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RESEARCH ARTICLE

Viral Co-Infections and Their Influence on IL-35 in Adult Type 1 Diabetes Mellitus

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Abstract

Background: Type 1 diabetes mellitus (T1DM) is an autoimmune disorder characterized by the destruction of insulin-producing beta cells in the pancreas, causing severe insulin deficiency and chronic hyperglycemia. Interleukin 35 (IL-35), a regulatory cytokine primarily produced by regulatory T cells, plays a crucial role in maintaining immune tolerance. Emerging evidence from several studies suggests that exposure to viral infections may trigger or exacerbate autoimmune processes associated with T1DM.

Objective: This study aimed to investigate the effect of viral co-infections on IL-35 levels in adult T1DM patients.

Methods: The study included 120 patients with T1DM, aged 17–58 years, and a control group of 90 age-matched healthy individuals. The ELISA technique was employed to assess the IL-35 and islet cell antibody (ICA) levels, while diagnostic tests were performed to identify different types of viral infections.

Results: There was a significant decrease in IL-35 levels in T1DM patients (145.26 ± 45.26 pg/mL) compared to healthy controls (312.37 ± 86.27 pg/mL). Viral infections were associated with differences in IL-35 levels. The results also revealed a positive correlation between IL-35 and ICA, while the correlation was negative with disease duration.

Conclusion: Our results showed that IL-35 levels were significantly decreased in adult T1DM patients. Additionally, viral infection was found to affect increasing IL-35 levels in T1DM patients, although these levels remained lower than those in the control group. The role of viral infection in modulating IL-35 levels warrants further investigation and underscores the need for additional research to investigate which viruses mostly affect IL-35 levels in diabetic patients and the potential mechanisms by which viral pathogens may influence the development of autoimmune diabetes.

1. Introduction

Type 1 diabetes mellitus (T1DM) is an autoimmune disorder in which the immune system mistakenly attacks and destroys the insulin-producing beta cells in the pancreas, causing severe insulin deficiency and, consequently, high blood glucose levels, which require lifelong insulin treatment [1]. The exact causes of T1DM are not fully understood; however, several studies have suggested a possible link between viral infections and T1DM by comparing the frequency of viral infections in patients and healthy individuals. Epidemiological research and meta-analyses have shown that enterovirus infections are the most common cause of this disease [2]. Previous studies have shown that some viruses target pancreatic beta cells, while others stimulate autoimmunity specific to these cells, both of which lead to the death of beta cells as a result of the pathological cellular events resulting from these infections, as well as contribute to the production of various and excessive inflammatory cytokines during viral infections [3]. Moreover, regulatory cytokines play a role in modulating the immune response and maintaining

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homeostasis, and any disruption of this regulation can lead to the breakdown of immune tolerance and the onset of autoimmune processes [4].

Interleukin 35 (IL-35) is a regulatory cytokine produced primarily by the regulatory T cells, which suppresses excessive or misdirected immune responses. Studies have indicated its critical role in protecting insulin-producing pancreatic beta cells from autoimmune attack [5]. Some studies have indicated that viral infections weaken this protective mechanism, making beta cells more susceptible to destruction by the immune system [6]. Therefore, this study aimed to investigate the role of different viral infections in modifying the concentration of IL-35 and its potential impact on T1DM.

2. Methods

The study population comprised 120 T1DM patients (17–58 years) recruited from the Endocrinology and Diabetes Center in Baghdad, Iraq. Among those diagnosed by the specialists were those who required insulin treatment since the moment of diagnosis. The diagnosis was confirmed by fasting blood sugar test (mg/dL) using Biorex Diagnostics kit (UK), glycated hemoglobin (HbA1c) test using A1c-3 cobas® c111 (Roche, Germany), and ICA (pg/mL) using the ELISA technique (SunLong Biotech, China) with assay range 2.5–80 pg/mL and sensitivity 0.5 pg/mL, after excluding the patients with type 2 diabetes mellitus, other autoimmune diseases, infectious diseases, and users of drugs that may affect the immune status.

The healthy control (HC) group was selected in parallel with the patient group based on age. The group consisted of 90 healthy individuals (17–48 years) free of any known diseases and not using any medication that could affect their immune status. To confirm their health status, they underwent erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) tests to exclude samples with results above the normal range.

