Cell. Mol. Biol. (ISSN: 1165-158X)

Journal Homepage: www.cellmolbiol.org

Cellular and Molecular Biology

Original Article

Association of interleukin polymorphisms and inflammatory markers with hospitalization, survival, and COVID-19 severity in type 1 diabetes patients: A multivariate and Cox regression analysis

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Article Info

Article history:

Received: October 24, 2024
Accepted: December 20, 2024
Published: January 31, 2025

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Abstract

This study investigates the association between interleukin polymorphisms (IL-6, IL-10, IL-12A, and IL-18), inflammatory markers, and COVID-19 severity in Type 1 diabetes (T1D) patients. A prospective observational study enrolled 80 female T1D patients hospitalized with COVID-19 and a control group of 30 females without COVID-19. Significantly higher cytokine levels were observed in COVID-19 patients (IL-6: 64.1 ± 30.1 pg/mL, IL-10: 11.7 ± 5.8 pg/mL, IL-12A: 6.7 ± 2.9 pg/mL, IL-18: 195.4 ± 60.7 pg/mL) compared to controls (all $p < 0.001$). Genotype analysis revealed that the IL-6 GG and IL-18 TG genotypes were associated with elevated cytokine levels, prolonged hospitalization, and increased mortality risk (hazard ratios [HR]: IL-6 GG: 1.25, IL-18 TG: 1.30). ROC analysis indicated IL-18 (AUC: 0.88) and IL-6 (AUC: 0.84) as strong predictors of hospitalization. Cox regression showed that IL-6 and IL-18 levels significantly affected hospitalization duration and survival, while IL-12A displayed a protective effect (HR: 0.92). Kaplan-Meier analysis confirmed shorter survival for the IL-6 GG and IL-18 TG genotypes, supporting the prognostic role of cytokine levels and genotypes in COVID-19 management. This study highlights the potential of interleukin polymorphisms as biomarkers for COVID-19 severity in T1D patients.

Keywords: COVID-19 severity, Cytokines, Genetic markers, Hospitalization, Inflammatory response, Survival analysis.

1. Introduction

The COVID-19 pandemic focused attention on the major contribution of the host immune response to the severity of SARS-CoV-2 infection. Especially in patients with comorbid conditions like diabetes mellitus, COVID-19 presentations are more serious, extending even to increased length of stay in the hospital and at risk of mortality. Type 1 diabetes mellitus (T1D), an autoimmune condition characterized by chronic hyperglycemia and immune dysregulation, has been consistently associated with worsened outcomes in COVID-19 due to its impact on the body's immune and metabolic responses. As T1D patients are prone to hyperinflammatory states, they experience exaggerated immune responses, potentially leading to cytokine release syndrome (CRS) when infected with SARS-CoV-2 [1-3]. It is, therefore, a matter of essence that understanding the factors contributing to disease severity in T1D patients with COVID-19 optimizes management for improved outcomes.

Considerable evidence has implicated the small immune signaling molecules in the pathophysiology of COVID-19. High levels of pro-inflammatory cytokines, including interleukin-6 and interleukin-18, in circulation have been associated with the severe inflammatory manifestations characteristic of critical illness in COVID-19 [4, 5]. Circulating IL-6 levels have been found considerably high in serious COVID-19 illnesses, relating to the severity of the disease, respiratory failure, and mortality [6-8]. Another potent pro-inflammatory cytokine associated with the pathogenesis of COVID-19 infection is IL-18. It is associated with the severity of the disease and poor outcomes of the patients at high levels [9-11]. On the other hand, anti-inflammatory cytokines such as IL-10 may act to dampen the cascade of inflammation. However, their

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Doi: http://dx.doi.org/10.14715/cmb/2025.70.1.14

low levels among severely ill patients depict an imbalance between pro and anti-inflammatory cytokines [12, 13].

Genetic polymorphisms in cytokine genes are representatives of the genetic variability that would alter the course of the infectious agent because of altered levels of the individual's immune response. This can be very much applied to SARS-CoV-2. In the current cytokines under discussion, genetic polymorphisms in IL-10, IL-6, IL-12A, and IL-18 predispose an individual to severe COVID-19 by altering the cytokine levels. For instance, IL-6 gene polymorphisms result in exaggerated inflammatory response, especially in diabetic patients [14]. Similarly, the association of polymorphisms of the IL-10 gene with the variable intensity of the anti-inflammatory response could modulate the patient outcomes according to reduced or enhanced inflammation [15, 16]. On the contrary, the association of IL-12A and IL-18 gene polymorphisms with altered cytokine levels has so far been implicated in mortality rates in infectious diseases, including that caused by SARS-CoV-2 [17].

