Turkish Journal of Biology

Volume 48 | Number 2 Article 4

4-26-2024

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Recommended Citation

Çelik, Süheyla Pınar; Parıltı, Damla Nur; Açık, Leyla; Yalçın, Mehmet Muhittin; Yetkin, İlhan; and Yunusov, Eldeniz (2024) "NAMPT, IL-6, and vaspin gene expressions and serum protein levels in type 2 diabetes mellitus and related complication," *Turkish Journal of Biology*: Vol. 48: No. 2, Article 4. https://doi.org/10.55730/1300-0152.2689
Available at: https://journals.tubitak.gov.tr/biology/vol48/iss2/4

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This article is available in Turkish Journal of Biology: https://journals.tubitak.gov.tr/biology/vol48/iss2/4

Turkish Journal of Biology

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Research Article

Turk J Biol
(2024) 48: 133-141
© TÜBİTAK
doi:10.55730/1300-0152.2688

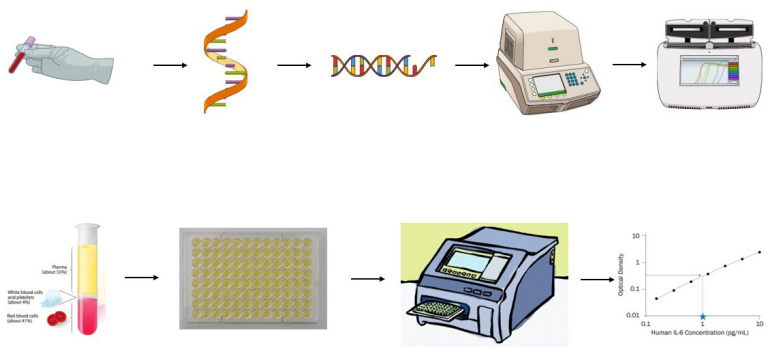
NAMPT, IL-6, and vaspin gene expressions and serum protein levels in type 2 diabetes mellitus and related complication

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Received: 26.12.2023 • **Accepted/Published Online:** 03.04.2024 • **Final Version:** 26.04.2024

Graphical abstract



RNA was extracted from blood samples obtained from participants (three distinct groups of patients with type 2 diabetes and a healthy control group). cDNA was synthesized from the extracted RNAs, and the resulting data were analyzed by examining gene expression levels using qPCR. Serum samples collected from the same participants were analyzed by ELISA method, and serum protein levels were determined. Statistical analyses of gene expression data and serum protein levels were performed to evaluate whether there were significant differences between the groups.

Background/aim: Type 2 diabetes mellitus (T2DM) is the most common type of diabetes and occurs due to insufficient insulin secretion or inability to use existing insulin and the effects of environmental factors. Although there are many studies on the pathophysiology of T2DM, the mechanisms contributing to the pathogenesis of insulin resistance and pancreatic beta-cell dysfunction have not been completely elucidated. Some adipokines secreted from adipose tissue, which are the primary regulators of insulin resistance, affect immune and inflammatory functions. Altered adipokine profiles have been observed in obesity and T2DM, leading to severe metabolic risks and changes in insulin sensitivity.

Materials and methods: This study used quantitative PCR and ELISA techniques to analyze samples from individuals without diabetes (control group) and with T2DM (macrovascular and microvascular complications and without complications) for at least 10 years.

Results: The mRNA expression and protein levels of NAMPT, IL-6, and vaspin genes were determined. While there was no significant difference in NAMPT, IL-6, and vaspin mRNA expression levels between diabetic groups, there was a significant decrease between the patient and control groups ($p < 0.001$). For serum protein levels, NAMPT protein levels decreased significantly in the uncomplicated group, while IL-6 and vaspin protein levels increased significantly in both microvascular and macrovascular complication groups ($p < 0.001$).

Conclusion: The correlations between gene expressions, clinical parameters, and protein levels are crucial to understanding the implications of the findings.