The incidence of viral infection was detected for cytomegalovirus, herpes simplex 1 and 2, hepatitis B, hepatitis C, rubella, and human immunodeficiency viruses using immunochromatography technique (+/−) (Biozek, The Netherlands), while Varicella zoster virus (negative control ≤ 0.10 and CUT OFF = negative control + 0.15) and the levels of IL-35 (assay range 16–900 pg/mL, sensitivity 4.5 pg/mL) were measured using the ELISA technique (SunLong Biotech, China). Samples with borderline results were excluded.

The data were analyzed using SPSS v. 20 software to compare study parameters in the study groups. The Kolmogorov–Smirnov Test of Normality was conducted, and all data were expressed as a mean ± SE. The T-test compared the two main groups (control and patient), while the ANOVA test was used to compare the three subgroups, namely, non-infected, single infection, and co-infection. Finally, Pearson's correlation coefficient was calculated to assess the strength and direction of the correlation between the factors.

3. Results

The distribution of the study samples was tested using the Kolmogorov–Smirnov Test of Normality. The K–S test statistic (D) was 0.14512, and the *P* value was 0.14459, which indicated normal distribution of the samples.

This study examined several health and metabolic parameters in T1DM patients and HC groups. Table 1 shows the significant differences in all parameters between the two groups ($P < 0.05$).

Diagnostic kits were used in this study to confirm and identify the presence of the following viruses: cytomegalovirus, rubella, hepatitis C, hepatitis B, HIV, varicella zoster, and herpes simplex 1 and 2. Based on the results of the diagnostic tests, study participants were classified into three categories: noninfected, single-infected, and coinfected with multiple viruses. Figure 1 shows the percentage in the main groups (T1DM patients and controls) based on the viral infection status. The noninfected and single viral infection subgroups with any of the studied viruses were significantly lower ([n = 32]26.6% and [n = 31]25.8%,

Table (1): The characteristic features of the T1DM and healthy control groups.

Parameters	Groups	N	Mean ± SE	*P*	T-test
Age	T1DM	120	28.47 ± 1.34	0.053	1.631
	HC	90	27.51 ± 0.63		
BMI	T1DM	120	23.25 ± 0.39	0.042*	1.744*
	HC	90	25.85 ± 0.43		
FBS	T1DM	120	246.18 ± 8.07	>0.001**	17.56**
	HC	90	98.33 ± 1.07		
HbA1c	T1DM	120	10.12 ± 0.18	>0.001**	24.76**
	HC	90	5.2 ± 0.03		
ICA	T1DM	120	16.25 ± 1.84	>0.001**	5.6**
	HC	90	0.94 ± 0.05		

BMI, Body mass index; FBS, Fasting blood sugar; HbA1c, Glycated hemoglobin; HC, Healthy control; ICA, Islet cell antibody; T1DM, Type 1 diabetes mellitus.

respectively) in the patient group than in the control group ([n = 36]40% and [n = 31]34.4%, respectively), while the coinfection was substantially higher in the T1DM group ([n = 57]47.5%) than the control group ([n = 23]25.5%).

This study compared IL-35 levels (mean ± SE pg/mL) between the T1DM and HC groups and found highly significant differences ($P < 0.001$) between the two. The effect of viral infection (noninfected, single-infected, and coinfection subgroups) on IL-35 levels in the T1DM group was examined and was found to be statistically significant ($P = 0.011$), while the HC group showed $P = 0.7$ (Table 2 and Figure 2).

Figure 2 shows the levels of IL-35 in both study groups (T1DM and HC) and their levels in the subgroups under the two main groups. The keys on the right indicate the numbers of each group.

Pearson's correlation coefficient was calculated to assess the strength and direction of the correlation between the factors (duration of disease, ICA, and IL-35). The results indicated a moderate positive correlation with statistical significance between IL-35 and ICA (R = 0.58; $P > 0.001$). In contrast, the correlations between the duration of the disease and both IL-35 and ICA were negative (r = −0.69 and −0.57, respectively) with high statistical significance (Figure 3).

