Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2020 Nov 12; 8(A):910-915.
https://doi.org/10.3889/oamjms.2020.5478
eISSN: 1857-9655
Category: *A - Basic Sciences*
Section: *Genetics*

The Association of Cytokine Genes Polymorphisms (IL1β+3954 C/T, IL18-137 G/C, and IL18-607 C/A) in Type 2 Diabetes Mellitus-tuberculosis

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Edited by: Slavica Hristomanova-Mitkovska
Citation: Sari MI, Tala ZZ. The Association of Cytokine Genes Polymorphisms (IL1β+3954 C/T, IL18-137 G/C, and IL18-607 C/A) in Type 2 Diabetes Mellitus-tuberculosis. Open Access Maced J Med Sci. 2020 Nov 12; 8(A):910-915. https://doi.org/10.3889/oamjms.2020.5478
Keywords: IL1β+3954 C/T; IL18-137 G/C; IL18-607 C/A; Polymorphisms; Type 2 diabetes mellitus-tuberculosis; Type 2 diabetes mellitus; Tuberculosis
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Received: 25-Sep-2020
Revised: 18-Oct-2020
Accepted: 02-Nov-2020

Funding: This study was supported by the Ministry of Research and Technology, Higher Education, Republic of Indonesia, a grant from Penelitian Dasar Unggulan Perguruan Tinggi in 2018 (Grant No. 84/UN5.2.3.1/PPM/KP-DRPM/2018) and 2019 (Grant No. 21/UN5.2.3.1/PPM/KP-DRPM/2019)
Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: The cytokine gene polymorphism is associated with the development of metabolic disorder conditions and infectious diseases such as Type 2 diabetes mellitus (T2DM) and tuberculosis (TB) disease.

AIM: The objective of the study is an attempt to examine the association of cytokine genes polymorphisms (IL1β+3954 C/T, IL18-137 G/C, and IL18-607 C/A) in T2DM-TB patients.

METHODS: The cytokine genes polymorphisms (IL1β+3954 C/T, IL18-137 G/C, and IL18-607 C/A) were investigated in 46 T2DM-TB patients, 46 T2DM patients, and 46 healthy controls. Cytokine genes polymorphism was carried out by the polymerase chain reaction-restriction fragment length polymorphism. Odds ratio (OR) with 95% confidence interval (CI) and p-value was calculated to determine the association between cytokine genes polymorphisms as the risk factor to T2DM-TB development.

RESULTS: No association between genotypes and alleles of cytokine genes polymorphisms (IL1β+3954 C/T, IL18-137 G/C, and IL18-607 C/A) in T2DM-TB compared to control group (p = 0.434; OR = 0.373; 95% CI = 0.068-2.028 and p = 0.444; OR = 0.387; 95% CI = 0.073–2.046), (p = 0.833; OR = 0.915; 95% CI = 0.400–2.092 and p = 0.864; OR = 1.061; 95% CI = 0.541–2.078), and (p = 0.815; OR = 0.896; 95% CI = 0.357–2.246 and p = 0.882; OR = 0.957; 95% CI = 0.534–1.715). This study also found no association between genotypes and alleles of cytokine genes polymorphisms (IL1β+3954 C/T, IL18-137 G/C, and IL18-607 C/A) with T2DM-TB compared to T2DM group (p = 1; OR = 0.652; 95% CI = 0.104–4.094 and p = 1; OR = 0.659; 95% CI = 0.108–4.041), (p = 0.189; OR = 1.786; 95% CI = 0.749–4.262 and p = 0.098; OR = 1.857; 95% CI = 0.887–3.889), and (p = 0.374; OR = 1.488; 95% CI = 0619–3.579 and p = 0.365; OR = 1.316; 95% CI=0.727–2.382).

CONCLUSION: There is no association of the cytokine genes polymorphisms (IL1β+3954 C/T, IL18-137 G/C, and IL18-607 C/A) in T2DM-TB compared to control and T2DM groups, and all cytokine genes polymorphisms not as the risk factor to T2DM-TB development in this population.