Key words: Type 2 diabetes mellitus, NAMPT, IL-6, vaspin, adipokines, complications

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1. Introduction

Diabetes mellitus (DM), caused by ineffectiveness or deficiency of the insulin hormone secreted by the beta islet cells of the pancreas, is affected by hereditary and environmental factors. It is a metabolic disease characterized by disorders in lipid, protein, and carbohydrate metabolism, accompanied by various complications and high blood glucose levels (fasting blood glucose ≥126 mg/dL, random plasma glucose ≥200 mg/dL, HbA1c ≥6.5%)[1] (Dilworth et al., 2021). The most common classifications are type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM), gestational diabetes mellitus (GDM), and other types of diabetes. While T1DM is an autoimmune disorder resulting from the destruction of β-cells, T2DM is the most common type of DM due to insulin resistance, insulin secretion deficiency, and environmental factors. GDM happens during pregnancy and is not usually observed after pregnancy. In addition to other specific types of diabetes, there are various genetic defects, additional diseases, and the use of certain drugs (Dilworth et al., 2021).

T2DM is a disease that consumes necessary health resources, contributing to 8.4% of the deaths worldwide. Despite significant diagnostic and treatment advances, T2DM remains associated with increased mortality and morbidity compared to that of the general population (Nanayakkara et al., 2021). Diabetes is one of the top 10 causes of death worldwide. By 2021, Türkiye had the highest number and prevalence of diabetes in the International Diabetes Federation (IDF) European region. DM is one of the most common chronic metabolic diseases. According to the TURDEP II study data, the prevalence of diabetes was determined to be 16.5%, indicating that there are 6.5 million adults in Türkiye affected by this disease. The prevalence of diabetes is higher in women than in men ($p = 0.008$) (Satman et al., 2013; $p = 0.008$) in Türkiye. Diabetes, which was ranked 6th among the top 10 causes of death in Türkiye in 2000, rose to 5th place in 2019.[2] According to IDF data, the diabetes-related death rate among individuals under the age of 60 years in Türkiye in 2021 was 4%. Approximately 83,220 people died of diabetes in 2021 (IDF, 2021).

In most cases, T2DM is associated with a variety of disabling and life-threatening complications, such as cardiovascular diseases, cerebrovascular diseases, nephropathy, and retinopathy (Rangel et al., 2019; Goyal et al., 2023). Although the exact causes of T2DM are not well understood, studies have shown that the development of T2DM is a multifactorial process involving a combination of genetic and nongenetic risk factors (Hansen, 2002). Studies have identified many risk factors for T2DM, such as ethnicity, family history of diabetes, age, sex, body mass index (BMI), obesity, sedentary lifestyle, overnutrition, low dietary fiber, central adiposity, alcohol, and smoking consumption, psychosocial stress factors, and environmental pollutants (Laakso, 2019; Abu-Shahba et al., 2021). These factors contribute to local or systemic low-grade chronic inflammation, which increases insulin resistance and leads to T2DM development (Abu-Shahba et al., 2021). Despite numerous studies that have been conducted on the pathophysiology of T2DM, the mechanisms underlying the pathogenesis of insulin resistance and pancreatic beta cell dysfunction still require further elucidation. In the context of T2DM, the identification of elevated circulating inflammatory factors such as cytokines, C-reactive protein (CRP), and chemokines in T2DM patients, as well as increased concentrations of some adipokines secreted by adipose tissue, associated with insulin resistance and pancreatic islet inflammation, creates a new field for understanding the pathophysiology, diagnosis, and treatment of T2DM (Cheng and Yu, 2022; Stanimirovic et al., 2022).

The cytokines secreted by adipose tissue are called adipokines, pivotal regulators of insulin resistance, and affect immune and inflammatory functions (Heo et al., 2019). Previous studies have evaluated the role of adipokines in the pathophysiological mechanism of T2DM and their potential as noninvasive biological candidates for managing T2DM, including diagnosis and treatment. As a typical example, patients with obesity and type 2 diabetes display altered adipokine profiles that lead to profound metabolic risks and changes in insulin sensitivity (Oh et al., 2016; Lee et al., 2019). Based on this, it has been hypothesized that therapeutically targeting inflammatory pathways may reduce the risk of T2DM or improve glycemic control in people with diabetes. NAMPT is an intracellular enzyme that plays an important role in ATP synthesis (Curat et al., 2006; Friebe et al., 2011). eNAMPT is secreted in many cell types, such as adipocytes and β cells, and functions as a proinflammatory cytokine in various signaling pathways such as IL-6/STAT3, PI3K/AKT. eNAMPT increases insulin secretion and protects from apoptosis in pancreatic β cells (Fukuhara et al., 2005). Interleukin-6 (IL-6) is a multifunctional cytokine secreted by many cell types. IL-6 also regulates and stimulates the release of chemotactic mediators, adhesion molecules, and acute phase proteins, leading to the release of other cytokines by enhancing the inflammatory response (Rodrigues et al., 2017). The role

