European Journal of Clinical Pharmacy

Print ISSN: 2385-409X **Online ISSN**: 3105-0409

Website: https://farmclin.com/

Original Research Article

Association of IL2Ra DNA Methylation and Epstein-Barr virus infection in type 1 Diabetes Mellitus patients

Article History:

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Received: 23-01-2026

Revised: 30-01-2026

Accepted: 24-02-2026

Published: 26-02-2026

Abstract: ***Background:*** Type 1 diabetes mellitus (T1DM) results from autoimmune damage of pancreatic β-cells, with autoreactive T lymphocytes playing a crucial role, and is characterized by the presence of islet autoantibodies. Subsequent hyperglycemia necessitates perpetual insulin replacement treatment. About fifty percent of the risk factors for T1DM are ascribed to hereditary predisposition, whereas other reasons are associated with epigenetic alterations and environmental influences. ***Objectives***: To evaluate the correlation between CPG methylation change of IL2Ra promoter and EBV infection on the risk of type 1 diabetes. ***Materials and methods***: thirty individuals suffering from type 1 diabetes mellitus who were hospitalized between December 2024 and March 2025 and eighteen healthy people served as the control group in this case-control study. Blood samples were taken and divided into two parts one for gel tubes, and the sera were split used for viral DNA extraction and quantitative polymerase chain reaction (QPCR) for determining the viral the desired level, and the other part was used for EDTA tube for human DNA extraction to estimate dna methylation test ***Results:*** The mean percentage of ILRa dna methylation considerably greater within the illness group when compared to the group of control subjects who were healthy .and the PCR analysis showed that 11 (26.79%) of the individuals with T1D had EBV DNA, while none of the controls had it (P<0.001). ***Conclusion*** dna methylation of IL-Ra as epigenetic mechanism contribute to genetic trigger , while EBV infection, an environmental triggers to the pathophysiology of T1DM.

Keywords: Type 1 diabetic mellitus; EBV , ILRa; DNA Methylation;.Epigenitic

INTRODUCTION

T1D is a multifactorial autoimmune disease that is characterized by the activation of T-cells mediate immunity against the insulin-producing islet cells, resulting in a significant reduction in the mass and function of the islet cells. thereby dysregulating circulating glucose level homeostasis., The disease presents clinically when 90% of the β-cells have been destroyed therefore there is no insulin in the body ,Antibodies against insulin, islet cells, glutamic acid decarboxylase, insulinoma-associated protein-2, zinc transporter-8, and other proteins are hallmarks of the autoimmune process(Kueh et al., 2024) Type 1 Diabetes (T1D), also referred to as juvenile diabetes or insulin-dependent diabetes, constitutes the second most common type of diabetes, representing about 5–10% of all diagnosed cases. Annually, over 65,000 new cases of T1D in children are reported globally, with a prevalence rate increasing by approximately 3% each year(Kandemir et al., 2024) Diabetes

mellitus type 1 (T1DM) is more common in Northern Europe and North America and less common in Asia. Progress in insulin treatment and diabetes control has led to a decline in mortality rates, although The risk of premature death is still higher for people with type 1 diabetes compared to the whole population (Sun et al., 2022).

Children, teenagers, and young adults are the most typically diagnosed with this kind of diabetes. T1DM requires lifelong insulin therapy to manage their illness. Patients may develop severe hyperglycemia and, ultimately, diabetic ketoacidosis, which can be life-threatening.(Ramos et al., 2023). (Van Belle et al., 2022)there are many different immune cells that are involved in the disease. Regarding this particular matter, T cells are of particular relevance. In fact, the death of β-cells is predominantly driven by cytotoxic CD8+ T cells, with assistance from CD4+ T helper cells providing support. (Šabanović, 2023)Antigen-presenting cells (APCs) comprise dendritic cells (DCs), macrophages located in the pancreatic islets. Self-antigens are presented to naive T cells by MHC molecules, facilitating the priming and expansion of pathogenic T cells and the generation of autoreactive CD4+ T cells. mature CD4+ T cells subsequently produce cytokines, which in turn activate beta-cell-specific cytotoxic CD8+ T cells,The activated T cells migrate to pancreatic islets through vascularization, stimulating macrophages and additional T cells, which contributes to the destruction of islet β-cells(Mauvais, 2025)(Hilliard et al., 2025)

The primary causes of Type 1 Diabetes Mellitus (T1DM) include genetic predisposition, with 50% of risk factors being hereditary, while the remainder is attributed to environmental influences and epigenetic alterations. Environmental influences can affect these alterations, interacting with genetic polymorphisms to determine illness progression(J. Zhang et al., 2023). Viral infections have been identified as probable environmental causes of T1DM. Animal studies suggest that infections can cause islet autoimmunity through many routes(Oliveira et al., 2023) In recent years, scientists have focused on the Epstein-Barr virus (EBV) as a probable cause of autoimmune disorders(França et al., 2022).

