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Investigating the Relation between *LCK* Gene Expression with Type 2 Diabetes Patients in Yazd Diabetes Research Center

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Abstract

Objective: Type 2 diabetes mellitus (T2DM) is characterized by insulin resistance and insulin secretory defect. Deficiency of cellular immunity is known as one of the factors involved in the pathogenesis of T2DM. lymphocyte-specific protein tyrosine kinase(LCK) is an important gene involved in the intracellular signaling pathways of lymphocytes. This study aimed at determining and comparing *LCK* gene expression levels in diabetic patients compared with the healthy controls.

Materials and Methods: In this case-control study, 60 people, including 30 T2DM and 30 healthy people were included. The expression levels of the *LCK* gene were measured by real-time polymerase chain reaction and the obtained data were analyzed by T-test in GraphPad Prism6 software.

Results: The expression level of the *LCK* gene was increased in diabetic samples compared with the healthy samples (P= 0.0001).

Conclusion: The results suggested that changes in the expression levels of *LCK* gene can play a role in the pathogenesis of T2DM.

Keywords: Type 2 diabetes mellitus, LCK gene, Gene expression

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Introduction

As the commonest chronic metabolic disease, type 2 diabetes mellitus (T2DM), affects many people globally and its prevalence rate is rising rapidly (1). It is estimated in many countries including the USA, India, and China, that have the most numbers of diabetic patients (more than 90% of these have T2DM), the diabetes prevalence will double in the next 20 years or so (2). Diabetes can lead to many complications, including heart and kidney disease and blindness (3). T2DM results from three major pathological defects, including insulin resistance, beta-cell apoptosis, and insulin secretion deficiency (4). The main goal in the treatment of T2DM is to restore glucose metabolism to normal levels, which is ideally possible through diet, exercise, and weight loss (5). Diabetic patients who cannot control their disease are prescribed hypoglycemic drugs, but often, they will need insulin (6).

Various studies have been published to determine different mechanisms involved in diabetes and impair the host's defense against pathogens. These mechanisms include dysfunction of immune cells, defects in phagocytosis, failure to kill microbes, and inhibition of cytokine production (7). Although the leading cause of T2DM is not known, it is believed that it can be caused by an interaction between environmental, genetic, and epigenetic factors (8,9). Previous studies showed that there is a strong relationship between diabetes and the immune system so that the genes which are playing role in the immune system can also play role in diabetes (10,11).

In recent years, the lymphocyte-specific protein tyrosine kinase (LCK) is known as one of the critical molecules that regulate T-cell functions (12). It was first identified as a member of the Src family of protein tyrosine kinases in the 1980s (13). The human *LCK* gene contains 13 exons and is located on chromosome 1p35.2. *LCK* is also known by other names such as the *LSK* and the protein kinase of the Src family. Earlier studies showed that *LCK* is involved in the different pathways related to the immune system such as the NF-kB pathway and is essential to T cell activation and development (14,15).

To determine the genes involved in the pathogenesis of T2DM and also to determine the pathways associated with its occurrence, this study aimed to evaluate the expression level of the *LCK* gene in patients with T2DM compared to controls.

Materials and Methods

Sample collection

Sixty people, including 30 T2DM and 30 healthy individuals were included in this study. The people aged 35 to 80 years referring to the Yazd Diabetes Research Center with fasting glucose of more than 126 mg / were considered T2DM cases and cases with fasting glucose of lower than 110 mg / dL were selected as control group. Inclusion criteria included the age of over 35 years and T2DM. Exclusion criteria included a history of liver diseases like hepatitis, a history of infectious and inflammatory diseases (lupus and rheumatoid arthritis), a family history of diabetes in first-degree relatives, a history of endocrine diseases such as hypothyroidism or hyperthyroidism, macrovascular and microvascular complications such as nephropathy, retinopathy, and coronary artery disease. From each participant, 5 ml of the whole blood without EDTA (for biochemical parameters and evaluation of gene expression) after 12h of fasting were collected.