Introduction

Type 2 diabetes mellitus (T2DM) is a metabolic disorder that has implications for the increased risk of tuberculosis (TB). Since the beginning of the 20th century, clinicians have observed an association between T2DM and TB [1]. T2DM was found in around 2–9% of the population in 22 countries with the highest burden of TB. Eight of ten countries with the highest incidence of T2DM in the world are also classified as the highest pulmonary TB burden [2]. T2DM patients have 2–3 times the risk of developing TB than people without T2DM. People with low immune systems due to chronic diseases such as T2DM have a higher risk of developing latent TB to active TB. The innate immune system is compromised by high blood glucose levels (BGLs), which increases the risk of developing infectious diseases such as TB [3], [4].

Increased BGLs in T2DM are associated with an inflammatory state characterized by cytokines such as interleukin (IL)-1, IL-6, IL-18, and tumor necrosis factor-α (TNF-α). Cytokines genes polymorphisms are thought to be one of the genetic factors involved in the pathogenesis of T2DM development. Previous studies have shown that polymorphism of IL18-607 C/A gene was associated with 2 h post-loading plasma glucose level in T2DM. Another previous study in the Chinese population showed that IL18-137 G/C gene polymorphism had a risk of T2DM with nephropathy. IL-18 was identified as an interferon (INF)-gamma inducing factor. IL-18 gene is located at 11q22.2-22.3, including six exons and five introns. IL18-607 C/A and IL18-137 G/C polymorphism are located at the promoter region of exon 1 in IL-18 gene [5], [6], [7]. Based on other studies, there is result showing that the IL-18 gene polymorphism also plays a role in the development mechanism of TB. A study by Han *et al.* (2010) showed an association between IL18-607 C/A gene polymorphism with TB [8].

The other cytokine gene polymorphism, that is, IL1β+3954 C/T is known to be associated with the development of TB in the Colombia population. The IL-1β gene is located on chromosome 2q13–q21. Single nucleotide polymorphisms (SNPs) in the IL1β promoter have been reported, some of which may regulate IL-1β expression. Allele T of the +3953 SNP, located at exon 5, was associated with a decrease in IL-1β production under inflammatory circumstances. ProIL1β is biologically inactive and must be converted to 17 kDa IL1β to function. IL1β is processed and released from cells by a mechanism involving caspase 1 [9], [10], [11].

The study regarding the association of the IL-18 gene and T2DM-TB showed that an association between IL18-607 C/A gene polymorphism with T2DM-TB but no showed an association between 137 G/C gene polymorphism [12]. To the best of our knowledge, no studies have yet reported the association of IL1β+3954 C/T with T2DM-TB. In this study, we analysis the association of the cytokine genes polymorphisms (IL1β+3954 C/T, IL18-137 G/C, and IL18-607 C/A) in T2DM-TB patients, T2DM patients, and healthy control groups at Medan city population, Indonesia.

Materials and Methods

Ethical agreement

Informed consent was given to each subject after receiving a simple description of the purposes and benefits of the study. The protocol of this study has been approved by the ethics committee of the Faculty of Medicine, Universitas Sumatera Utara (USU) (KEPK-FK USU-RSUP HAM No. 227/2018 and 447/2019) following the Second Declaration of Helsinki. The study was conducted from March 2018 to June 2019.

Study design and subjects

A case-control design was conducted on subjects in several places. T2DM-TB subjects were recruited from health-care facilities for pulmonary disease in Medan city, T2DM patients were recruited from USU Hospital Medan, and the healthy control were employees of USU medical faculty in Medan, Indonesia. A total of 138 subjects consisting of 46 T2DM-TB patients, 46 T2DM patients, and 46 healthy controls. The diagnosis of T2DM-TB and T2DM patients was based on the Indonesian Lung Doctor Association [13] and Indonesian Endocrinology Society [14]. Characteristics of subjects in this study, including age, gender, family history of T2DM, and smoking habit, were collected through interviews using questionnaires.

Blood samples, BGLs examination, and DNA extraction

Blood samples (5 ml) were collected in plain and ethylenediaminetetraacetic acid tubes from the median cubital vein under aseptic conditions. The BGLs were tested in the USU hospital according to the routine procedure in the hospital. The criteria for BGLs of healthy control were <126 mg/dl.