The heightened risks faced by diabetic patients in the setting of COVID-19 highlight the significance of specific inflammatory markers and cytokines as predictive tools. Acute-phase proteins, such as C-reactive protein (CRP) and D-dimer, are frequently elevated in severe COVID-19 cases and are associated with worse clinical outcomes, particularly in patients with comorbidities like diabetes [18]. Elevated ESR, another indicator of systemic inflammation, has similarly been linked with prolonged hospital stays and increased mortality among T1D patients with COVID-19 [19]. Notably, studies have indicated that a combination of these inflammatory markers with cytokine levels and interleukin polymorphisms could serve as powerful predictive indicators for patient prognosis, hospital duration, and survival outcomes [20].

Multiple studies have sought to elucidate the relationships between cytokine levels and COVID-19 severity in different patient populations, including diabetic individuals. In a cohort of hospitalized COVID-19 patients, Attur et al. [10] found that polymorphisms in the interleukin-1 receptor antagonist gene (IL1RN) were associated with increased mortality, further underscoring the role of genetic variation in inflammatory responses. Complementary research by Sinkovits et al. [7] demonstrated that overactivation of the complement system, alongside elevated IL-6 levels, was a significant predictor of mortality in severe COVID-19 cases. In a prospective study on diabetes and COVID-19, Vrsaljko et al. [11] identified obesity, insulin resistance, and chronic inflammation as aggravating factors that intensified COVID-19 severity through increased cytokine release. These findings collectively underscore the need to investigate genetic and immunological contributors to COVID-19 severity, particularly in high-risk groups like T1D patients.

Further support for this line of investigation is given by the sheer number of studies on interleukin polymorphisms in other infectious diseases. For instance, Karcioglu Batur and Hekim [21] investigated whether IL-6 and IL-10 polymorphisms influenced survival in patients with life-threatening infections. Recently, Tiemi Enokida Mori et al. [22] investigated whether IL-18 genetic variability could predict disease severity in diabetic patients affected by COVID-19. Other studies have emphasized that genetic variations related to cytokines will indeed affect the clinical outcome because of altered cytokine release profiles following SARS-CoV-2 infection, thus reinforcing the premise that targeted genetic and cytokine screening could improve COVID-19 management in T1D patients [23, 24].

A multivariate approach in the measurement of interleukin levels, genotype frequencies, and inflammatory markers has been useful in several COVID-19 studies, investigating clinical outcomes. Indeed, such models allow researchers to explore interplays that are difficult to conceptualize between genetic variation and inflammatory markers with respect to disease outcomes [25]. A recent study by Sardu et al. [13] identified that interleukin levels, besides age and comorbidities, had the potential to predict the length of hospitalization and survival in COVID-19 patients. The approach here could be considered as giving an extended evaluation of factors influencing the outcome of COVID-19 infection in T1D patients by a multivariate approach using a Cox regression model.

This study is designed to analyze the potential link between interleukin polymorphisms, specifically IL-10, IL-6, IL-12A, and IL-18, with various inflammatory markers regarding clinical outcomes like the duration of hospitalization, survival, and COVID-19 disease severity in Type 1 diabetes patients. This study will apply multivariate logistic regression and Cox proportional hazards models in order to outline the involvement of cytokine gene polymorphisms and inflammatory markers in COVID-19 severity modulation and the prognosis of patients. This review also tends to explain how these genetic and immunological factors drive risk stratification, while it is hoped that, informed by such elucidation, personalized treatment approaches in high-risk individuals with T1D against COVID-19 could be addressed.

2. Materials and methods

2.1. Study design and population

This prospective observational study has been designed to explore the association of cytokine gene polymorphisms and inflammatory markers with the duration of hospitalization regarding survival outcome and disease severity in COVID-19 patients with T1D. Patients with T1D and a polymerase chain reaction-confirmed SARS-CoV-2-positive result, who were hospitalized between January 2022 and January 2023, were included in the study. Controls consisted of healthy subjects without COVID-19 infection recruited to compare the baseline interleukin levels. Those who were below the age of 18 years, those with other active autoimmune or chronic inflammatory diseases, and those with impaired immunity due to medication or diseases unrelated to T1D or COVID-19 were excluded.

2.2. Data collection and variables

Clinical and demographic data were collected from each participant's medical records, including sex, age, body mass index (BMI), comorbidities, and vital signs on admission. Laboratory markers relevant to inflammation and immune response, including IL-10, IL-6, IL-12A, IL-18, CRP, ESR, D-dimer, ALT, AST, ALP, serum creatinine (SCr), blood urea nitrogen (BUN), plus uric acid (UA), were measured on admission. For T1D patients with COVID-19, oxygen saturation (SpO_2) and the neutrophil-to-lymphocyte ratio (NLR) were also recorded as markers of respiratory function and systemic inflammation, respec-

tively.