RNA isolation and quantitative real time-PCR

To evaluate the expression level of *LCK* gene in the blood samples of the patients and healthy people, the total RNA was first extracted from the blood using YTzol (Yekta Tajhiz Azma, IRAN). The purity and concentration of RNA were analyzed using a spectrophotometer (NanoDrop, Thermo

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Fisher) and OD 260/280. Complementary DNA (cDNA) of the *LCK* gene was synthesized using the protocol of the cDNA synthesis kit from SMO BIO Co. Gene expression was performed by thermal cycler (Applied Biosystem, ABI, Step One Plus, USA) that flowed by one cycle as a pre-denaturation step at 95 ˚C for 120 s and 40 cycles including a denaturation step at 95 ˚C for 5 s and an annealing step at 60 ˚C for 45 s. Then, expression levels were evaluated using $2^{-\Delta\Delta Ct}$. In our study, *GAPDH* gene was used as an internal control. Primers of *GAPDH* gene and *LCK* gene were ordered and prepared by Fanavaran Gene Company. The primer sequences were as follows: *LCK* forward, 5`- AGCAGAGCAGCGAGTGGT -3` and reverse, 5`- GATGAGGAAGGAGCCGTGA GTG -3` with a product size of 185 bp; *GAPDH* forward, 5`- AATCCCATCACCATC TTCCA -3` and reverse, 5`- TGGACTCCAC GACGTACTCA -3` with a product size of 82 bp.

Statistical analysis

$2^{-\Delta\Delta CT}$ method was used to investigate the relative expression of genes in each group. Statistical analyses were considered to compare the mean relative expression of its target gene (*LCK*) in the groups (diabeticand healthy samples) using GraphPad Prism6 software and two independent sample T-test regarding significant difference of $P< 0.05$ for this study and the diagrams were drawn using GraphPad Prism6 software. All reactions were done in duplicate.

Ethical considerations

The Institutional Ethics Committee of the Shahid Sadoughi University of Medical Sciences, Yazd approved the study design (IR.IAU.YAZD.REC.1400.009), and also the written informed consent was obtained from each participant before blood sampling.

Results

Evaluation of gender ratio in patient and control groups

In this study, the sex ratio of control and patient individuals was measured and the statistical test showed that there was no significant difference between control and patient individuals ($P= 0.99$) (Table 1).

Comparison of FBS in two groups

Fasting blood sugar (FBS) levels were measured in patients and controls. The mean FBS levels in patients were 185.19 and in controls were 105.9. So, there is a significant difference between two groups ($P< 0.0001$).

Gene expression of LCK

Gene expression alteration of this gene in blood samples of diabetic and control individuals shows that the *LCK* gene expression in the blood of diabetics is 1.42 times higher than the control subjects. As shown in Figure 1, the *LCK* gene expression level in healthy people showed a significant difference with diabetic people ($P= 0.0001$).

Discussion

The progression of T2DM in humans has been clearly shown to be associated with several genetic and epigenetic changes (16). Although the molecular mechanism involved by these genetic changes is not yet completely understood, our knowledge proves that several genes are activated or inactivated in this disease (17).

The role of the *LCK* gene in type 1 and 2 diabetes has also been identified in several studies. Several microarray expressions studies have been performed in people with T2DM compared to healthy individuals.

Table 1. Evaluation of gender ratio

The actual number in each group	Women		Men		Total
	Number	%	Number	%	
Control	21	70	9	30	30
Patient	20	66	10	34	30
Total	41	68.33	19	31.66	60