The analysis of cytokine genes polymorphisms started from the DNA extraction from each subject's blood samples at Biomolecular Laboratory, Medical Faculty of USU. A total of 200 μL of blood from 138 patients were isolated using the Promega extraction kit (USA). The DNA isolation procedure was carried out according to the manual kit. The isolated DNA stored at −80°C for further analysis. The quality and quantity of the DNA samples were evaluated by agarose gel electrophoresis and ultraviolet (UV) spectrophotometer Nanodrop with an absorbance of 260/280 and 260/230.

Genotyping

Genotyping of cytokines was obtained using the polymerase chain reaction (PCR) technique. PCR solution of IL1β+3954 C/T gene consists of 23 μL (12.5 μL Go Taq master mix from Promega, USA, each 1 μL reverse primer and forward primer 8.5 μL nuclease-free water), and then 2 μL of isolated DNA was added. The PCR products for IL1β+3954 C/T gene were further analysis by the restriction fragment length polymorphism (RFLP) and digested with restriction enzymes.

PCR solution of IL18-137 G/C and IL18-607 C/A genes consisted of F1 solution and F2 solution. The F1/F2 solution was made with a volume of 23 μL (12.5 μL Go Taq master mix, each 1 μL reverse primer, F1/F2 primer, and control primer, 7.5 μL nuclease-free water), and 2 μL of isolated DNA was added. PCR primers, PCR conditions, and restriction enzymes for each gene were shown in Table 1, as described previously [15], [16].

PCR and RFLP products were analysis using agarose gel (UltraPure™) electrophoresis technique of 2% (PCR) or 4% (RFLP) at 100 volts in 1 h. Then, the electrophoresis results were visualized using Gel Doc 1000 (Bio-Rad, USA) with UV light and the fragment of each gene was shown in Table 2.

Statistical analysis

All of the statistical analyses were performed with SPSS version 24. The distribution of genotypes and alleles of cytokine genes frequency was calculated by direct counting. Hardy-Weinberg equilibrium (HWE) from cytokine genes in all groups studied was calculated using Chi-square goodness-of-fit test. The association between cytokine genes polymorphisms as a risk factor to T2DM-TB was assessed using the Chi-square test

Table 1: Primer, PCR conditions, and restriction enzyme of cytokine genes

Polymorphism	Primers and PCR conditions	Restriction enzyme
IL1β+3954 C/T	(F) 5'-GTT GTC ATC AGA CTT TGA CC-3' (R) 5'-TTC AGT TCA TAT GGA CCA GA-3' (Macro gene, USA) 95°C 4 min., 30× (95°C 30 s; 59°C 30 s; 72°C 30 s), 72°C 4 min	Taq I (65 °C, 45 min) (Time-Saver Qualified enzyme. New England Biolabs, USA)
IL 18-137 G/C	(R): 5'-AGG AGG GCA AAATGC ACT GG-3' (F1): 5'-CCC CAA CTT TTA CGG AAG AAA AG-3' (F2): 5'-CCC CAA CTT TTA CGG AAG AAA AC-3' (CTRL): 5'-CCA ATA GGA CTG ATT ATT CCG CA-3' (Macrogen, USA) 94°C 3 min., 40× (94°C 20 s; 54°C 20 s; 72°C 20 s), 72°C 5 min	-
IL 18-607 C/A	(F1): 5'-GTT GCA GAA AGT GTA AAA ATT ATT AC-3' (F2): 5'-GTT GCA GAA AGT GTA AAA ATT ATT AA-3' (R): 5'-TAA CCT CAT TCA GGA CTT CC-3' CTRL): 5'-CTT TGC TAT CAT TCC AGG AA-3' (Macrogen, USA) 94°C 3 min., 40× (94°C 20 s; 50°C 20 s; 72°C 20 s), 72°C 5 min	-

PCR: Polymerase chain reaction.

or Fisher's exact test with an odds ratio (OR) and confidence interval (CI) 95%.