Epstein-Barr virus (EBV), a member of the Herpesviridae family and the Gammaherpesvirinae subfamily, is also known as human herpesvirus type 4 (HHV-4). The encapsulated double-stranded DNA virus known as EBV is composed of around 180 thousand base pairs, has a diameter of 150–200 nm, and translates for 80–100 proteins.(Huang et al., 2023) The equilibrium between viral replication and host immune responses is maintained by EBV's lifelong persistence in the memory B cells of the infected host after initial infection, accompanied by occasional reactivations in saliva that lack clinical significance(Torne & Robertson, 2024) The Epstein-Barr virus induces autoimmune diseases as environmental triggers via multiple mechanisms. Initially, immune regulatory proteins associated with immune evasion may be produced by cells infected with EBV.Secondly, a range of cytokines and inflammatory mediators may be generated in response to EBV. By activating the TLR3 signalling system, virus-encoded EBER can elicit a substantial release of proinflammatory factor(Silva et al., 2024)(Guojiang, 2023)

Epigenetic regulations denote alterations in gene expression that do not entail nucleotide changes, yet may be inheritable. The primary epigenetic mechanisms encompass DNA methylation, post-translational changes of histones, and RNA-mediated gene silencing(Lossi et al., 2024)

Studies demonstrate that epigenetic modifications significantly contribute to the pathogenesis of type 1 diabetes (T1D). such as DNA methylation, might alter gene transcription and translation, potentially leading to the development of autoimmune diabetes(Of & Lactate, 2024), including the destruction of pancreatic beta cells. Additionally, these modifications may serve as potential biomarkers for the early detection and progression prediction of T1DM(DeLorenzo & Powder, 2024).

.DNA methylation primarily occurs in CpG dinucleotides and involves the attachment of a methyl group to the fifth carbon of cytosine, resulting in 5-methylcytosine (Mattei et al., 2023). Both strands of the DNA include this palindromic CpG sequence, which is formed when the S-adenosyl methionine donor (SAM) transfers its methyl group to the DNA. Enzymes known as DNA methyltransferases (DNMTs) are responsible for this transfer(Gresh et al., 2024) The majority of CpG dinucleotides in the mammalian genome are methylated. The impact of DNA methylation is contingent upon its location within genes; however, it is crucial for the modulation of their expression in both gene regulatory regions and gene bodies(Wilcox et al., 2025). Defective DNA methylation of genes associated with beta cell formation, maturation, differentiation, and insulin manufacturing may predispose individuals to Type 1 Diabetes Mellitus (T1DM).Additionally, certain environmental triggers can promote overexpression of particular cytokines in the islets, leading in hyper -methylation of DNMTs and INS gene and inhibiting insulin transcription(Maccalman et al., 2024)

This review will illustrate the impact of DNA methylation on the the IL2RA promoter region between T1DM patients and healthy controls, and how these variations may affect the risk and progression of T1DM(Pahkuri et al., 2023)

IL2RA (interleukin-2 receptor alpha chain, CD25), sometimes referred to as CD25, is situated on chromosome 10p15. constitutes a component of the high-affinity IL-2 receptor complex. IL2RA is constitutively expressed on regulatory T cells, a subset of T cells with a significant capacity to inhibit autoreactive T cells(Lokau et al., 2024)

The α chain of the IL-2 receptor complex binds to IL-2, a crucial factor in the proliferation of T regulatory cells, with great affinity. The expansion of CD4+ FOXP3+ Tregs dependent on IL-2/IL-2RA is essential for sustaining immunological homeostasis. In Type 1 Diabetes Mellitus (T1DM), the impairment of CD4+ FOXP3+ regulatory T cells (Tregs) mostly results from inadequate induction and maintenance of these cells, along with impairments in IL-2/IL-2RA signalling(Kyrgios et al., 2023) Six positive regulatory regions (PRR) and two negative regulatory elements (NRE) situated between 29 kb and +3.6 kb from the transcriptional start site (TSS) are involved in the control of IL2RA production in response to stimuli Every one of these locations has multiple CpGs that are known to change gene expression through modifying transcriptional protein binding or permitting methyl-CpG binding domain protein binding. Evidence suggests that variations in DNA methylation play a significant role in the subset-specific transcription of cytokine genes in T cells(Cerna, 2023) For these reasons prior research demonstrated the DNA methylation status of six CpGs situated in the proximal promoter of IL2RA in T1D patients, alongside the genetic variations contained inside the adjacent 180 kb region of chromosome 10p15.1(Yahaya et al., 2024).