1 American Diabetes Association (ADA) (2017). Diagnosing diabetes and learning about prediabetes [online]. Website: http://www.diabetes.org/diabetes-basics/diagnosis/?loc=db-slabnav [accessed 14 March 2023].

2 World Health Organization (2020). The Global Health Observatory. Global health estimates: Leading causes of death [online]. Website: https://www.who.int/data/gho/data/themes/mortality-and-global-health-estimates/ghe-leading-causes-of-death [accessed 14 March 2023].

of IL-6 in type 2 diabetes and insulin resistance has not yet been clarified. While some studies have shown its negative effects on insulin (Klover et al., 2003; Lagathu et al., 2003), others have indicated that it is probably necessary to maintain glucose balance (Matthews et al., 2010; Wallenius et al., 2002). Vaspin (serpina12), isolated from adipose tissue of Otsuka Long-Evans Tokushima fatty (OLETF) rats, is an adipocytokine released from visceral adipose tissue. Vaspin, a serine protease inhibitor, is effective in insulin resistance, inflammation, and obesity (Hida et al., 2005). NAMPT, IL-6, and vaspin are significantly associated, potentiating each other's effects. Based on the information mentioned above, we investigated NAMPT, IL-6, and vaspin gene expression, as well as serum protein levels, on T2DM development, diabetes complications, and the significant impact potential of these adipokines on T2D pathogenesis. We examined T2DM patients with micro- and macrovascular complications, those without complications, and a healthy control group living in Türkiye.

2. Method

2.1. Collection of type 2 diabetes and healthy control samples

We used data from the Gazi University Hospital Endocrinology and Metabolic Diseases Clinic, comprising individuals aged 18–70 diagnosed with T2DM for at least 10 years, categorized into groups with macrovascular complications (coronary artery disease, peripheral artery disease, and cerebrovascular diseases) (n = 40), microvascular complications (nephropathy, retinopathy, and neuropathy) (n = 40), and those without complications (n = 40), and healthy individuals (n = 40) aged 18–70 years with no known diabetes diagnosis. Blood and serum samples were collected. It was confirmed that the study participants had received COVID-19 vaccines and had not been diagnosed with COVID-19 until at least three months ago. Our study was approved by the Keçiören Training and Research Hospital Clinical Research Ethics Committee (decision no: 2012-KAEK-15/1873) and was conducted in accordance with the principles of the Declaration of Helsinki. Individuals who volunteered to donate blood were informed about the study, and their written and verbal consent was obtained.

2.2 NAMPT, IL-6, and vaspin gene expression analysis

The manufacturer's protocol isolated total RNA using a HighPure RNA isolation kit (Roche Diagnostics, Mannheim, Germany). All samples were electrophoresed on a 1% agarose gel to assess total RNA integrity and visualized using a UV gel documentation system. Reverse transcription-polymerase chain reaction (RT-PCR) was performed using the Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics) in a reaction volume of 20 µL according to the manufacturer's instructions. The expression levels of target genes were analyzed using a RealTime PCR device (Roche Diagnostics, LightCycler 480 Instrument II) and LightCycler 480 Basic Software 1.5.1 (Roche Diagnostics). β-actin was used as a housekeeping gene. The RT-PCR mix consisted of 20 µL, comprising of 5 µL of cDNA product, 10 µL of 2X LightCycler 480 Probes Master Kit (Roche Diagnostics), 1 µL of TaqMan Gene Expression Assay (Roche Diagnostics) (Table 1), and 4 µL of water containing RNase (Roche Diagnostics). RT-PCR reactions were performed in triplicate, with a nontemplate negative control.