Table 2: DNA fragments of cytokine genes

Polymorphism	PCR amplicon	Homozygous wild-type	Homozygous mutant	Heterozygous
IL1β+3954 C/T	249 bp	CC 135+114 bp	TT 249 bp	CT 249+135+114 bp
IL 18-137 G/C	261 bp Control: 446 bp	GG F1: 261+446 bp F2: 446 bp	CC F1: 446 bp F2: 261+446 bp	GC F1:261+446 bp F2:261+446 bp
IL 18-607 C/A	196 bp Control: 301 bp	CC F1: 196+301 bp F2: 301 bp	AA F1: 301bp F2: 196+301 bp	CA F1:196+301 bp F2:196+301 bp

PCR: Polymerase chain reaction.

Results

In this study, the clinical and laboratorial characteristics of T2DM-TB patients, T2DM patients, and controls are summarized in Table 3.

Table 3: The clinical and laboratorial characteristics of T2DM-TB patients, T2DM patients, and controls

Characteristic	Control	T2DM	T2DM-TB
Age (years), (mean ± SD)	42.52 ± 13.15	55.95 ± 12.11	53.50 ± 8.27
Gender (n; %)			
Male	25 (54.3)	25 (54.3)	32 (69.6)
Female	21 (45.7)	21 (45.7)	14 (30.4)
Family history of T2DM			
No	37 (80.4)	15 (32.6)	37 (80.4)
Yes	9 (19.6)	31 (67.4)	9 (19.6)
BGLs (mg/dl) (mean ± SD)	118.98 ± 28.75	301.11 ± 100.35	304.33 ± 68.77
Cigarette smoking (n; %)			
Never	37 (80.5)	28 (60.9)	19 (41.3)
Former	3 (6.5)	12 (26.1)	20 (43.5)
Current smoker	6 (13.0)	6 (13.0)	7 (15.2)

T2DM: Type 2 diabetes mellitus, TB: Tuberculosis, BGL: Blood glucose level.

The distribution of genotype and allele of cytokines and analysis of HWE in the population studied are summarized in Table 4.

The IL1β+3954 C/T gene, wild type CC genotypes was found the most in the T2DM-TB group (controls vs. T2DM vs. T2DM-TB = 89.1% vs. 93.5% vs. 95.7%), while CT genotype was found the most in the control group (controls vs. T2DM vs. T2DM-TB = 10.9% vs. 6.5% vs. 4.3%). There is no TT genotype of IL1+3954 C/T in this population studied. C allele of IL1β+3954 C/T gene was found the most in T2DM-TB group than the other groups, while T allele of IL1β+3954 C/T gene was found the most in the control group than the others groups.

Table 4: Distribution of genotype and allele of cytokines and analysis of Hardy-Weinberg equilibrium in the population studied

Cytokine genes	Control n (%)	T2DM n (%)	T2DM-TB n (%)
IL1β+3954 C/T			
Homozygote wild type/CC	41 (89.1)	43 (93.5)	44 (95.7)
Heterozygote mutant/CT	5 (10.9)	3 (6.5)	2 (4.3)
Homozygote mutant/TT	0 (0)	0 (0)	0 (0)
C allele	87 (94.6)	89 (96.7)	90 (97.8)
T allele	5 (5.4)	3 (3.3)	2 (2.2)
HWE; X2 (p-value)	0.151 (0.696)	0.052 (0.819)	0.022 (0.880)
IL 18-137 G/C			
Homozygote wild type/GG	26 (56.5)	33 (71.7)	27 (58.7)
Heterozygote mutant/GC	18 (39.2)	12 (26.1)	15 (32.6)
Homozygote mutant/CC	2 (4.3)	1 (2.2)	4 (8.7)
G allele	70 (76.1)	78 (84.8)	69 (75.0)
C allele	22 (23.9)	14 (15.2)	23 (25.0)
HWE; X2 (p-value)	0.260 (0.609)	0.005 (0.940)	0.782 (0.376)
IL 18-607 C/A			
Homozygote wild type/CC	12 (26.1)	17 (37.0)	13 (28.3)
Heterozygote mutant/CA	28 (60.9)	25 (54.3)	27 (58.7)
Homozygote mutant/AA	6 (13.0)	4 (8.7)	6 (13.0)
C allele	52 (56.5)	59 (64.1)	53 (57.6)
A allele	40 (43.5)	33 (35.9)	39 (42.4)
HWE; X2 (p-value)	2.615 (0.105)	1.512 (0.218)	1.872 (0.171)

T2DM: Type 2 diabetes mellitus, TB: Tuberculosis, HWE: Hardy-Weinberg equilibrium.