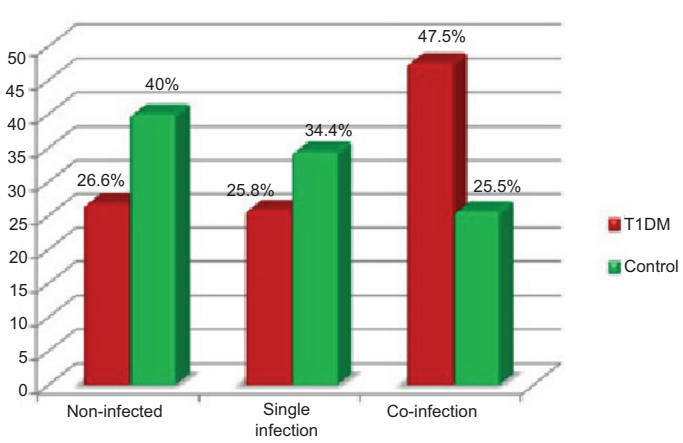

Figure (1): The viral infection status in the study groups.

Discussion

The inability to regulate blood sugar levels leads to high HbA1c due to high levels of antibodies that destroy insulin-producing beta cells, resulting in a low body mass index (BMI) [7].

The significant age difference between the two groups was purely a coincidence, due to the strict controls for

Table (2): The levels of IL-35 in the T1DM and control groups, compared with IL-35, based on the viral infection status.

Groups	N	Viral infections						*P*	
		Non-infected		Single infection		Co-infection			
		IL-35 Mean ± SE	N	IL-35 Mean ± SE	N	IL-35 Mean ± SE	n		
T1D	120	117.34 ± 28.4	32	161.03 ± 44.7	31	153.31 ± 47.27	57	0.011*	>0.001**
		145.26 ± 45.26							
HC	90	311.98 ± 84.19	36	326.64 ± 92.74	31	293.75 ± 85.85	23	0.702 NS	
		312.37 ± 86.27							

HC, Healthy control; IL-35, Interleukin 35; NS, Not significant; SE, Standard error; T1D, Type 1 diabetes.

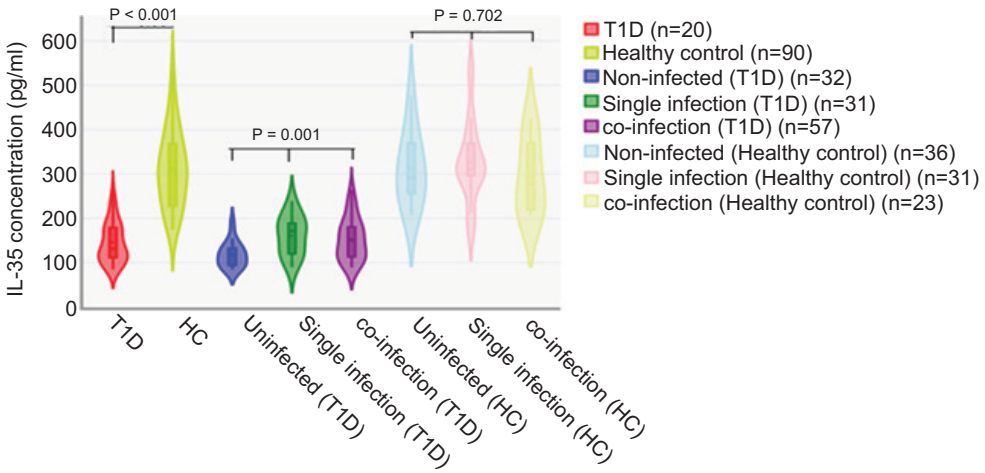

Figure (2): The levels of IL-35 and effects of viral infection in the studied groups.

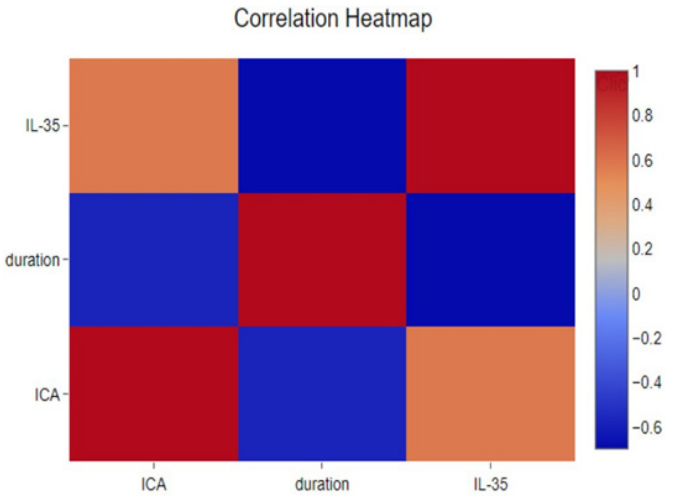

Figure (3): Pearson's correlation between ICA, IL-35, and T1DM duration.

selecting the individuals in the control group in terms of not having any disease. In addition, managing the disease in diabetic patients through treatment and healthcare led to an increased lifespan [8].