MATERIALS AND METHODS

Case and control study designed included type 1 diabetic mellitus admitted to center for endocrinology and diabetes Babylon province from December 2024 to March 2025 , Patients' specimens whole blood were collected from two groups: 26 female patients and 24 male patients..

The patient's age range between (2 -35) years old who were diagnosed by physician according to inclusion criteria that were done throw several clinical and laboratory investigation The data were gathered from their personal records and direct patient-questionnaire, which included information such as patient number, family medical history and the treatment they're undergoing, the studya lso involved Control groups specimens were collected from 18 people who were healthy.

Ethical approval:
in our study All samples of the will be accepted after obtaining verbal consent from the Ethics Committees of Babylon health office unit in Babylon province. in addition this research must be authorised by Babylon University's medical college's ethical research committee and the Babylon Health Directory under reference number (No: 9786 in 2024).

patients and control groups samples involved whole blood specimens were collected in gel and clot activator vacuum tubes with collected blood were centrifuged to separate serum at supernatant, which were transferred into Eppendorf tubes 1.5 ml and stored in a deep freezer at -20 °C to be used in ELISA technique and for molecular analysis.

Molecular analysis of EBV :
Serum sample was used to extract the viral genome for each group by using DNA genomic extraction kit (Geneaid /Taiwan) Cat..NO.GB100 according to instruction protocol within the Kit.

Detection of Epstein Barr virus by real time (Probe qPCR):
This kit uses TaqMan probe real-time fluorescent PCR technology with particular primers that were developed for a highly conserved area of the EBV genome. It amplified the DNA by using fluorescent PCR technology. The PCR master mix had 5 µl of isolated DNA in it. The EBV reagent fluid had 19.5 µl of primers and the EBV reaction enzyme had 0.5 µl. All of these chemicals, along with the other ones needed for the PCR reaction, were put into PCR tubes. These included stabiliser, dNTPs, reaction buffer (1x) with 1.5 mM MgCl2, and top DNA polymerase (1U). The ultimate volume of each reaction tube was 20 µL. The fluorescent technology signal of real-time PCR was used to directly analyse the setting of the baseline and cycle threshold. A positive result was found when the CT value of the detection FAM channel was about ≤35 and the curve of reaction showed exponential growth negative result was found when there were no CT PCR

RESULTS

thermo cycle conditions were done as shown in table (1).

step	Cycle numbers	temperature	time	Fluorescent signal
1	2 cycle	95c	2minute	no
2	40 cycle	94c	18sec	no
		55c	40sec	yes

Extraction of genomic DNA and bisulfite conversion:

One expert approach for extracting genomic human DNA from whole blood is the (Geneaid kit/Taiwan). It takes about 300 µL of blood and produces about 4-6 ng of highly pure genomic material. In this technique, DNA is bound to silica in a spin column, and then a succession of washing buffers are used to remove impurities,10 µl of DNA from each sample was utilised for Bisulfite modification following the technique outlined by the Methylamp™ One Step DNA Modification Kit (Epigentek, USA). This kit transforms all unmethylated cytosines into uracil, while leaving methylated cytosines unaltered..

Methylation Sensitive-High Resolution Melting (MS-HRM)

The current study used the MS-HRM assay with methylation-independent primers (MIP) show in table 2 to evaluate the methylation of the IL2RA gene ,This primer encodes promoter region and its designed according to (Pinzón-Reyes, 2020)) and it's manufactured by Macrogen company in Korea. The MS-HRM analyses were performed using a Stratagene MX3005 Real-Time PCR detection system (Agilent, USA) PCR was performed in a final volume of 20µl, which consist from: 4 µl HOT FIREPol® EvaGreen® HRM Mix kit (Solis BioDyne, Estonia), 1µl of Forward and reverse primers, 5 µl modified DNA and 10 µl H_2O PCR grade.To generate a standard curves, fully methylated and unmethylated human control DNA (EpiTect® methylated and unmethylated human control DNA kit) (Qiagen,Italy) were mixed in different proportions to obtain the following ratios of methylation: 0%, 10%, 25%, 50%, 75%, 100%

Table 2 primer sequences of IL-2Ra gene promoter

Primer sequences of IL-2Ra gene promoter	
Forward sequences	**-5`-TGTGGGCTGGGGTTGATGAG- 3`**
Reverse sequences	**- 5` - ATATTGGAGGCTGCCTGACC- 3`**

