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EVALUATING THE GENE EXPRESSION LEVEL OF IRF4 GENE IN TYPE 1 DIABETES

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Abstract

Aims

In the present study, we aim to clarify the expression level of the IRF4 gene in TID patients compared with healthy controls.

Materials and Methods

We obtained PBMC samples from patients with T1D and healthy individuals aged 12–19 years, performed real-time PCR gene expression, and compared the results of T1D patients with those of healthy individuals.

Results

The PBMCs procured from T1D donors and healthy ones represent a unique opportunity to identify IRF4 gene expression levels. The data analysis identified the limited significance of the expression level between T1D patients and healthy controls.

Conclusion

Our findings point to the IRF4 gene as a potential target and therapeutic candidate against T1D, despite its limited role in supporting the immune-tolerance system.

Introduction

Mononuclear cell infiltration leads to dysfunction of ins ulin
producing beta cells in type 1 diabetes, which is an autoi mmune disorder [1].
Studies on NOD mice, an extensively researched animal model for type 1 diabetes, have shown that a breakdow n in immunological control leads to the destruction of b eta cells.
Upon failure, the innate immune system is triggered, lea ding to an increase in autoreactive CD4+, CD8+ T cells, & B cells that produce autoantibodies [2, 3]. Nevertheless, we must still finalise the precise mechanis ms that govern this autoimmune process.
Interferon regulatory factor 4 (IRF4), belonging to the I RF family, acts as a transcriptional factor governing bot h innate and adaptive immune responses [4].
Contrary to other factors that activate the immune respo nse, lymphocytes mainly produce IRF4, and interferons do not stimulate its production.
On the other hand, it is induced by antigen receptor-mediated stimulation, which includes the use of plant le ctins, CD3, or IgM crosslinking [5].
Several studies have confirmed that IRF4 plays various roles in the development of lymphoid cells and their im mune response [6, 7].
Studies indicate that at the molecular level, IRF4 functio ns as a "pioneer transcriptional factor."
It facilitates the accessibility of chromatin for certain tra nscriptional factors associated with various cell lineages and promotes gene expression to support the differentia tion of effector T cells.
Effector CD4+ T cells, including Th2, Th9, Th17, follic ular Th cells, and cytotoxic T cells, rely on IRF4 for thei r development.
Additionally, it contributes to the regulation of effector r egulatory T cells, several stages of B cell maturation, an d the growth and function of dendritic cells.
Research conducted on mice that had the IRF4 gene rem oved demonstrated the prevention of autoimmune illnes ses such as experimental autoimmune encephalomyelitis , experimental colitis, and lupus nephritis.
This indicates that IRF4 plays a significant role in the pr ogression of these disorders.
Studies using animal models of autoimmune disorders, s uch as MRL/lpr mice and NOD mice [8], have demonstr ated that the absence of IRF4 hinders the normal develo pment of CD4+ T cells, particularly Th17 cells.
This enhances the animals' resistance to illness.
Nevertheless, it is still unclear whether particular subset s of T helper cells, such as Th17 cells, have a predomina nt impact on the progression of autoimmune pathogenes is in type 1 diabetes.
Both our own research and the studies conducted by oth er scientists demonstrate that the lack of IL-
17 does not have an impact on the likelihood of develop ing diabetes in NOD mice [35, 36].
Researchers have discovered that IRF4 plays a crucial r ole in preventing the differentiation of CD8+ T cells.
It achieves this by regulating the function of transcriptio nal factors such as T-bet and Blimp-
1, which are essential for the production of effector cells [9].
Furthermore, the connection between antigens and T cel l receptors (TCRs) regulates the synthesis of IRF4, whic h is essential for the mobility and metabolic functioning of activated CD8+ T cells, and this process is influenced by the dosage [9].
IRF4 is a candidate gene located in the idd14 susceptibil ity region of NOD mice, which is thought to have a role in the development of type 1 diabetes.
We discovered a notable rise in the expression of IRF4 i n NOD mice aged 7 weeks and above
who have encountered inflammation in the islets, as wel l as in diabetic NOD mice.
These findings indicate that increased IRF4 expression may have a considerable impact on the development o f diabetes in NOD mice that are already prone to the dis ease [10].
This study aims to assess the extent of IRF4 gene expres sion in individuals diagnosed with type 1 diabetes in co mparison to a group of healthy individuals serving as co ntrols.