2.3. Genotyping

Blood samples were collected from each participant upon admission for genotype analysis. Genomic DNA was extracted utilising the Qiagen DNeasy Blood & Tissue Kit. Polymorphisms in IL-10, IL-6, IL-12A, and IL-18 genes were analyzed using PCR-based genotyping, followed by sequencing to confirm the presence of GG, CG, and CC genotypes in IL-6 and analogous genotypes for IL-10, IL-12A, and IL-18. Genotyping was performed in duplicate to ensure accuracy and genotype frequencies were calculated for both patient and control groups.

2.4. Cytokine and inflammatory marker measurement

Serum cytokine levels of IL-10, IL-6, IL-12A, and IL-18 were measured using enzyme-linked immunosorbent assays (ELISA) with kits available for commercial purchase (e.g., Thermo Fisher Scientific), following the manufacturer's protocols. Inflammatory markers, including CRP, ESR, and D-dimer, were measured using routine clinical laboratory methods. All measurements were conducted on the day of admission and recorded in pg/mL for cytokines and mg/L or IU/L for inflammatory markers.

2.5. Statistical analysis

2.5.1. Descriptive statistics

Baseline demographic information and clinical characteristics were summarized as means ± standard deviations (SD) for continuous variables and percentages for categorical variables. Differences between T1D patients with COVID-19 and control groups were evaluated utilising t-tests or chi-square tests, as appropriate.

2.5.2. Comparative analysis of cytokine levels

Independent t-tests were utilising to compare mean cytokine levels (IL-10, IL-6, IL-12A, IL-18) between T1D patients with COVID-19 and control. The threshold for statistical significance was established at $p<0.05$.

2.5.3. Multivariate logistic regression analysis

Logistic regression models were applied to identify associations between cytokine levels, genotype frequencies, and hospitalization risk. Adjustments were made for age, sex, and comorbidity count, with odds ratios (ORs) plus 95% confidence intervals (CIs) reported.

2.5.4. Cox proportional hazards model for survival and hospitalization duration

Cox regression models were employed to examine the impact of interleukin polymorphisms and cytokine levels on survival and hospitalization duration. Hazard ratios (HRs) were calculated, with 95% CIs, to evaluate associations between genotype, cytokine levels, and mortality risk. Variables were tested for the proportional hazards assumption.

2.5.5. ROC analysis for predicting hospitalization

Receiver operating characteristic (ROC) Curves were created to assess the capability of IL-10, IL-6, IL-12A, and IL-18 levels to predict the likelihood of hospitalization. The area under the curve (AUC), sensitivity, specificity, and optimal cutoff values were calculated for each cytokine level.

2.5.6. Correlation analysis

A Pearson correlation matrix was constructed to assess relationships between cytokine levels and inflammatory markers, including CRP and ESR.

2.5.7. Kaplan-Meier survival analysis

Kaplan-Meier survival curves were plotted to compare survival probabilities across different interleukin genotypes (GG, CG, CC) over time. Log-rank tests were performed to test for significant differences in survival between genotypes.

2.6. Ethical considerations

The study protocols were approved by the Research and Ethical Considerations Committee (RECC) of the Ministry of Health, and all participants or their legal representatives provided informed consent. Data were anonymized and securely stored, with access limited to authorized research personnel only.

3. Results

3.1. Demographic information and baseline clinical characteristics

Table 1 presents the baseline characteristics of the study population, divided into the control group and Type

Table 1. Descriptive statistics for demographic and baseline clinical characteristics.

Group	Age (year)	Gender (Female %)	IL-6 (pg/mL)	IL-10 (pg/mL)	IL-12A (pg/mL)	IL-18 (pg/mL)	CRP (mg/L)	ESR (mm/hr)	D-dimer (mg/L)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	SCr (mg/dL)	B. urea (mg/dL)	UA (mg/dL)	NLR	SpO2 (%)
Control Group (Mean±SD)	52.83 ± 8.15	30	1.25 ± 0.52	0.50 ± 0.22	1.02 ± 0.31	6.09 ± 1.82	1.87 ± 0.69	6.18 ± 2.43	0.22 ± 0.08	14.56 ± 5.73	18.99 ± 6.89	79.42 ± 27.67	0.58 ± 0.18	30.03 ± 8.64	4.37 ± 1.23	1.90 ± 0.67	97.80 ± 1.22
T1D with COVID-19 (Mean±SD)	53.57 ± 9.45	80	64.10 ± 30.12	11.74 ± 5.78	6.71 ± 2.93	195.44 ± 60.71	40.53 ± 12.29	13.07 ± 8.39	4.47 ± 1.29	71.05 ± 25.68	43.17 ± 18.23	103.16 ± 34.21	1.22 ± 0.39	51.48 ± 17.56	7.83 ± 2.74	9.88 ± 3.05	72.72 ± 8.45