Table 3 Recommended qPCR cycling protocol:

step	**Cycle number**	**Temperature**	**Time**
1 Initial activation	1	95c◦	12min
Denaturation	40	95c◦	20sec
Annealing		60c◦	30sec
Extension		72c◦	30sec
HRM	1	95c◦	1min
		70 c◦	30sec

continuous acquisition to 95°C at one acquisition per 0.2°C

Statistical analysis

We used statistical tools like SPSS 23 (IBM, Chicago, USA) to collect and analyse the data. & Microsoft Office Excel 2022We did a Kolmogorov-Smirnov normality test after figuring out the mean and standard deviation to solve a statistical problem. The independent sample t-test is a typical way to find the mean difference between two groups, but it only works if the variable follows a normal distribution. The ANOVA test only goes one way. It is often used to find the difference in means between groups, as long as the variable follows a normal distribution. Percent methylation of each test sample was determined by interpolation of the data generated from the polynomial linear regression analysis of the standard curve from each gene

RESULTS

Detection of EBV virus by Real time qPCR

EBV genome were done depended on results of Real time qPCR are shown in table (4).The results was positive 11/30 (36.0%) of T1DM patients have EBV virus genome, while all healthy
control don't have the EBV genome, and there was a highly significant difference (p>0.05)

Table 4: Real-time PCR results

	DM Patients *n* = 30	Healthy control *n* = 18	*P*
Real time qPCR			
Positive, *n* (%)	11 (36.0%)	0	0.002 ¥ S
Negative, *n* (%)	39 (64.0%)	18 (100.0%),	

n: number of incidents; S: significant at P>0.05; ¥: Chi-square test.

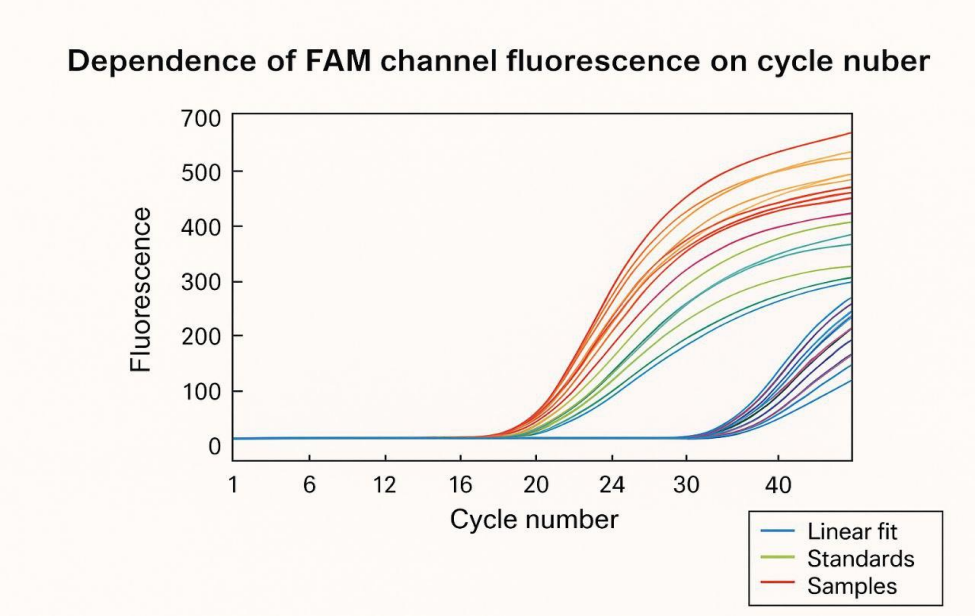


figure (1) Detection of Epstein Barr virus by RT-Qpcr

Estimation the methylation rate of IL-2Ra gene promoter region:

The comparison of methylation rate of IL-Ra gene among group studies (T1D group with EBV negative , T1D group with positive EBV, and healthy group) according to diagnosis of EBV virus and methylation analysis has been carried out and the results were demonstrated in table (5), the present results show the mean levels of methylation rate were (82.73 ,65.18 ,40.57) respectively , higher in both T1DM whom positive EBV patient and T1dm whom negative EBV in compared to healthy control and the difference was highly significant (P < 0.001)

Table (5): General comparison of methylation rate of IL2Ragene among three studied groups

Gene	Group	N	Methylation level %		P value
			Mean	SD	
IL2Ra	**EBV+ve**	11	82.73	7.66	**a. <0.001*** **b. <0.001*** **c. <0.001***
	T1D	19	65.18	3.78	
	Control	18	40.57	10.94	

a: comparison means of all three studied groups, b: comparison means between control and **EBV+ve**group, c: comparison means between control and **T1D**. Anova test used for comparison all three studied groups, while Dunnett's test used for statistical analysis against control group.