2.3. Investigation of NAMPT, IL-6, and vaspin protein levels

The manufacturer's protocol was used to determine protein levels in serum samples using a Human ELISA Kit (Bioassay Technology Laboratory, Shanghai, China). The samples were centrifuged to observe the absence of precipitation in the serum samples. According to the kit protocol, 50 µL standards were prepared at five concentrations (320, 160, 80, 40, and 20 ng/L) using 640 ng/L of the original standard solution and standard diluent. A standard diluent was used as blank. Serum samples (40 µL) were collected, and 10 µL of the antibody was added. Blanks, standards, and samples were added to each well of a 96-well plate. Except for the blank well, 50 µL of streptavidin-HRP was added to the wells and incubated at 37 °C for 1 h. The whole plate was washed five times with 300 µL of wash buffer. Then, 50 µL of substrate A and 50 µL of substrate B were added to all wells and incubated at 37 °C for 10 min. Finally, 50 µL of stop solution was added, the plate was read in a plate reader at 450 nm.

Table 1. TaqMan Gene Expression Assays ID.

Name	**Assay ID**
TaqMan Gene Expression Assays, Gene NAMPT/Human	Hs00237184_m1
TaqMan Gene Expression Assays, Gene IL-6/Human	Hs00174131_m1
TaqMan Gene Expression Assays, Gene Vaspin/Human	Hs01100128_m1
TaqMan Expression Assays, Gene ACTβ/Human	Hs01060665_g1

2.4. Statistical analysis

When evaluating clinical features between type 2 diabetes cases and controls, the Tukey test was used after ANOVA to determine the difference between groups. Tamhane's T2 test was used after Welch's ANOVA. While calculating the statistics for NAMPT, IL-6, and vaspin plasma protein levels and NAMPT, IL-6, and vaspin gene expression levels between the groups, the normal distribution of the data was first checked. It was determined that the data did not show a normal distribution, and since there were more than two groups, the analysis was performed with the independent-samples Kruskal-Wallis test. This analysis was conducted using SPSS software (version 16.0; SPSS, Inc., Chicago, IL, USA). Differences were considered statistically significant at $p \leq 0.05$. Significance values were adjusted for multiple testing using Bonferroni correction.

3. Results

Blood and serum samples were collected from 20 women and 20 men (mean age ± SD 62.30 ± 6.23 years) with macrovascular complications, 27 women and 13 men with microvascular complications (mean age ± SD 59.05 ± 6.83 years), and 26 women and 14 men without complications (mean age ± SD 58.65 ± 6.91 years). Controls were divided into four main groups comprising 26 females and 14 males (mean age ± SD 48.48 ± 7.93 years). NAMPT, IL-6, and vaspin expression levels were analyzed using RT-PCR, and serum protein levels were analyzed using ELISA. The relative quantification (RQ) of NAMPT, IL-6, and vaspin was calculated using the delta-delta Ct (ΔΔCt) method adjusted for the expression level of β-actin. The RQ value for the calibrator was equal to one (Livak and Schmittgen, 2001).

3.1. Clinical data

The clinical, biochemical, and genetic characteristics of the participants included in this study are summarized in Table 2. There was no significant difference between the diabetic and control groups in terms of sex ($p > 0.05$). The mean BMI of patients with T2DM without complications was 28.95 kg/m2, with microvascular complications was 29.16, with macrovascular complications was 29.84, and in the healthy control group, was 26.59 (interquartile range 40–52, n = 40). The mean duration of T2DM was 10 years. There was no significant difference between the diabetic and control groups in terms of sex, BMI, TG level, and T2DM duration ($p > 0.05$). However, a significant

Table 2. Clinical, biochemical, and genetic features of the subjects.