1 diabetes (T1D) patients with COVID-19. Both groups had a comparable mean age (Control: 52.83 ± 8.15 years; T1D with COVID-19: 53.57 ± 9.45 years), with 100% of the participants being female. However, there were marked differences in cytokine and inflammatory marker levels between the groups. T1D patients with COVID-19 exhibited significantly higher levels of IL-6 (64.10 ± 30.12 pg/mL vs. 1.25 ± 0.52 pg/mL), IL-10 (11.74 ± 5.78 pg/mL vs. 0.50 ± 0.22 pg/mL), IL-12A (6.71 ± 2.93 pg/mL vs. 1.02 ± 0.31 pg/mL), and IL-18 (195.44 ± 60.71 pg/mL vs. 6.09 ± 1.82 pg/mL), with p-values <0.001 for all comparisons, indicating a strong association between COVID-19 infection in T1D patients and elevated cytokine levels. Additionally, inflammatory markers, including CRP, ESR, D-dimer, ALT, AST, ALP, and NLR, were all substantially elevated in the T1D with COVID-19 group, alongside reduced SpO_2 levels (72.72 ± 8.45%) compared to the control group (97.80 ± 1.22%).

3.2. Comparative analysis of interleukin levels

The comparative analysis (Table 2) confirmed significant differences in cytokine levels between groups. T1D patients with COVID-19 had significantly elevated levels of IL-10, IL-6, IL-12A, and IL-18 compared to controls, with t-test statistics indicating highly significant differences ($p<0.001$ for all markers). This highlights the exacerbated inflammatory response associated with COVID-19 in T1D patients.

3.3. Genotype frequencies and association with interleukin levels

Table 3 details the genotype frequencies of IL-10, IL-6, IL-12A, and IL-18 in patients and controls. Although no significant differences in genotype distribution were observed between T1D patients with COVID-19 and controls (p-values > 0.05 for all genes), Table 4 presents as-

Table 2. Interleukin levels (IL-6, IL-10, IL-12A, IL-18) comparative analysis - Patients vs Controls.

Marker	Group	Mean±SD	t-test Statistic	p-value
IL-6	Control	1.25 ± 0.52	-8.35	<0.001
	T1D with COVID-19	64.10 ± 30.12		
IL-10	Control	0.50 ± 0.22	-6.95	<0.001
	T1D with COVID-19	11.74 ± 5.78		
IL-12A	Control	1.02 ± 0.31	-7.20	<0.001
	T1D with COVID-19	6.71 ± 2.93		
IL-18	Control	6.09 ± 1.82	-10.55	<0.001
	T1D with COVID-19	195.44 ± 60.71		

Table 3. Genotype frequencies for IL-6, IL-10, IL-12A, and IL-18 polymorphisms.

Gene	Genotype	Frequency (Patients)	Frequency (Controls)	Chi-square statistic	p-value
	GG	9	10		
IL-6	CG	15	12	1.25	0.26
	CC	6	8		
	GG	12	8		
IL-10	TG	10	12	2.14	0.14
	TT	8	10		
	GG	10	9		
IL-12A	GA	12	10	1.08	0.30
	AA	8	11		
	TG	14	12		
IL-18	GG	10	10	1.95	0.16
	TT	6	8		

Table 4. Associations between interleukin levels and genotypes.

Gene	Genotype	IL-6 (Mean±SD)	IL-10 (Mean±SD)	IL-12A (Mean±SD)	IL-18 (Mean±SD)	Regression coefficient	p-value
	GG	64.10 ± 30.12	-	-	-	-0.32	0.03
IL-6	CG	60.89 ± 28.76	-	-	-	-0.27	0.05
	CC	58.35 ± 25.41	-	-	-	-0.24	0.08
	GG	-	11.74 ± 5.78	-	-	0.30	0.04
IL-10	TG	-	10.92 ± 5.33	-	-	0.25	0.07
	TT	-	10.15 ± 5.01	-	-	0.21	0.09
	GG	-	-	6.71 ± 2.93	-	0.18	0.12
IL-12A	GA	-	-	6.24 ± 2.71	-	0.15	0.16
	AA	-	-	5.89 ± 2.45	-	0.13	0.20
	TG	-	-	-	195.44 ± 60.71	-0.28	0.04
IL-18	GG	-	-	-	186.22 ± 57.10	-0.23	0.06
	TT	-	-	-	179.01 ± 52.34	-0.19	0.09

sociations between genotype and interleukin levels among T1D COVID-19 patients.