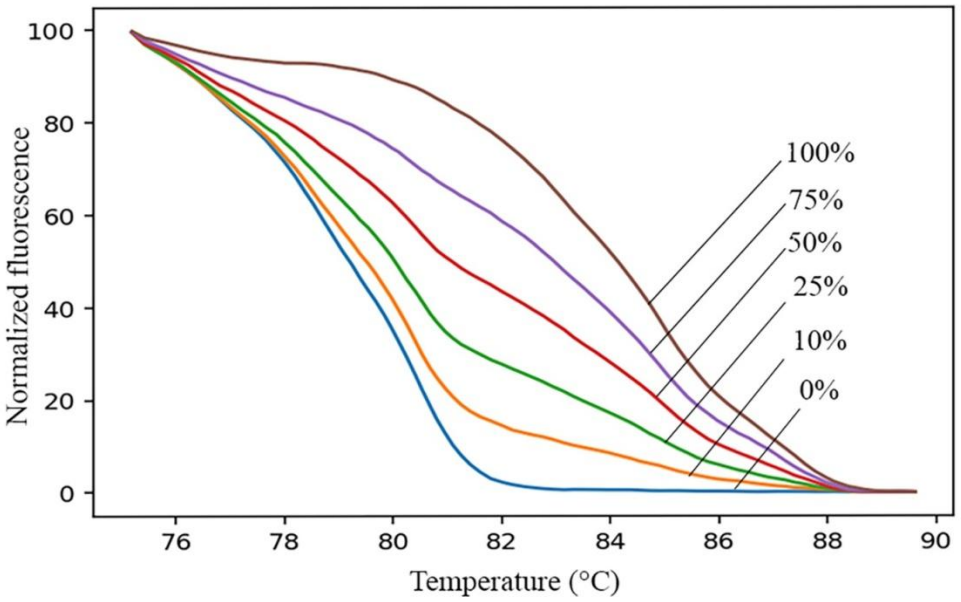


Fig. (2): MS-HRM standard curve to detect the methylation level of the IL2Ra gene promoter. Standard curves are shown, from top to bottom: 100%, 75%, 50%, 25%, 10%, and 0% of standard methylated DNA