	Study group				
Characteristic	Without complication (n = 40)	Microvascular complication (n = 40)	Macrovascular complication (n = 40)	Healthy group (n = 40)	p
Sex (F:M)	14:26	13:27	20:20	14:26	0.290
Age (years)	**58.65 ± 6.91**	**59.05 ± 6.83**	**62.30 ± 6.23**	**48.48 ± 7.93**	**<0.001**
BMI (kg/m^2)	28.95 ± 4.33	29.16 ± 5.74	29.84 ± 4.65	26.59 ± 3.62	0.073
T2D duration (years)	15 ± 5.11	16.05 ± 5.11	15.28 ± 5.69	-	0.658
FBG (mg/dL)	**157.4 ± 53.24**	**160.50 ± 63.33**	**176.58 ± 70.43**	**89.58 ± 8.14**	**<0.001**
HbA_{1c} (%)	**7.84 ± 1.90**	**8.20 ± 1.77**	**8.82 ± 1.81**	**5.75 ± 0.29**	**<0.001**
HDL-C (mg/dL)	**52.75 ± 13.83**	**51.68 ± 12.44**	**43.05 ± 8.27**	**54.60 ± 12.95**	**<0.001**
LDL-C (mg/dL)	**123.13 ± 79.26**	**122.38 ± 37.47**	**96.90 ± 34.81**	**145.85 ± 36.33**	**<0.001**
T-C (mg/dL)	**205.28 ± 44.67**	**205.43 ± 48.56**	**173.53 ± 43.55**	**225.85 ± 43.95**	**<0.001**
TG (mg/dL)	159.00 ± 79.26	160.23 ± 86.28	167.23 ± 84.36	127.13 ± 61.86	0.106
Creatinine	**0.71 ± 0.17**	**0.80 ± 0.32**	**0.89 ± 0.42**	**0.69 ± 0.17**	**0.011**
NAMPT (ng/L)	**11.52 ± 4.49**	**16.31 ± 4.81**	**16.91 ± 5.90**	**17.47 ± 3.81**	**<0.001**
IL-6 (ng/L)	**20.28 ± 5.23**	**27.41 ± 6.81**	**27.03 ± 7.97**	**20.28 ± 4.81**	**<0.001**
Vaspin (ng/L)	**22.33 ± 5.30**	**29.39 ± 7.88**	**28.87 ± 7.63**	**22.36 ± 2.53**	**<0.001**
NAMPT	**0.55 ± 0.58**	**0.61 ± 0.62**	**0.56 ± 0.60**		**<0.001**
IL-6	**0.51 ± 1.00**	**0.31 ± 0.65**	**0.33 ± 0.74**		**<0.001**
Vaspin	**0.70 ± 0.74**	**0.62 ± 0.70**	**0.85 ± 0.96**		**<0.001**

BMI, body mass index; FBG, fasting blood glucose; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; T-C, total cholesterol; TG, triglycerides; NAMPT, nicotinamide phosphoribosyltransferase; IL-6, interleukin-6.

difference was observed between the diabetic patients and the control group regarding age, HbA1c, HDL-C, and LDL-C levels ($p < 0.001$). HbA1c values were significantly higher in the group with macrovascular complications (8.82%) than in the group without complications (7.84%). Additionally, HDL-C levels were considerably lower in the macrovascular complications group (43.05 mg\dL). LDL-C levels showed significant differences between the patient groups and control groups. They were found to be considerably lower in the macrovascular complication group (96.90 mg/dL) compared to the microvascular complication group (122.38 mg/dL) and the uncomplicated group (123.13 mg/dL). As expected, patients with diabetes had higher FPG levels than those in the control group ($p < 0.001$).

3.2. Gene expression analysis

We found that NAMPT, IL-6, and vaspin mRNA expression significantly decreased at all investigated groups without complications (NAMPT: 0.55 ± 0.58 fold change, $p < 0.001$; IL-6: 0.51 ± 1.00 fold change, $p < 0.001$; vaspin: 0.70 ± 0.74 fold change, $p < 0.001$), with microvascular complications (NAMPT: 0.61 ± 0.62 fold change, $p < 0.001$; IL-6: 0.31 ± 0.65 fold change, $p < 0.001$; vaspin: 0.62 ± 0.70 fold change, $p < 0.001$), and with macrovascular complications (NAMPT: 0.56 ± 0.60 fold change, $p < 0.001$; IL-6: 0.33 ± 0.74 fold change, $p < 0.001$; vaspin: 0.85 ± 0.96 fold change, $p < 0.001$) (Table 2, Figure 1).