Material and Methods:

Study design:

This is a case-control study, taking blood samples from 30 patients with diabetes mellitus type 1 (case) and 30 healthy people (control) after signing informed consent.

We collected blood samples from February 18th, 2023, until the end of March, 2023.

The Diabetic Clinic and Endocrine Centre at Merjan Medical City Hospital in Babylon Province diagnosed all of the study's patients.

Isolation of peripheral blood mononuclear cells:

We will dilute the blood sample by 2:1 with sterile PBS. Next, we separate and dissolve the PBMC (peripheral blood mononuclear cells) in PBS after two washes using Ficoll-Hypaque centrifugation.

RNA extraction and real-time PCR are used to measure gene expression.

We performed RNA extraction and cDNA synthesis using the AddPrep Total RNA Extraction Kit and the AddScript cDNA Synthesis Kit, respectively, following the company's instructions. We measured the expression level of IRF4 using specific primers and real-time PCR techniques. We use the expression of GAPDH genes as an internal control.

Inclusion criteria

1: Patients suffering from type 1 diabetes mellitus.

2: Healthy individuals without a history of diabetes mellitus type 1 or any autoimmune or inflammatory diseases, as well as their family,

3: Patients between the ages of 12 and 19.

Exclusion criteria

Patients with diabetes mellitus type 1 who also have other diseases are not eligible.

Sampling

We matched 30 patients with diabetes mellitus type 1 and 30 healthy individuals in terms of age and sex for this study. We explain the plan and obtain informed consent before taking 10 cc of blood samples from the patients, which we then transfer to the lab for further steps.

Statistical considerations and data analysis

We will perform all data analyses using Statistical Software for Social Scientists (SPSS) Version 25. A Mann-Whitney test was performed. We also performed the (2-CT) method using GRAPH PAD PRISM 9 software. We set the level of confidence at 95% for this study.

All results will be expressed as mean ± standard deviation (SD), while p values < 0.05 will be considered statistically significant.

3. Results

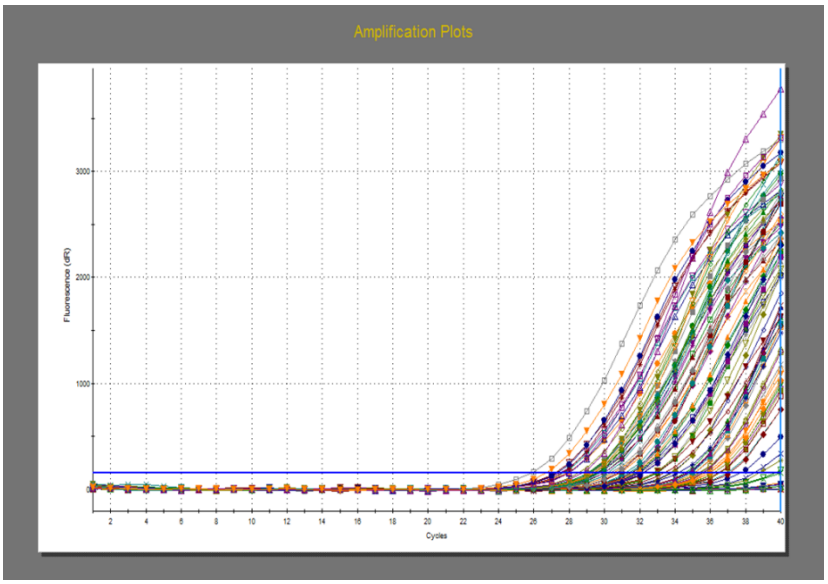

Figure 1: IRF4 gene propagation plot using the system Mx3005P Stratagene.