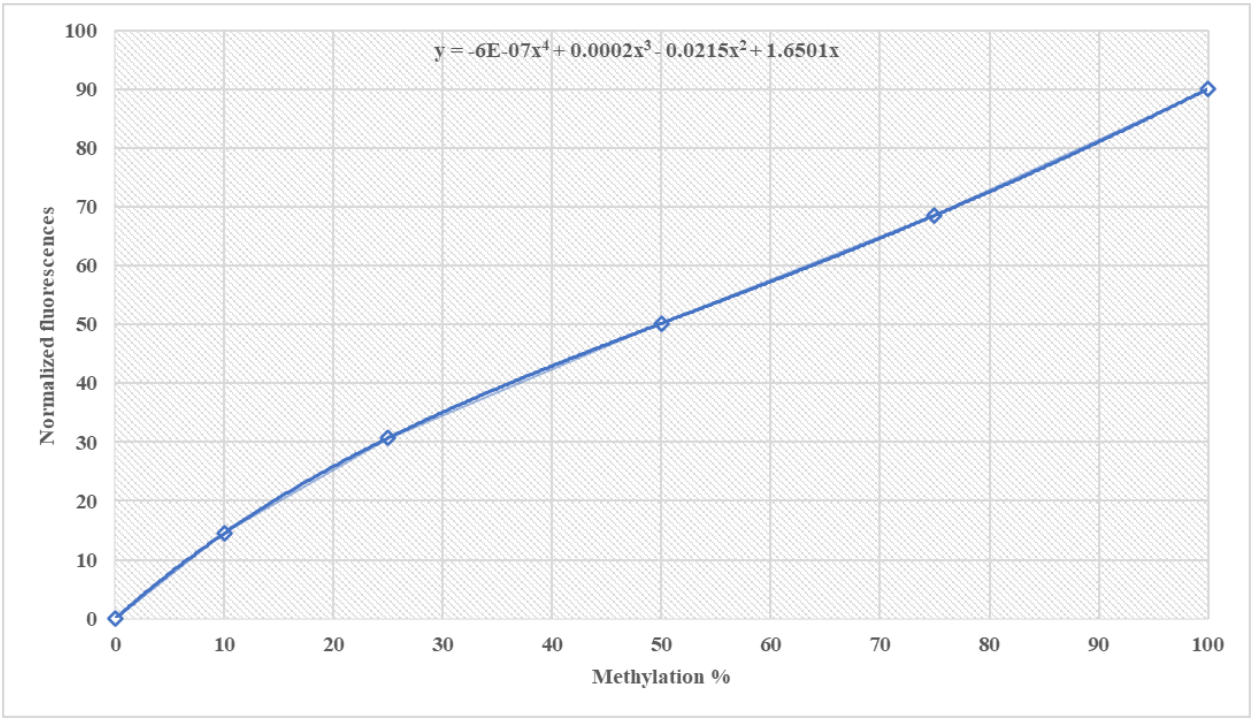
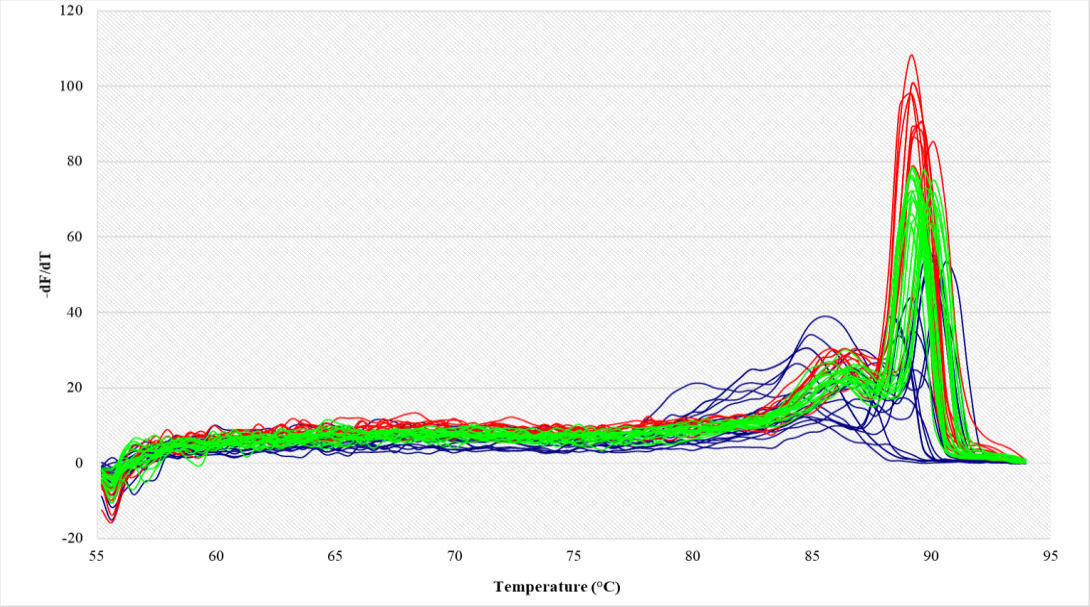


Fig. (3): Polynomial MS-HRM standard curve fit with equations extracted by peak height method.



Fig(4) HRM Difference plot of the three groups; Control group (Navy blue), EBV+ve Group (Red) and type 1 DM T1D (Green).

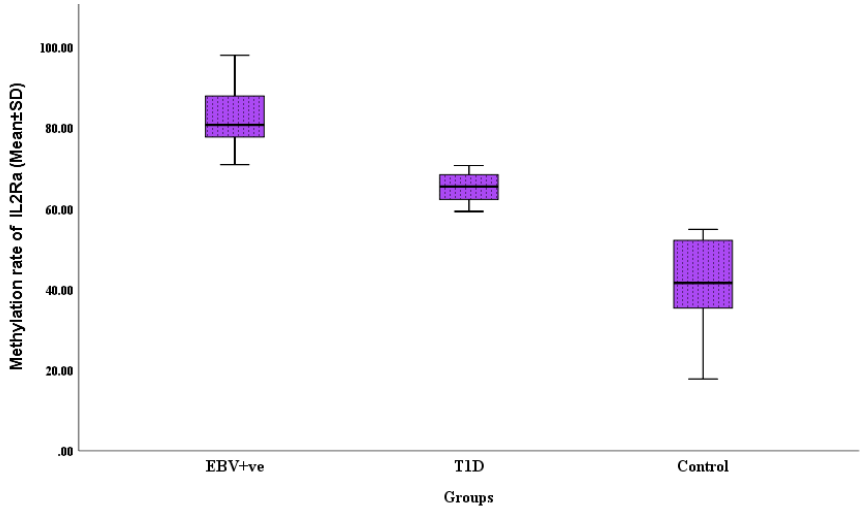


Fig. (5): Box plot illustrates the methylation rate of IL2Ra among three groups.