3.2. Analysis of serum protein levels

NAMPT, IL-6, and vaspin protein levels in all investigated groups were 11.52 ± 4.49, 20.28 ± 5.23, 22.33 ± 5.30 in

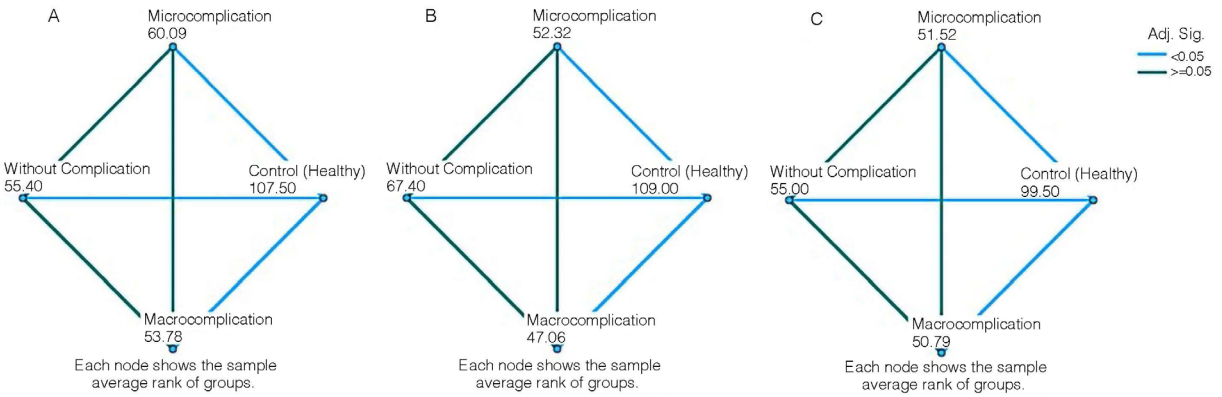


Figure 1. Statistical relationship between gene expression levels without complication, microvascular complications, macrovascular complications, and control groups (A: NAMPT gene expression, B: IL-6 gene expression, C: vaspin gene expression).

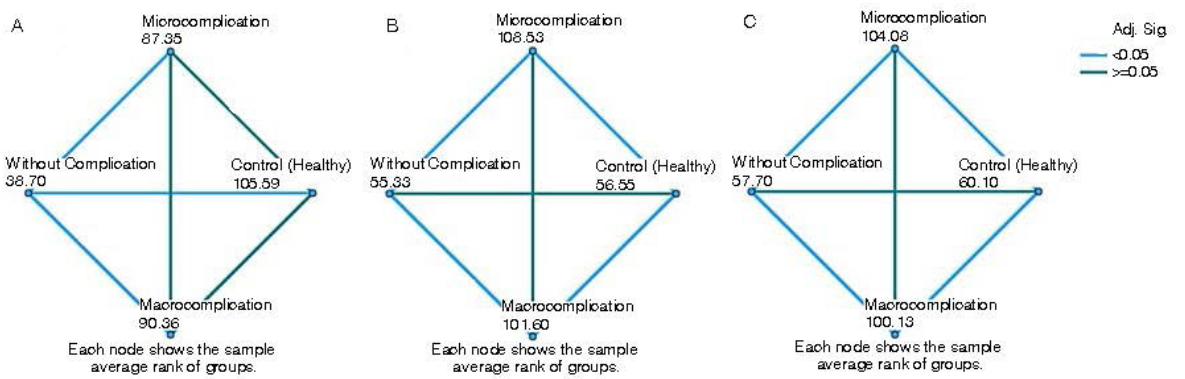


Figure 2. Statistical relationship between serum protein levels without complication, microvascular complications, macrovascular complications, and control groups (A: NAMPT protein level, B: IL-6 protein level, C: vaspin protein level).

the group without complications, and 16.31 ± 4.81, 27.41 ± 6.81, 29.39 ± 7.88 in microvascular complications, 16.91 ± 5.90, 27.03 ± 7.97, 28.87±7.63 in macrovascular complications, and 17.47 ± 3.81, 20.28 ± 4.81, 22.36 ± 2.53 in the control group, respectively (Table 2, Figure 2). We found that NAMPT serum protein levels without complications were significantly lower than those in all other groups and the control groups. On the other hand, IL-6 and vaspin at micro/macrovascular complications were considerably higher than those in the groups without complications and the control group.