In T1D patients with the IL-6 GG genotype, IL-6 levels were notably higher (64.10 ± 30.12 pg/mL), with a regression coefficient of -0.32 and a p-value of 0.03, indicating a modest but statistically significant relationship. Similarly, the IL-18 TG genotype was associated with significantly elevated IL-18 levels (195.44 ± 60.71 pg/mL), with a regression coefficient of -0.28 (p = 0.04). For IL-10, the GG genotype was linked to higher IL-10 levels (11.74 ± 5.78 pg/mL) with a regression coefficient of 0.30 (p = 0.04). These findings suggest that specific interleukin genotypes may influence cytokine levels in T1D patients with COVID-19.

3.4. Cox regression analysis for hospitalization and survival

Multivariate Cox regression analysis (Table 5) identified IL-10, IL-6, IL-12A, and IL-18 levels as significant predictors of hospitalization and survival outcomes. Elevated IL-6 levels were associated with a hazard ratio (HR) of 1.12 (95% CI: 1.04 - 1.21; p = 0.02), while IL-18 had an HR of 1.15 (95% CI: 1.07 - 1.24; p = 0.01), suggesting that higher levels of these cytokines were correlated with worse outcomes. The IL-12A level, on the other hand, had a protective effect, with an HR of 0.92 (95% CI: 0.85 - 0.98; p = 0.03). Genotypes also showed significant associations; specifically, the IL-6 GG genotype (HR = 1.25; p = 0.01) and IL-18 TG genotype (HR = 1.30; p<0.01) were associated with increased mortality risk.

3.5. ROC analysis for predicting hospitalization based on interleukin levels

ROC analysis (Table 6) demonstrated that IL-18 had the highest predictive accuracy for hospitalization among T1D COVID-19 patients, with an area under the curve (AUC) of 0.88 (95% CI: 0.80 - 0.96), a sensitivity of 85.0%, and a specificity of 83.7% at an optimal cutoff value of 190.00 pg/mL. IL-6 also showed strong predictive capability with an AUC of 0.84 (95% CI: 0.76 - 0.92), sensitivity of 82.5%, and specificity of 80.2% at a cutoff value of 60.00 pg/mL.

3.6. Correlation analysis of inflammatory markers and interleukin levels

Table 7 provides the correlation matrix between inflammatory markers and interleukin levels. IL-6 showed a strong positive correlation with IL-18 (r = 0.65) and CRP (r = 0.56), suggesting that elevated IL-6 levels are associated with increased inflammatory responses. Similarly, IL-18 correlated positively with CRP (r = 0.62) and ESR (r = 0.58), indicating its central role in the inflammatory cascade in T1D COVID-19 patients.

3.7. Multivariate logistic regression for risk of comorbidities

The multivariate logistic regression model (Table 8) identified that higher IL-6 and IL-18 levels were signifi-

Table 5. Multivariate Cox regression for hospitalization and survival.

Variable	Hazard ratio (HR)	95% CI	p-value
IL-6 Level	1.12	1.04 - 1.21	0.02
IL-10 Level	1.08	1.01 - 1.16	0.04
IL-12A Level	0.92	0.85 - 0.98	0.03
IL-18 Level	1.15	1.07 - 1.24	0.01
Genotype			
Genotype IL-6 (GG)	1.25	1.05 - 1.48	0.01
Genotype IL-10 (GG)	1.18	1.02 - 1.36	0.03
Genotype IL-12A (GG)	0.95	0.80 - 1.12	0.18
Genotype IL-18 (TG)	1.30	1.12 - 1.51	<0.01
Age	1.05	1.02 - 1.08	<0.001
Comorbidity Count	1.10	1.04 - 1.17	<0.01

Table 6. ROC analysis for predicting hospitalization based on interleukin levels

Interleukin level	AUC	95% CI	Sensitivity (%)	Specificity (%)	Optimal cutoff value
IL-6	0.84	0.76 - 0.92	82.5	80.2	60.00 pg/mL
IL-10	0.78	0.70 - 0.86	75.3	72.9	10.50 pg/mL
IL-12A	0.72	0.64 - 0.80	70.0	68.4	6.00 pg/mL
IL-18	0.88	0.80 - 0.96	85.0	83.7	190.00 pg/mL

Table 7. Correlation matrix between inflammatory markers and interleukin levels.

Marker	IL-6	IL-10	IL-12A	IL-18	CRP	ESR
IL-6	1.00	0.42	0.37	0.65	0.56	0.40
IL-10	0.42	1.00	0.34	0.41	0.29	0.36
IL-12A	0.37	0.34	1.00	0.53	0.25	0.28
IL-18	0.65	0.41	0.53	1.00	0.62	0.58
CRP	0.56	0.29	0.25	0.62	1.00	0.44
ESR	0.40	0.36	0.28	0.58	0.44	1.00

cantly associated with an increased risk of comorbidities in T1D COVID-19 patients, with odds ratios (OR) of 1.15 ($p<0.01$) and 1.25 ($p<0.001$), respectively. IL-12A levels, conversely, showed a protective effect (OR = 0.89; p = 0.01). Genotypes were also relevant, with the IL-6 GG (OR = 1.20; p = 0.01) and IL-10 GG (OR = 1.10; p = 0.04) genotypes being associated with a higher comorbidity risk.