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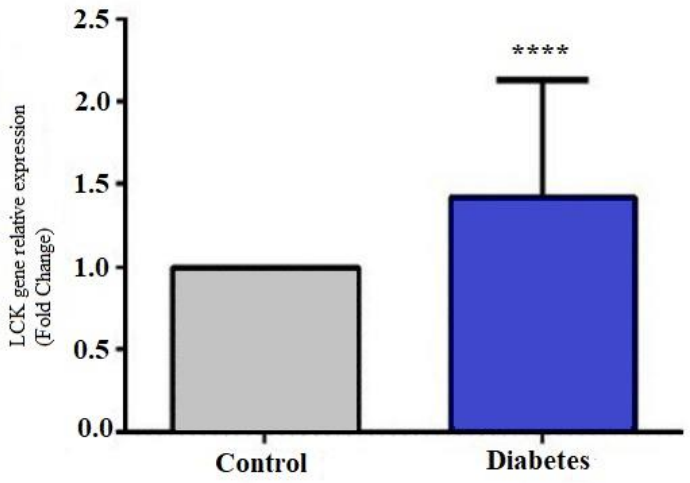


Figure 1. Comparison of the expression levels of *LCK* gene between healthy control and T2DM patients. (*P*= 0.0001)

In Cui et.al. study, the overall expression of genes was measured using the microarray technique in people with T2DM compared to healthy individuals. One of the genes that showed increased expression was the *LCK* gene (18). In the Grayson et.al. study, the overall expression of genes was also measured using the microarray technique in people with metabolic syndrome, heart disease, rheumatoid arthritis, and T2DM compared to healthy individuals, which ultimately shows the *LCK* gene expression increased in patients (19).

These studies led us to use a more accurate technique to measure *LCK* gene expression in people with T2DM in compared to healthy individuals and the results of this study showed that *LCK* gene expression was significantly increased in people with T2DM in compared to healthy people. The results of this study are in line with previous studies on the expression of *LCK* in T2DM diseases (18,19).

The functional mechanism that is disrupted as a result of increased expression of the *LCK* gene is probably related to the activation of T cells. When T cell receptor (TCR) interacted with major histocompatibility complex (MHC), leads to immunoreceptor tyrosine-based activation motif (ITAM) phosphorylation at the end of CD3 chains by *LCK*. Activated CD3 which binds to the ZAP70 protein, causes linker for activation of T cell (LAT) to be phosphorylated. LAT is a membrane protein that interacts directly with proteins such as growth factor receptor-bound protein 2 (Grb2) and phospholipase C γ1 (PLC γ 1) and indirectly to SOS and ITK adapter proteins. Activated PLCy1 protein converts phosphatidylinositol (4,5) bisphosphate (PIP2) to diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3). IP3 induces intracellular calcium secretion, and DAG activates the protein kinase C (PKC) pathway which ultimately activates NF-kB in T cells. NF-kB as a transcription factor, transported to the nucleus and enhanced the expression of inflammatory proteins such as interleukin 6 (IL-6), interleukin 1A (IL-1A), and tumor necrosis factor-alpha (TNF-alpha). Intracellular calcium flow, on the other hand, activates calcineurin; a protein that phosphorylates nuclear factor of activated T-cells (NFAT); transmitting it to the nucleus and expressing proinflammatory genes such as interleukin 2 (IL-2) and interleukin 1B (IL-1B) (15,20,21).

NF-kB signaling pathway plays an important role in insulin resistance. Increased NF-kB expression leads to increased expression of chemokines and cytokines in T cells, which

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ultimately lead to pancreatic beta-cell damage and insulin resistance. In addition, many studies have shown that NF-kB has increased expression in T2DM (15,20).

According to previous studies, the role of *LCK* gene in the pathways of inflammation and regulation of T cells has been proven. On the other hand, T2DM is also associated with the path of inflammation and improper activation of T cells. Therefore, increased LCK gene expression may be involved in the pathogenesis of T2DM by activating inflammation-related pathways as well as changes in T cell activation, although further cellular and molecular studies are needed to substantiate this claim.

Conclusions

From our study, we conclude that restrictive lung disease (low lung volume) is significantly associated with prediabetes. This study contributes evidence for a prospective relationship between lung volume and the incidence of newly diagnosed prediabetes among subjects with normal glucose metabolism at baseline.

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Conflict of Interest

The authors declare that they have no conflicts of interests

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