4. Discussion

Diabetes is a chronic and broad-spectrum metabolic disorder characterized by hyperglycemia, which occurs due to relative or absolute insulin deficiency or "insulin resistance" developed against the effect of insulin in peripheral tissues, affecting many organs and causing multisystemic involvement. T2DM accounts for 90–95% of all diabetes cases and mainly occurs after the age of 30. Patients are often obese or overweight. Exposure to chronic hyperglycemia has a significant impact on the quality of life and overall life expectancy by causing microvascular events such as nephropathy, retinopathy, neuropathy, as well as macrovascular events such as peripheral arterial disease, coronary artery disease, and stroke.

In our study, the patients were overweight or obese. Although the duration of T2DM was statistically similar, glucose and HbA1c levels were higher in the groups that developed complications. In T2DM, blood glucose, and HbA1c levels are used to evaluate diagnosis and treatment effectiveness. Additionally, their relationship with micro- and macrovascular complications has been confirmed by studies (Stratton et al., 2000; Seshasai et al., 2011).

Lipid abnormalities are also common in patients with diabetes and contribute to an increased risk of these complications. In our study, LDL-cholesterol, the treatment target, was similar or lower in the complication groups than in the other groups. This was due to more intensive cholesterol-lowering treatment of patients who developed complications.

In the present study, we report for the first time the role of NAMPT, IL-6, vaspin gene, and protein levels in Turkish T2DM patients with micro- and macrovascular complications, without complication, and a healthy control group. Furthermore, this is the first time that people with such long-term diabetes (10 years) have been studied in three separate complication groups. The results of our data specify that NAMPT, IL-6, and vaspin gene expression levels significantly decreased in all groups compared to control groups. The results of the present study indicate that NAMPT serum levels decrease considerably in the group without complications compared to all other groups and controls. Similarly, Alshahrani et al. (2019) stated that NAMPT expression is reduced in obese patients with T2DM, and metformin treatment reverts NAMPT expression to normal levels. However, Kieswich et al. (2016) showed that serum levels increased for monomeric eNAMPT in diabetic mice. In our study, protein levels and gene expression were lower in individuals with diabetes than in the controls. Previous studies have shown that these low levels may contribute to the development of cardiovascular complications (Hausenloy, 2009; Lovren et al., 2009; Naz et al., 2017). In a study by Catalán et al. (2011), the expression levels of NAMPT in blood monocytes were examined, revealing higher rates in obese individuals with T2DM compared to those with nonobese diabetes. The expression levels in nonobese individuals with T2DM were similar to those in controls (Catalán et al., 2011). Additionally, high gene expression was observed in studies involving obese individuals, and similar gene expression was noted in nonobese individuals and controls. This can be explained by the fact that the individuals participating in our study were not obese.

On the other hand, IL-6 and vaspin levels show a significant increase in micro/macrovascular complications compared to those in groups without complications and the control group. In another study, Jian et al. (2014) reported that obese diabetic patients had increased serum vaspin levels. Results from our research and other studies suggest that vaspin plays a role in human insulin resistance (Genc et al., 2011). It can inhibit a protease that plays a role in the degradation of a hormone with direct or indirect glucose and lipid-lowering effects (Genc et al., 2011). Vaspin gene expression has been shown to decrease with loss of body weight and progression of diabetes, and vaspin serum levels return to normal with insulin and pioglitazone treatment (Hida et al., 2005). In different studies, vaspin levels in individuals with diabetes have been found to be both lower (Jian et al., 2014; Sathyaseelan et al., 2016) and higher (Bilir et al., 2016; Montazerifar et al., 2018). Irregular production of IL-6 and long-term exposure lead to inflammation, which induces insulin resistance and overt T2DM. There is a mechanistic relationship between the stimulation of IL-6 and insulin resistance. IL-6 causes insulin resistance by impairing the phosphorylation of insulin receptors and receptor substrate-1, inducing the expression of SOCS-3, a potential inhibitor of insulin signaling (Rehman et al., 2017).