DISCUSSION

Globally, the incidence of type 1 diabetes varies, and a number of studies have demonstrated that the rising incidence of T1D is a significant community health matter. The prevalence of T1D is increasing by 0.34% annually.(Ogrotis et al., 2023) South and East Asia, as well as the Middle East and North Africa, were the third and fourth most impacted regions in the world, respectively. A recent study conducted by the International Diabetes Association (IDF) found that 8.2 out of 1000 Iraqis aged 0 to 19 had type 1 diabetes.(Ogle et al., 2025)

T1DM occurs when the immune system mistakenly attacks and kills pancreatic β-cells, resulting in a complete lack of insulin production. The disease becomes clinically apparent when 90% of the β-cells are destroyed(Boldison, J., & Wong, 2022)(Šabanović, 2023)

Based on immunological studies, autoimmune illnesses are characterised by imbalance of the effectors and regulatory immune responses. This leads the immune system to shift into a pro-inflammatory state, create autoantibodies, and destroy tissue.(Xiang et al., 2023) The process of eliminating β-cells involves various parts of the immune system, such as APCs, DCs, macrophages, B lymphocytes, NK cells, CD4+ and CD8+ cells, and natural killers(Scherm et al., 2022)..

According to previous studies, the "environmental factor" that causes the autoimmune reactions in type 1 diabetes mellitus typically comes first. The autoimmune response to β-cells can be activated by a range of situations, according to the scientific community's current consensus(Van Belle et al., 2022)Viral infections considered as as potential environmental triggers of T1DM, Some of these studies have established that infections might trigger islet autoimmunity via several distinctive mechanisms(Rewers, 2023) EBV infection has been linked to the onset of autoimmune illnesses, as this virus exhibits several immune evasion mechanisms and immune-modulating proteins, suggesting its potential association with the development of T1D(Zhao et al., 2024) Our data demonstrate in table (4) and figure (1) that 11 out of 30 (36.0%) of T1DM patients have the EBV virus genome, but none of the healthy controls have. The difference was very significant (p=0.02). This shows that EBV and T1DM are very closely related., and the study is consistent with earlier findings. (Mohammed, 2022)(Hassan et al., 2023) PCR detection of the EBV genome supports the concept of a link between EBV and T1D., Nonetheless, a number of distinct situations are amenable to inclusion. As a first step, infection with EBV increases inflammatory cytokine production, which in turn increases immune cell cytotoxicity and tissue damage. Secondly, when EBV moves from B lymphocytes to pancreatic tissue, it triggers a local immune response that damages beta cells(Zeng et al., 2024)

Inherited genetic factors significantly contribute to the pathophysiology of T1D. Over sixty susceptibility loci associated with Type 1 Diabetes have been found through genetic investigations. Furthermore, other genetic variations have been identified as being related with T1D, including INS, CTLA4, PTPN22, and IL2RA(Tait, 2024).in this study we will focus on gene loci IL-2ra dna methylation and its effect on T1DM disease

The connection between the external environment and our internal genetic framework is found in the evolving domain of epigenetic. , the examination of how environmental factors influence gene expression without changing the DNA sequence, acts as the crucial connection between the exposome and the onset of autoimmune diseases(Vojdani & Vojdani, 2021) Epigenetic alterations function as molecular switches, activating or deactivating genes. These switches can be activated by numerous environmental variables, including pollution, nutrition, stress, and infections(Banushi et al., 2025) Epigenetic changes in autoimmune illnesses can disrupt immune responses, resulting in the generation of autoantibodies and damage to healthy organs(Bagni et al., 2025) One epigenetic mechanism that has frequently been linked to transcriptional regulation is DNA methylation(Qiao et al., 2025)Dysregulated DNA methylation has recently been discovered in autoimmune conditions including (T1DM ,psoriasis , rheumatoid arthritis , systemic lupus)(Mei et al., 2022).

IL2RA (IL-2 receptor a-chain, CD25) is a component of the IL-2 receptor complex that has a high affinity. IL2RA is always present on regulatory T cells, which are a type of T cell that can strongly suppress autoreactive T cells .In other T cells, IL2RA is only present when it is needed. There are different versions of IL-2RA that are linked to T1D(Pahkuri et al., 2023) The methylation of the IL2RA gene promoter and its correlation with the onset of Type 1 Diabetes Mellitus (T1DM) is a critical research focus, given IL2RA's essential function in immunological control. Alterations in the IL2RA gene, especially regarding its methylation patterns, have been associated with susceptibility to T1DM. This association primarily arises from the gene's impact on regulatory T cells (Tregs), which are crucial for sustaining immunological tolerance(Shouse et al., 2024)(El et al., 2021) DNA hypermethylation in the IL2Ra promoter region and upstream of the transcription start site correlates with gene silence where IL-2 signaling appears to play a significant role in the pathophysiology of T1D.(Pinzón-Reyes, 2020).