The levels of IL-35 were significantly lower in the T1DM group compared to the HC group, and these findings were consistent with those of the previous studies [9,10]. The low levels of IL-35 play a crucial role in mediating T1DM by increasing the differentiation of effector T cells and Th17 cells, with scarce regulatory T cells that are crucial in maintaining self-tolerance in autoimmune diseases.

T1DM occurs as a result of the gradual immune-mediated deterioration of the β-cells, triggered by Th1 cells and differentiation into subtypes such as Th2, Th17, Th9, Th22, follicular Th, and Treg cells, causing obvious hyperglycemia [11]. Due to metabolic and inflammatory environmental factors, there will be direct and indirect effects on IL-35 production. The additional reasons that lead to decreased IL-35 levels include dysfunction and the number of regulatory T cells (the main producers of IL-35). This dysfunction and destruction may be the result of an autoimmune attack on these cells that produce IL-35 in addition to the destruction of the beta cells [12]. The autoimmune response may also disrupt the regulation of signaling pathways in the context of T1DM, causing changes in the expression of many transcription factors, cytokines, or other regulatory molecules that control the production of IL-35 [13]. The further decrease in IL-35 in T1DM ultimately leads to the collapse of the immune tolerance and the development of an immune response against pancreatic beta cells [14].

Several studies have indicated the role of different viruses in the development of T1DM [3,15]. This may be due to molecular mimicry, differences in the inflammatory response, or modulation of the immune response, resulting from different viral infections, which contribute to the induction of the autoimmune response and cause the termination of self-tolerance [15,16]. On the other hand, these results indicate that T1DM patients are more susceptible to viral infections due to hyperglycemia [17].

As for IL-35 level, the present study found a significant difference between the three subgroups (noninfected, single infection, and co-infection subgroups) of T1DM patients. This may be due to various factors such as the individual's immune status and the type, and duration and severity of the viral infection. From Table 2, IL-35 is generally lower than in the control group. The increase in IL-35 levels observed in subgroups with single and coinfection may be the result of the immune response to viral components such as viral nucleic acids and its proteins [18,19], leading to a pro- or anti-inflammatory response that directly or indirectly stimulates the production of IL-35 by regulatory T cells in an attempt by the immune system to suppress the immune response and limit the damage caused by the viral infection [20].

The ICA has a positive correlation with IL-35, and these two markers have a negative correlation with disease duration due to the progressive destruction of beta cells leading to a decline in ICA and IL-35 production (as a compensatory or regulatory response) [10,21]. The ICA is a crucial marker for predicting and diagnosing T1DM, especially the onset of the disease (especially in children), and its high levels indicate the activity of the autoimmune response and beta cell destruction [22]. Several studies have shown that ICA levels decrease with increasing T1DM duration [22,23] due to the exhaustion of the immune response and the lack of antigenic targets as a result of the destruction of beta cells (reduced antigenic stimulus) [24]. Our results differ from other studies that use children as a study sample regarding IL-35 and the nature of the correlations between the parameters, and this can be attributed to the difference in the immune status between children and adults [22,25].

Conclusion

The study concludes that (1) IL-35 levels decrease in adult patients with T1DM compared to healthy subjects, and (2) viral infection has an effect on increasing levels of IL-35 in T1DM patients, while IL-35 levels remained lower than those in the control group. The role of viral infection in modulating IL-35 levels warrants further investigation and emphasizes the need for additional research to determine which viruses affect IL-35 levels the most in diabetic patients and the potential mechanisms by which viral pathogens may influence the development of autoimmune diabetes.

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Conflicts of interest

No Conflicts of interest.

Compliance with ethical standards

In vitro study. The study was conducted in accordance with research ethics and based on the principles of the Declaration of Helsinki for human research, as reviewed by the Ethics Committee of the College of Science, Department of Biology, Al-Mustansiriyah University Ref.:1221/00016M.

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Authors' Contributions

Ali Fakhir Hashim (Methodology, Investigation, Writing, and Funding Acquisition). **Alia Essam Mahmood ALubadi** (Conceptualization, Reviewing and Editing, and Supervision).

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