Elevated plasma levels of proinflammatory cytokines such as IL-6 were observed in T2DM patients compared to non-T2DM individuals (Kozakova et al., 2019; Randeria et al., 2019). According to the results of the metaanalysis study, T2D patients had high levels of IL-6, and the risk of developing T2D was increased in individuals with elevated IL-6 levels (Bowker et al., 2020).

NAMPT and vaspin play roles in the intricate molecular processes related to T2DM and obesity. Their involvement in metabolic regulation, inflammation, and insulin sensitivity highlights their potential significance as targets for therapeutic interventions or biomarkers for assessing metabolic health. However, the precise mechanisms and therapeutic implications of these proteins in T2DM and obesity are areas of ongoing research (Garten et al., 2015). In addition to NAMPT and vaspin, interleukin-6 (IL-6) is a well-known pleiotropic cytokine and adipokine that plays crucial roles in metabolism and immunity in various biological systems and organs (Kang et al., 2019).

Both genetic and environmental factors influence gene expression. Interactions between genetic variants and environmental influences may contribute to the observed differences in gene expression. Differences in the characteristics of study populations, such as age, sex, ethnicity, and lifestyle factors, can contribute to variability in gene expression. Furthermore, the statistical power of a study, influenced by factors such as sample size, can affect its ability to detect actual differences. In addition to that, IL6 expression can vary in different tissues. If the studies focused on other tissues or cell types, this could result in variations in the findings. Additionally, T2DM is a heterogeneous condition with various underlying mechanisms contributing to its development. Gene expression patterns may differ among subtypes of T2DM patients. Subgroup analyses based on disease duration, severity, or response to treatment might provide more insights.

Investigating the molecular mechanisms behind T2DM helps researchers and healthcare professionals better understand the underlying causes and pathophysiology of the disease. Molecular studies identify specific molecular pathways and targets that play a role in T2DM. This information is valuable for developing targeted therapies that can address the root causes of the disease, potentially leading to more effective and personalized treatments. Biomarkers are measurable indicators of a biological state and can be used for early diagnosis, prognosis, and disease monitoring. This capability can lead to earlier intervention and better management of the condition. Molecular insights into T2DM contribute to the development of new drugs and therapies. Researchers can design more effective drugs with fewer side effects by targeting specific molecules or pathways.

Upregulated adipokines in the development of obesity and T2DM generally have proinflammatory effects, leading to a chronic inflammatory state and contributing to metabolic dysfunction. In contrast, antiinflammatory adipokines benefit adiposity and insulin activity (Cheng and Yu, 2022). However, there are conflicting data regarding the circulating levels of some adipokines in T2DM. Therefore, instead of focusing on a single factor, studying the relationship between multiple adipokine balances and metabolic diseases would be more helpful in understanding the pathogenesis of T2DM. In this study, we investigated cytokines secreted by adipose tissue, which have promising functions in T2DM and obesity because they are key regulators of insulin resistance and affect immune and inflammatory processes.

This study determined that NAMPT, mRNA expression, and protein levels were lower than those of the control, and mRNA and protein results were consistent. In the case of IL-6 and vaspin, mRNA expression levels were lower; however, protein levels were higher. These contradictory results may be obtained because of the long mRNA half-life due to the drug treatments received by patients with diabetic complications. We conclude that IL-6 and vaspin mRNA half-life increased for unknown reasons, resulting in an unexpected increase in protein expression.

Funding

This study was funded by the Gazi University Scientific Research Projects Coordination Unit (grant number:05/2020-03). Leyla AÇIK received research support from Gazi University BAP.

Acknowledgments

This article was produced from the doctoral thesis of the first author, Süheyla Pınar ÇELİK.

Contribution of authors

Süheyla Pınar ÇELİK, Leyla AÇIK, Mehmet Muhittin YALÇIN, and İlhan YETKİN contributed to the study design and conception of the manuscript revision, read, and approved the final version of the manuscript. Süheyla Pınar ÇELİK, Damla Nur PARILTI, Leyla AÇIK, Mehmet Muhittin YALÇIN, Eldeniz YUNUSOV, and İlhan YETKİN performed material preparation and analysis.

Conflict of interest

The authors declare that they have no conflict of interest.

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