On the other hand, in the IL18-137 G/C gene, wild type GG genotype was found the most in the T2DM group (control vs. T2DM vs. T2DM-TB = 56.5% vs. 71.7% vs. 58.7%), whereas GC was found the most in the control group (control vs. T2DM vs. T2DM-TB = 39.2% vs. 26.1% vs. 32.6%) and CC genotypes were found the most in the T2DM-TB group (control vs. T2DM vs. T2DM-TB = 4.3% vs. 2.2% vs. 8.7%). G allele of the IL18-137 G/C gene was found the most in T2DM group than the other groups, while C allele of IL18-137 G/C gene was found the most in T2DM-TB group than the others groups.

Table 5: Cytokine genes polymorphisms and the risk of T2DM-TB in the population studied

Cytokine genes	T2DM-TB vs. control		T2DM-TB vs. T2DM	
	p-value	OR (95% CI)	p-value	OR (95% CI)
IL1β+3954 C/T				
CC	-	1 (reference)	-	1 (reference)
CT+TT	0.434	0.373 (0.068–2.028)	1	0.652 (0.104-4.094)
C	-	1 (reference)	-	1 (reference)
T	0.444	0.387 (0.073–2.046)	1	0.659 (0.108-4.041)
IL 18-137 G/C				
GG	-	1 (reference)	-	1 (reference)
GC+CC	0.833	0.915 (0.400–2.092)	0.189	1.786 (0.749–4.262)
G	-	1 (reference)	-	1 (reference)
C	0.864	1.061 (0.541–2.078)	0.098	1.857 (0.887–3.889)
IL 18-607 C/A				
CC	-	1 (reference)	-	1 (reference)
CA+AA	0.815	0.896 (0.357–2.246)	0.374	1.488 (0619–3.579)
C	-	1 (reference)	-	1 (reference)
A	0.882	0.957 (0.534–1.715)	0.365	1.316 (0.727–2.382)

T2DM: Type 2 diabetes mellitus, TB: Tuberculosis, OR: Odds ratio, CI: Confidence interval.

In the IL18-607 C/A, wild type CC genotype was found the most in the T2DM group (control vs. T2DM vs. T2DM-TB = 26.1% vs. 37.0% vs. 28.3%), whereas CA genotype was found the most in the control group (control vs. T2DM vs. T2DM-TB = 60.9% vs. 54.3% vs. 58.7%) and AA genotype was found the most in the control

and T2DM-TB group (control vs. T2DM vs. T2DM-TB = 13.0% vs. 8.7% vs. 13.0%). C allele of the IL18-607 C/A gene was found the most in T2DM group than the other groups, while A allele of IL18-607 C/A gene was found the most in control group than the others groups. The frequency distribution of genotype cytokines in all groups was in an agreement under the HWE law ($p > 0.05$).

Table 5 showed that genotype (CT+TT vs. CC) and allele (T vs. A) of IL1β+3954 C/T were no difference in T2DM-TB group compared to control group ($p = 0.434$; OR = 0.373; 95% CI = 0.068–2.028 and $p = 0.444$; OR = 0.387; 95% CI = 0.073–2.046) and no difference in T2DM-TB group compared to T2DM group ($p = 1$; OR = 0.652; 95% CI = 0.104–4.094 and $p = 1$; OR = 0.659; 95% CI = 0.108–4.041).

The IL18-137 G/C genotype (GC+CC vs. GG) and allele (C vs. G) were also no difference in T2DM-TB group compared to control group ($p = 0.833$; OR = 0.915; 95% CI = 0.400–2.092 and $p = 0.864$; OR = 1.061; 95% CI = 0.541–2.078) and no difference in T2DM-TB group compared to T2DM group ($p = 0.189$; OR = 1.786; 95% CI = 0.749–4.262 and $p = 0.098$; OR = 1.857; 95% CI = 0.887–3.889).