3.8. Feature importance for predicting hospitalization

Random forest and Elastic Net models in Table 9 reveal that IL-18 levels were the most important predictor of hospitalization (Random Forest Importance: 0.25, Elastic Net Coefficient: 0.20), followed by IL-6 levels. Age and comorbidity count were also significant but had less predictive power than IL-6 and IL-18.

3.9. Survival analysis by genotype

The Cox Proportional Hazards model in Table 10 indicated a significant increase in mortality risk for T1D patients with COVID-19 who carried the IL-6 GG genotype (HR = 1.30; p = 0.01) and IL-18 TG genotype (HR = 1.25; $p<0.01$). Genotypes for IL-10 (GG) also showed an increased mortality risk (HR = 1.15; p = 0.04). These findings underscore the influence of specific interleukin genotypes on mortality risk in T1D patients.

3.10. Hospitalization duration analysis by genotype

Table 11 shows that the IL-6 GG genotype was associated with longer hospitalization duration (HR = 0.82; p = 0.02), as was the IL-18 TG genotype (HR = 0.78; $p<0.01$). Other genotypes, such as IL-10 GG, showed a non-significant trend toward longer hospitalization duration.

3.11. Kaplan-Meier survival analysis

Kaplan-Meier analysis in Table 12 demonstrated that the IL-6 GG genotype group had a median survival time of 60 days with significantly lower survival probabilities at 30, 60, and 90 days (0.85, 0.65, and 0.45, respectively) compared to other genotypes (Log-Rank Test p-value = 0.02). Similar trends were observed for the IL-18 TG genotype, reinforcing the association between certain genotypes and poorer survival outcomes in T1D COVID-19 patients.

4. Discussion

This study provides significant insights into the role of interleukin polymorphisms and cytokine levels in predicting COVID-19 severity, hospitalization duration, and survival among patients with Type 1 diabetes (T1D). The findings reveal that elevated levels of pro-inflammatory cytokines, particularly IL-6 and IL-18, as well as specific interleukin genotypes, are strongly associated with worse outcomes in T1D patients hospitalized with COVID-19. These associations underscore the importance of genetic and inflammatory profiles in shaping disease progression, reinforcing the need for targeted interventions in high-risk populations.

4.1. Elevated cytokine levels and inflammatory response in T1D patients with COVID-19

Our analysis found that T1D patients with COVID-19 displayed markedly elevated IL-6, IL-10, IL-12A, and

Table 8. Multivariate logistic regression for risk of comorbidities.

Variable	Odds ratio (OR)	95% CI	p-value
IL-6 Level	1.15	1.06 - 1.25	<0.01
IL-10 Level	1.05	0.98 - 1.12	0.11
IL-12A Level	0.89	0.82 - 0.96	0.01
IL-18 Level	1.25	1.14 - 1.37	<0.001
Genotype IL-6 (GG)	1.20	1.05 - 1.38	0.01
Genotype IL-10 (GG)	1.10	1.02 - 1.20	0.04

Table 9. Feature importance in predicting hospitalization (Random forest and elastic net models).

Feature	Random forest (Importance)	Elastic net (Coefficient)
IL-6 Level	0.20	0.12
IL-10 Level	0.15	0.10
IL-12A Level	0.12	-0.08
IL-18 Level	0.25	0.20
Age	0.18	0.15
Comorbidity Count	0.10	0.08

Table 10. Survival analysis for genotype impact on COVID-19 outcomes using Cox proportional hazards model to assess the impact of genotypes on survival in type 1 diabetic patients with COVID-19.

Genotype	Hazard ratio (HR)	95% confidence interval	p-value	Interpretation
IL-6 (GG)	1.30	1.10 - 1.54	0.01	Significant increase in mortality risk for GG genotype
IL-6 (CG)	1.12	0.95 - 1.32	0.18	Not significant
IL-10 (GG)	1.15	1.01 - 1.31	0.04	Slightly increased mortality risk for GG genotype
IL-10 (TG)	1.02	0.89 - 1.17	0.76	Not significant
IL-12A (GG)	0.90	0.78 - 1.05	0.17	No significant difference in mortality risk
IL-12A (GA)	1.08	0.94 - 1.25	0.28	Not significant
IL-18 (TG)	1.25	1.08 - 1.45	<0.01	Significant increase in mortality risk for TG genotype
IL-18 (TT)	1.05	0.91 - 1.22	0.53	Not significant

Table 11. Cox proportional hazards model for hospitalization duration by genotype.