This study demonstrate in table (5) clearly different in methylation rate of IL-2Ra ,highly methylation rate found in T1DM positive for EBV (82.73%) ,T1DM negative EBV(65.18%) and minimum level of methylation (40.57%) observed in healthy group ,the difference was clinically significant ($p<0.001$), which described the possible interfere between EBV ,epigenetic alteration and T1DM incidence, methylation of IL-2Ra gene promoter specifically T1D patient with EBV positive ,reflect the evidence that the viral trigger , epigenetic regulation and autoimmunity all these factor are linking together ,in which the EBV contribute to autoimmune by several mechanism such as molecular mimic (Tyler, 2022)(Lemos et al., 2023) One interesting thing about EBV is that it can take over host DNA methyltransferases detect by(L. Zhang et al., 2022) could clarify why EBV-positive T1D patients have a lot more *IL-1Ra* methylation (82.73%) than EBV-negative patients (65.18%) and healthy controls (40.57%). The result improved that T1DM with positive EBV altered the epigenetic mechanism in order to become in silencing gene that are responsible for immune –regulatory , that make the immune response unbalance and expose to autoimmune chance ,several studies and observation find methylation of variant site in distinct regulatory gene that linked to T1DM which provided more conformity function DNA methylation in the diseases' complications.(J. Zhang et al., 2023)(Leong

& Lung, 2021) Past studies demonstrate that single nucleotide polymorphism in IL-2Ra gene have critical change effect on level of DNA methylation in certain types of lymphcyre have high risk for t1d development (Pahkuri, 2023) In cohort study notbly change at cpg island of IL2Ra promoter region indicated the epigenetic dysregulation that accelerate the genetic factor for t1d progressively(Belot et al., 2023)..

Predominantly IL-2RA as regulatory receptor that act as anti-inflammatory mediators , as aresult the hypermethylation of promoter region which effect on the IL-1β/IL-1Ra balance lead to inflammation that evoke the immune response that mediate β-cell death.(Belot et al., 2018)(Shouse et al., 2024) our result alongside with other find that explain the epigenetic dysregulation decreases the expression of IL-2Ra(Cerna, 2023) that assess the infection pathway the attributed by EBV infection could accelerate the development of the diseases ,these finding magnify the role of viral and epigenetic change with abnormal immune response together in T1DM progressive

CONCLUSION

The result of this research revealed there is positive correlation between hypermethylation of IL-2Ra gene promoter regions with viral infection specifically EBV and the occurrence of T1DM , the finding clarify that is highly methylation level found in EBV-positive T1DM (82.73%) ,subsequently EBV-negative T1DM (65.18%) and apparently healthy control (40.57%), this statistical level suggest that EBV may modulate gene function that complicate immunological dysfunction by inhibiting IL-2Ra and induced inflammation . which evoke beta cell death ,this concept are supported by observation the employ DNA methylation to connect EBV autoimmunity, (L. Zhang et al., 2022) another applied to T1DM where inhibited lead to IL-1B mediate inflammation result from repress of IL-2Ra(Al, 2018; Dinarello, 2018),it is improved that EBV has potent effect on epigenetic modulation ,the variation of methylation among studied groups is clinically significant , this may implies the role of EBV as environmental trigger, upon our finding anr previous published the connection between these factors in susceptibility of T1DM ,in which also create new medicine approach to diminish these risk factor such as antiviral that indicate EBV or therpy that target methylation to eliminate it ,in to ensure that EBV infection play critical role in epigenetic change linked to T1DM risk , nemoureous studies and researches should be done to evaluate the methylation change for determine the causes behind that .

ETHICAL DECLARATIONS

Ethics Approval and Consent to Participate

The study accepted after obtaining verbal consent from the ethical research committee in the college of medicine , Babylon university , and Babylon health directory under reference number (No: 9786 in 25/11/2024).

Acknowledgements

I am grateful to medical college of Babylon university and the Diabetes and Endocrinology Center in, hilla city.

Consent for Publication

Medical data is included.

Availability of Data and Material

The datasets produced and/or analyzed during the present study can be obtained from the corresponding author upon reasonable request.

Conflict of interest

The authors declare that there is no conflict of interest.

Funding

self funding.

Authors' Contributions

Al-Shibly IKA and Al-Hamdani MH contributed to the design and implementation of the research. Jalil HM was contributed to all the laboratory work, the data analysis, and the writing of the manuscript. Al-Shibly IKA was contributed to reviewing and proofreading of the manuscript. All authors read and approved the final version of the manuscript.

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