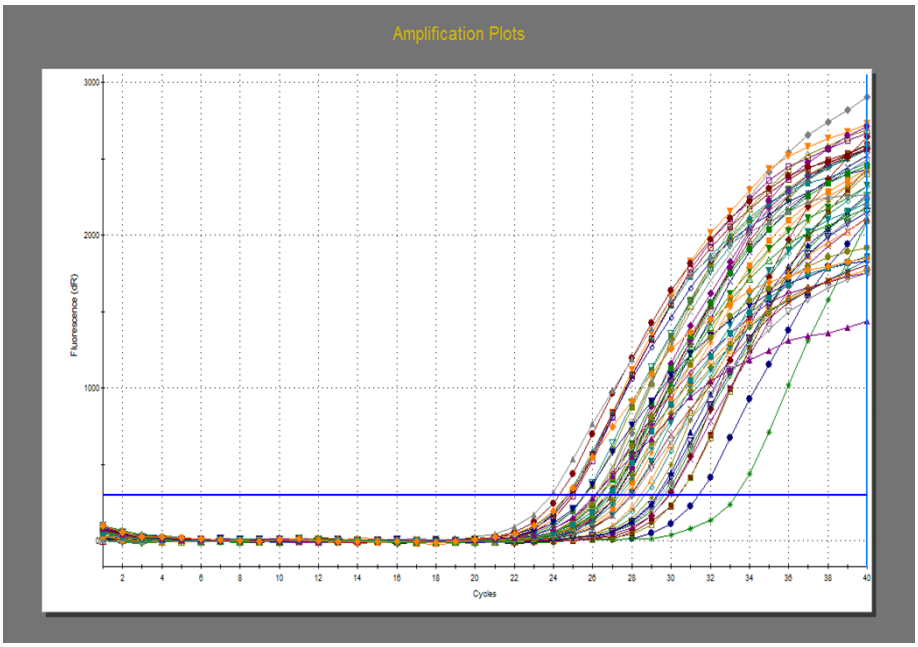

Figure 2: GAPDH gene propagation plot using the system Mx3005P Stratagene.

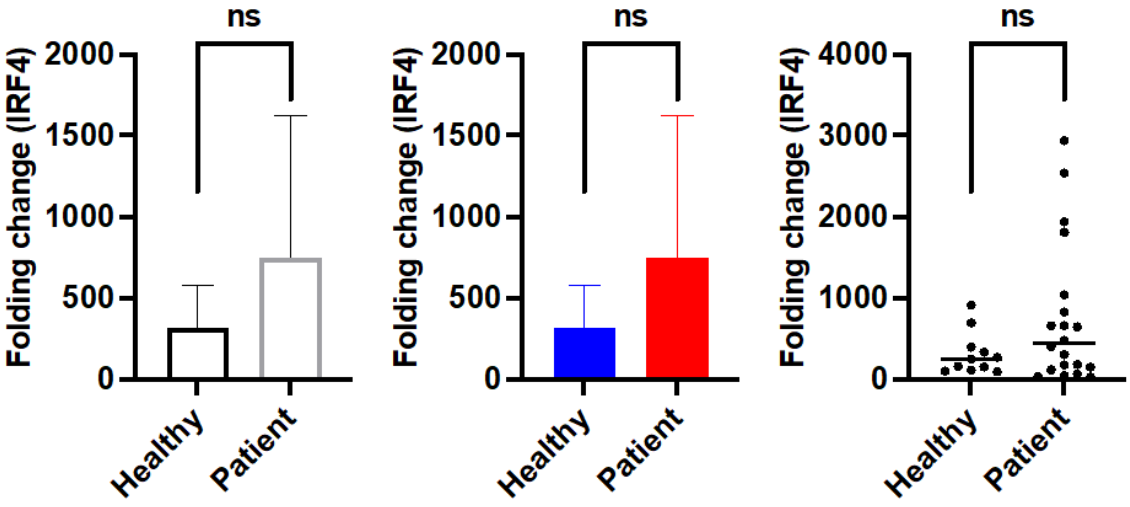

Figure 3: Statistics using GRAPH PAD PRISM 9 software

IRF4 Descriptive analysis

	Healthy	Patient
Number of values	**30**	**30**
Minimum	**90.51**	**25.99**
25% Percentile	**110.7**	**122.3**
Median	**249.0**	**439.7**
75% Percentile	**396.2**	**985.2**
Maximum	**910.2**	**2937**
Mean	**314.5**	**750.4**
Std. Deviation	**265.2**	**872.3**
Std. Error of Mean	**79.97**	**195.1**
Lower 95% CI	**136.4**	**342.1**
Upper 95% CI	**492.7**	**1159**