In this study also found that the IL 18-607 C/A genotype (CA+AA vs. CC) and allele (A vs. C) were no difference in T2DM-TB group compared to control group ($p = 0.815$; OR = 0.896; 95% CI = 0.357–2.246 and $p = 0.882$; OR = 0.957; 95% CI = 0.534–1.715) and no difference in T2DM-TB group compared to T2DM group ($p = 0.374$; OR = 1.488; 95% CI = 0619–3.579 and $p = 0.365$; OR = 1.316; 95% CI = 0.727–2.382).

Discussion

In this study, genotyping of cytokine, that is, IL1β+3954 C/T, IL18-137 G/C, and IL18-607 C/A in T2DM-TB, T2DM, and control group study has been carried out. The results of the Chi-square goodness-of-fit test analysis which examined the distribution of genotype frequencies of all groups were in accordance with the HWE law ($p > 0.05$). HWE law states that, in a population, the genotype and allele frequencies are constant from generation to generation. Many factors that can change the frequency of genotype and allele in a population are non-random mating, mutation, migration, genetic drift, and natural selection [17]. Medan city is the third-largest city in Indonesia, with a large population of various ethnicities. As a result, mating between individuals happens randomly and caused the distribution of the genotype of cytokines studied were in accordance with the HWE law.

In this current study showed that no association between genotypes and alleles of IL1β+3954 C/T, IL18-137 G/C, and IL18-607 C/A gene polymorphism with T2DM-TB compared to control group and T2DM-TB compared to T2DM group. The association of IL1β+3954 C/T gene polymorphism with TB and latent TB has been reported in the literature before, with a different result [9], [18], [19], [20], but the associations of IL1β+3954 C/T gene polymorphism with T2DM-TB have not reported.

Several studies regarding the IL18-137 G/C and IL18-607 C/A gene polymorphism in T2DM-TB have been reported, and only the IL18-607 C/A gene polymorphism that has an associated T2DM-TB. The IL18-137 G/C gene polymorphism is not associated with T2DM-TB [10]. Several previous studies regarding the IL-18 cytokine gene polymorphism in T2DM have shown an association of IL18-607 C/A gene with 2 h post-loading plasma glucose levels in type 2 diabetes and IL18-137 G/C gene polymorphism has a risk of T2DM with nephropathy [6], [7]. However, the results of this study are not consistent with another study [21]. A previous study by Han *et al*. (2010) showed an association between IL18-607 C/A gene polymorphism with TB [8], but different result in a study by Zhou and Sheng (2019) showed there is no association between IL-18 polymorphisms (−137C/G and −607A/C) with TB [22].

Cytokines are a number of substances secreted by certain cells of the immune system. The cytokines based on the main producing cell type, divided into monokines and lymphokines. These cells will produce types of cytokines such as IL-1β, IL-6, IL-8, IL-12, and TNF-α, and IL-2, IFN-γ, IL-4, IL-5, IL-6, and IL-10. Cytokines are synthesized based on the nucleotide sequence in the gene. SNPs in cytokine genes may change the recognition sites of transcription factors, activation of transcriptions, and changes in cytokine production levels. Levels of cytokines have an important role in the body's immune responses. SNIPs of cytokine genes also cause differences in individuals' immune responses [5], [23], [24], [25].

The differences in individuals' immune responses are thought to underlie the pathogenesis of T2DM-TB disease. Individuals' immune responses, apart from being influenced by genetics, are also influenced by environmental factors [26], [27]. The results of other investigators suggest a risk of latent TB activation in T2DM patients who are not a primary TB infection [19].

Further research needs to be done to assess the relationship with levels and polymorphism of the cytokine genes as the risk factors for the occurrence of T2DM-TB from T2DM conditions or from control subjects. It is also necessary to analyze the environmental factors, history of previous infectious disease, and history of using TB drugs in future studies. A larger number of samples and widespread population are expected in subsequent studies to analyze the relationship of cytokine genes polymorphisms with the incidence of T2DM-TB.

Conclusion

This study showed that cytokine gene polymorphisms (IL1β+3954 C/T, IL18-137 G/C, and IL18-607 C/A) have not shown any association with T2DM-TB compared to control and T2DM groups, and all cytokine genes polymorphisms not as the risk factor to T2DM-TB development in this population.

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