Genotype	Hazard ratio (HR)	95% Confidence interval	p-value	Interpretation
IL-6 (GG)	0.82	0.70 - 0.96	0.02	GG genotype associated with longer hospitalization (HR < 1)
IL-6 (CG)	0.95	0.80 - 1.13	0.52	Not significant
IL-10 (GG)	0.88	0.75 - 1.04	0.13	GG genotype tends to increase hospitalization length, but not significant
IL-10 (TG)	1.05	0.92 - 1.21	0.44	Not significant
IL-12A (GG)	1.10	0.95 - 1.27	0.22	No significant impact on hospitalization duration
IL-12A (GA)	1.12	0.98 - 1.28	0.10	Not significant
IL-18 (TG)	0.78	0.67 - 0.92	<0.01	TG genotype significantly associated with longer hospitalization
IL-18 (TT)	0.92	0.80 - 1.06	0.25	Not significant

Table 12. Kaplan-Meier survival analysis by genotype for type 1 diabetes patients with COVID-19 comorbidities.

Genotype	Median survival time (Days)	Survival probability at 30 days	Survival probability at 60 days	Survival probability at 90 days	Log-rank test p-value	Hazard ratio (HR)	95% CI for HR	Cumulative incidence at 30 days	Cumulative incidence at 60 days	Cumulative incidence at 90 days
GG	60	0.85	0.65	0.45	0.02	1.00 (reference)	-	0.15	0.35	0.55
GA	75	0.90	0.70	0.50	0.02	0.85	(0.65 - 1.10)	0.10	0.30	0.50
AA	80	0.92	0.72	0.55	0.02	0.78	(0.60 - 1.02)	0.08	0.28	0.45

IL-18 levels compared to controls (Table 2), suggesting a hyperinflammatory state characteristic of cytokine release syndrome (CRS). The increased IL-6 levels observed in T1D patients align with previous studies, indicating that IL-6 is a critical driver of COVID-19 severity and mortality [1, 26]. IL-6 facilitates a cascade of inflammatory responses, leading to acute-phase protein production, endothelial dysfunction, and coagulation activation—all of which are exacerbated in diabetic patients due to chronic low-grade inflammation [27]. The elevated IL-18 levels, similarly, highlight its role in amplifying the inflammatory response in severe COVID-19 cases [22]. The correlation matrix (Table 7) showing strong associations between IL-6, IL-18, and acute-phase markers such as CRP and ESR emphasizes the synergistic nature of cytokines and inflammation in promoting adverse outcomes. This finding aligns with other research showing that IL-6 and IL-18 are predictive of clinical deterioration in high-risk COVID-19 populations [5, 28].

4.2. Impact of interleukin genotypes on cytokine levels and disease outcomes

The study identifies specific genotypes in IL-10, IL-6, IL-12A, and IL-18 as influential factors in cytokine expression and COVID-19 outcomes. T1D patients with the IL-6 GG genotype exhibited higher IL-6 levels, which were associated with prolonged hospitalization and higher mortality risk (Table 10). This genotype has been implicated in previous research as a genetic marker for an exaggerated inflammatory response, potentially predisposing individuals to hyperinflammation during infections [6, 7]. The IL-18 TG genotype, likewise, was associated with significantly elevated IL-18 levels and worse clinical outcomes, including increased mortality and extended hospitalization (Table 11). These findings are consistent with studies that have linked IL-18 polymorphisms to inflammation-related diseases and adverse outcomes in viral infections [29]. Given the strong association between genotype and cytokine expression, genotyping could serve as a valuable tool for identifying patients at higher risk of severe COVID-19 complications.

4.3. Prognostic value of cytokine levels in predicting hospitalization and survival

The ROC analysis demonstrated that IL-18 and IL-6 levels are robust predictors of hospitalization and mortality in T1D COVID-19 patients, with AUCs of 0.88 and 0.84, respectively (Table 6). These findings align with prior studies indicating that elevated IL-6 and IL-18 levels are reliable markers of poor prognosis in COVID-19 [30]. The strong predictive capacity of these cytokines may stem from their role in perpetuating the inflammatory response and inducing tissue damage, particularly in individuals with T1D who are already predisposed to inflammation and endothelial dysfunction [12, 13]. The ability of IL-6 and IL-18 levels to distinguish between severe and non-severe cases underscores the potential for using these cytokines as part of a prognostic panel to guide clinical management and prioritize resources for high-risk patients.

4.4. Genetic variability and COVID-19 severity: Clinical implications

Our findings that specific interleukin genotypes influence cytokine levels and, by extension, COVID-19 outcomes have significant clinical implications. The IL-6 GG and IL-18 TG genotypes were associated with increased mortality risk and prolonged hospitalization duration, which suggests that genotyping could identify T1D patients likely to experience severe COVID-19 outcomes.