Mann Whitney test	
P value	**0.1770**
Exact or approximate P value?	**Exact**
P value summary	**ns**
Significantly different (P < 0.05)?	**No**
One- or two-tailed P value?	**One-tailed**
Sum of ranks in column A,B	**153 , 343**
Mann-Whitney U	**87**

4. Discussion

There is limited direct evidence establishing a connection between IRF4 and the disease. Nevertheless, research has shown that IRF4 plays a significant role in various autoimmune illnesses, suggesting its possible contribution to T1DM. For instance, a study discovered abnormal expression of IRF4 in various adult lymphoid neoplasms, where it functions as an oncogene [11]. This indicates that IRF4 deregulation may contribute to the development of autoimmune conditions, such as T1DM. Furthermore, scientists have observed IRF4 expression in dermatopathic lymphadenopathy (DL). In DL cases, the researchers documented the presence of MUM1/IRF4, a unique form of IRF4 [12]. Despite DL's lack of a direct connection to T1DM, this discovery suggests that IRF4 may play a role in the immune response associated with chronic inflammatory diseases.

Melo AP et al. (2022) conducted a pioneering investigation to analyse numerous genetic variations in pro-inflammatory genes linked to obesity in Brazilian youngsters. According to their findings, variations in the IRF4 transcription factor gene may have a significant impact on the occurrence of childhood overweight and raise the likelihood of these children being susceptible to type 1 diabetes [13].

Research has demonstrated that the combination of IRF4 and AhR generates a complex that acts as a transcription factor. ITK signalling in both humans and mice affects the production of IRF4, which is essential for the differentiation of Tr1 cells. Research based on the study of mechanisms has shown that activin-A signalling is responsible for initiating The activation process of the transcription factor IRF4 begins. The aryl hydrocarbon receptor and another receptor work together to selectively attach to specific promoter regions (IL-10 and ICOS) and regulate gene production in human CD4+ T cells. IRF4 inhibits IL-10 and ICOS activation by activin-A, resulting in a decrease in the suppressive ability of human act-A-iTr1 cells. The act-a-iTr1 cells, together with IRF4 & AhR, constitute a transcriptional network. They facilitate the proliferation of human Tr1 cells and inhibit allergic responses induced by Th2 cells [11].

Torabi et al. (2023) found that Type 1 Diabetes Endotype 1 (T1DE1) exhibits excessive expression of the IRF4 gene [15]. The IRF4 gene serves as a transcription factor that facilitates T lymphocyte differentiation upon antigen stimulation. It has previously been linked to the onset of type 1 diabetes in both people and animals.

Figure 3 shows that the IRF4 gene has a minimal impact on TID patients' expression levels in comparison to healthy individuals.

Conclusive evidence now links the IRF4 gene to numerous autoimmune diseases, indicating its possible role in T1D. However, our research indicates that it has a limited effect on immune tolerance maintenance and warrants consideration as a potential target and therapeutic option for T1D.

Acknowledgment

Merjan educational hospital, center of diabetes & the directorate of research and development, Hilla, Babylon Provence, Iraq

Tehran University of Medical Sciences

Ethical approval: The study's techniques, which involved human participants, followed the rules established by the institutional research committee and were in accordance with the Helsinki Declaration and its subsequent amendments, as well as other comparable ethical norms. The study has received ethical approval from the Tehran University of Medical Sciences, with the code IR.TUMS.CHMC.REC.1398.055.

Participants consent: Informed consent was acquired from all individual participants after providing a detailed explanation of the study's procedures and objectives.

Publication consent: Patients provided informed consent for the release of their data.

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