for the C allele, it was 135bp.

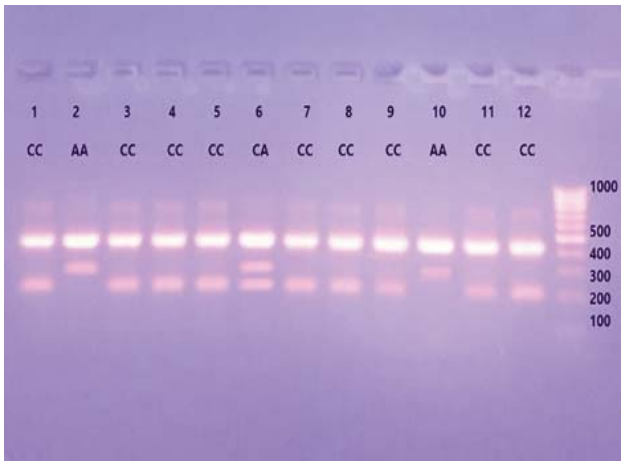

Figure-2: Gel electrophoresis of polymerase chain reaction (PCR) product of -607C/A genotyping. Lane 1 represents homozygous wild, lane 6 represents heterozygous, and lane 2 represents homozygous mutant genotype. Product size was 440 base pair (bp). For mutant A allele, the product size was 278bp, and for wild type C allele, the product size was 208bp.

was found in 9(10.5%) patients and 3(3.7%) controls, while the heterozygous genotype was found in 27(31.4%) patients and 9(11%) controls (p<0.001). The mutant allele C was found in 45(26.2%) patients and 15(9.1%) controls (p<0.001) (Figure 1). For 607 C/A, mutant homozygous genotype was found in 14(16.3%) patients and 5(6.1%) controls, the heterozygous genotype was found in 19(22.1%) patients and 7(8.5%) controls (p<0.001). The mutant allele A was found in 47(27.3%) patients and 17(10.4%) controls (p<0.001) (Figure 2). IL18-137 G/C and IL18-607C/A mutant alleles had ORs 3.52 (CI: 1.91-6.63) and 3.25 (CI: 1.77-5.83), respectively, as risk factors for the T2DM occurrence (Table 2).

The association of 137 G/C and 607 C/A was significant with IL18 (p<0.05), but not with IL18BP (Table 3).

There was an association of interleukin18-137G/C homozygous mutant genotype with the occurrence of retinopathy among type 2 diabetes mellitus patients (p=0.046), but risk allele C was not associated with retinopathy (p>0.05).

Discussion

The effect of high level of inflammatory cytokine is associated with diabetes as a causative agent of insulin resistance (IR) resulting from hyperglycaemia and leading to complications that are mostly microvascular[11].

An elevated IL18 serum level has been proposed to influence beta cell dysfunction and hyperglycaemia[12]. Furthermore, lower response to antidiabetic drugs have been reported after stimulation of IL18 production[13]. The action of IL18 as a pleiotropic cytokine was balanced and

regulated by specific new family protein IL18BP, which represents an inhibitor for the signalling of IL18. The high affinity between IL18 and IL18BP leads to the formation of complex blocks[14].

The correlation of IL BP with hyperglycaemia, indicated by HBA1c levels, was reported to be positive in patients with type 1 diabetes mellitus, suggesting a potential link between IL18 and the metabolic activity in such patients[15]. The current study reported low level of IL18BP in T2DM patients.

The elevated levels of free circulating IL18 are related with metabolic syndrome in the presence of the G allele of 137 G/C at the region of promoter rs187238, suggesting an inherited origin[17].

The current study found that higher median level of IL18 was related to the presence of SNPs 607 C/A and 137 G/C. This explains the hypothesis of the current study that gene polymorphism might be involved with the pathogenesis of T2DM through the induction of higher levels of IL18 that activate cells to promote inflammation, IR and interferon (IFN) gamma production, and further the loop of inflammation.

The genetic polymorphism of the IL18 at the promoter region rs1946518 of allele 607 C/A showed a related effect on immune-response with the risk of several diseases[18]. The individuals carrying 137 G allele or carrying 607 C allele had elevated IL18 action and high levels of resistance against infectious diseases[19].

In contrast, a study found no association between 137 G/C and 607 C/A gene polymorphisms and the risk of T2DM. This might be due to small sample size of the study or because of ethnic differences[20]. Similar findings were reported by a study in Slovenia[21]

The presence of 137 C allele or 607 A allele may increase the risk of developing diabetes in the current study. There was an inverse relation between IL18 and IL18BP levels in T2DM patients, which may be attributed to the hyperglycaemia pathological effect.

Limitation: The current study has limitations as the sample size was not calculated, which could have affected the power of the study.

Conclusion

The presence of 137 G/C and 607 C/A IL18 gene polymorphism might be considered a risk factor for developing T2DM. These were associated with increased levels of free circulating IL18 rather than inadequate regulatory IL18BP.

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Conflict of Interest: None.

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