These genotypes could serve as predictive markers, allowing clinicians to implement more intensive monitoring and early intervention strategies for genetically predisposed individuals [14, 31]. This approach is particularly relevant in T1D patients, where genetic predispositions may exacerbate the risk of COVID-19 complications, as has been demonstrated in other studies linking cytokine gene polymorphisms to susceptibility and mortality in infectious diseases [15, 32].

4.5. Implications for targeted therapies and precision medicine

The association between elevated IL-6, and IL-18 levels, and adverse COVID-19 outcomes highlights the therapeutic potential of cytokine-targeting interventions in T1D patients with COVID-19. Anti-IL-6 therapies, such as tocilizumab, have shown promise in reducing inflammatory responses in severe COVID-19 cases [16, 33]. Given the pronounced IL-6 elevation in T1D COVID-19 patients, these therapies could mitigate hyperinflammation and improve clinical outcomes. Similarly, targeting IL-18 through emerging immunomodulatory agents could offer a therapeutic avenue for reducing the inflammatory burden in genetically susceptible individuals [34]. Such targeted therapies would align well with the principles of precision medicine, offering tailored interventions based on each patient's genetic and inflammatory profile.

In our study, we found a significant association between interleukin polymorphisms and COVID-19 severity in patients with Type 1 diabetes. The elevated levels of cytokines such as IL-6 and IL-18 in COVID-19 patients align with previous research indicating that inflammatory markers play a crucial role in the pathophysiology of severe COVID-19 cases. For instance, Albeizanee et al. (2025) emphasize the importance of the antioxidant glutathione system components during COVID-19 infection, suggesting that oxidative stress may exacerbate inflammatory responses, thereby influencing disease severity in diabetic patients[35].

Moreover, the findings from Nalaini et al. (2023) [36] regarding brain complications in COVID-19 patients underscore the systemic effects of cytokine storms, which are characterized by elevated levels of pro-inflammatory cytokines like IL-6 and IL-10. This supports our observation that specific interleukin genotypes correlate with increased hospitalization duration and mortality risk[2]. The neuroinflammatory processes highlighted in their review may also contribute to the overall severity of COVID-19 in T1D patients, suggesting a multifaceted interaction between metabolic and inflammatory pathways.

Additionally, Malik and Dhuldhaj (2023) provide an overview of available drug therapies for COVID-19 and their side effects, which is particularly relevant given that our study identified IL-6 and IL-18 as strong predictors of hospitalization. The therapeutic implications of targeting these cytokines could be pivotal in managing severe cases among T1D patients[37]. Understanding the interplay between genetic predispositions and inflammatory responses can guide future therapeutic strategies aimed at mitigating severe outcomes in this vulnerable population.

In summary, our findings reinforce the potential role of interleukin polymorphisms as biomarkers for assessing COVID-19 severity in Type 1 diabetes patients, while also highlighting the need for further research into targeted therapies that address both metabolic and inflammatory aspects of this disease.

4.6. Limitations and future research

While this study presents strong associations between interleukin polymorphisms, cytokine levels, and COVID-19 outcomes, it has some limitations. First, the study focused exclusively on female T1D patients, limiting the generalizability of findings to other demographic groups. Additionally, while we identified significant relationships between genotype and cytokine expression, the underlying mechanisms linking genetic variation to cytokine dysregulation in COVID-19 remain unclear. Future research could expand on these findings by exploring the mechanistic pathways through which interleukin polymorphisms influence COVID-19 progression. Longitudinal studies would also be valuable to assess the long-term effects of COVID-19 in T1D patients, particularly regarding how genetic and cytokine factors impact recovery and post-acute sequelae of SARS-CoV-2 infection.

5. Conclusion

This study underscores the critical role of interleukin polymorphisms and cytokine levels in determining COVID-19 severity, hospitalization, and mortality in T1D patients. Elevated IL-6 and IL-18 levels, along with specific genotypes such as IL-6 GG and IL-18 TG, were strongly associated with adverse outcomes, emphasizing the need for genetic and cytokine-based risk stratification. These findings support the integration of cytokine profiling and genotyping into COVID-19 management protocols for high-risk populations, facilitating personalized treatment approaches that could ultimately improve outcomes in T1D patients with COVID-19. By bridging genetic predispositions with cytokine expression, this study contributes to the growing body of evidence advocating for precision medicine in the fight against COVID-19.

Acknowledgments

Thanks, and gratitude to the Ministry of Health for providing the capabilities to collect samples and helping us with the equipment in this work.

Authors' contributions

M. ABDULLAH and F. ALHADDAD prepared the article draft. C. PAMBUK and S. MARGHALI edited the current manuscript.

Conflicts of interest

The authors announce that they have no conflicts of interest.

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