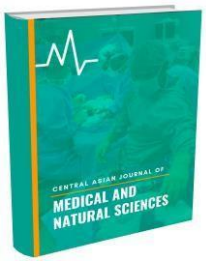

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Article

Functions of Some Sirtuins and Their effect on Type 2 Diabetes Mellitus in Iraqi Patients

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Abstract: This paper intended to estimate the interrelationship among the SIRT1 gene expression and type 2 diabetes mellitus (T2DM) in a sample of individuals from Wasit City, Iraq in collaboration with AL-Karama Teaching Hospital. A total of 60 participants (40 diabetic patients and 20 healthy controls), aged between 40 and 70 years, were enrolled. Blood samples were analyzed using RT-PCR to assess gene expression and ELISA to measure protein concentration in serum .SIRT1 gene expression was significantly upregulated in diabetic patients, as indicated by a lower ΔCt value, with strong statistical significance (P = 0.001). No significant difference in SIRT1 protein levels in the serum among the diabetic and controlling groups (P = 0.85). No significant gender-based differences were noticed in SIRT1 protein concentrations . The findings suggest a notable increase in SIRT1 gene expression in individuals with T2DM, potentially indicating a regulatory role of this gene in the disease. However, the lack of corresponding changes in protein levels warrants further studies to clarify the underlying mechanisms.

Keywords: SIRT1gene, T2DM, Real-TimePCR

1. Introduction

(T2DM) is among the commonest metabolic syndromes globally. Its onset is mainly attributed to two critical factors: damaged insulin secretion via pancreatic β-cells and the resistance of insulin-responsive tissues to insulin action [1]. Type 2 diabetes is witnessing a growing and alarming spread worldwide. Its causes are diverse and can be separated into adjustable risking factors like excessive calorie consumption, physical inactivity, and chronic stress—and non-adjustable ones, including genetic predisposition and aging. Overweight and obesity, often resulting from various social, economic, and cultural factors, are among the most significant contributors to the development of this disease [2], [3]. The development of T2DM is resulted out of interacting genetic predisposition, environmental influences, and behavioral factors. Type 2 diabetes arises due to the body's resistance to insulin and is more ubiquitous than type I diabetes, which results from inadequate insulin secretion. Additionally, around 5–15% of pregnant females experience gestational diabetes, a form of the disease that occurs exclusively during getting pregnant [4], [5].

In the past, diabetes mellitus was considered a rare disease, often identified by symptoms such as frequent urination . Today, however, it has become one of the most widespread diseases globally and is associated with various metabolic disorders [6]. It is estimated that around 8.8% of the global population between the ages of 20 and 79 is

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affected by this condition. The continuous rise in diabetes cases is largely attributed to lifestyle-related factors, including unhealthy eating habits, inadequate healthcare services, and a lack of physical activity [7].

In 2021, approximately 537 million were reportedly having diabetes, with more than 90% of cases being type 2. It is projected that this total is going to go up to 783 million by 2045. Managing type 2 diabetes involves nonpharmacological approaches like adoption of a wholesome diet and having engaged in consistent physical activity, in addition to pharmacological treatments that include medications or insulin injections [8], [9], [10]. However, drug therapies may lead to undesirable side effects, particularly in the digestive system, and can be financially burdensome for many patients. This highlights the need for safer alternative treatments. As a result, there is a growing need to develop new antidiabetic medications that target the root causes of the disease. Sirtuins protrude as a promising class of proteins in this regard, as research indicates their significant connection to diabetes and its risk factors [11]. Numerous scientists worldwide are actively studying these proteins to better understand their roles and potential as novel therapeutic targets. Moreover, various studies have shown that physical activity and calorie restriction can enhance overall health and activate sirtuins, which may help slow the progression of the disease [12]. Type 2 DM (T2DM) is typically categorized by defectively functioning insulin secretion from pancreatic beta cells. In typical settings, too much insulin release by pancreatic β-cells is generally adequate to effectively support insulin activity and consistently sustain normal glucose tolerance. Being obese, having nutrition disorders, hypertension, lifestyle, and genetic/hereditary factors were strongly involved in resisting insulin and type 2 DM via their directly disruptive effects on glucose metabolism [13], [14], [15]. The core task of sirtuins is to specifically deacetylate the imperative proteins for cellular homeostasis that broadly normalize myriad of processes for protein, carbohydrate, and lipid metabolism, mitochondrial homeostasis, and programmed cell death mechanisms like apoptosis and autophagy. Interestingly, adipocyte SIRT1 actively controls systemic glucose homeostasis and insulin sensitivity through continuing cross-talk with adipose-resident macrophages [16]. The cytoplasm and nucleus are the localiztions of SIRT2, and could extensively be shown in numerous tissues, like the brain, muscle, pancreas, liver, kidney, and adipose tissues. SIRT2 interacts with many histone and non-histone protein substrates, including tubulin and histone [17], [18], [19]. SIRT2 is involved in multiple cellular functions, including genomic integrity, cell growth, differentiation, and energy metabolism, and decreased SIRT2 activity was associated in cancer, neurodegeneration and metabolic diseases. Prior research uncovered that SIRT2 is effective in numerous physiological processing to maintain metabolic homeostasis, like inflammation, oxidative stress and mitochondrial function, as well as adipocyte differentiation, fatty acid oxidation, gluconeogenesis, and insulin sensitivity [20], [21], [22]. A small number of reports unveiled that SIRT2 makes anti-inflammatory and antioxidative tension impacts and optimizes mitochondrial functioning in metabolic-related tissues, like skeletalmuscle. SIRT2 is found in either the cytoplasm and the nucleus, and could broadly be found in numerous tissues, like the brain, muscles, pancrease, liver, kidneys, and adipose tissue. It copes with a range of both histone and non-histone protein substrates, such as tubulin and histone H4 [23], [24]. SIRT2 contributes to several cellular functioning, like integrity of genome, growth of cell, variation, and energy metabolism. Decreased SIRT2 activation was associated with cancer, neurodegenerative diseases, and metabolic disorders. Previous research also unveiled that SIRT2 plays a significant part in these biological processes . SIRT3 plays a positive aspect in glucose metabolism by enhancing insulin sensitivity and lowering blood glucose levels [25]. Preserving mitochondrial healthiness is undoubtedly critical to avoid insulin resisting and T2DM during getting elder. SIRT3 is located in the mitochondria. SIRT3 could undoubtedly be a core mitochondrial deacetylase and function in deacetylating and modify the enzymatic activations of numerous mitochondrial proteins [26]. The mammalian sirtuins as a whole

encompass numerous subcellular distributions, with a subset of sirtuins exist principally in nuclear (SIRT1, SIRT6, and SIRT7), cytosolic (SIRT2), or mitochondrial (SIRT3, SIRT4, and SIRT5) compartments. Impairments in the pathways dominated by SIRT1 and SIRT3 could undoubtedly cause varying metabolic and neurodegenerative diseases, like being obese, T2DM, nonalcoholic fatty liver, Alzheimer, as well as cancer. SIRT1 and SIRT3 are renowned of increasing mitochondrial biogenesis and improving mitochondrial functioning [27], [28], [29]. SIRT3 could be a key regulatory controller of mitochondrial protein acetylation levels and its biological activations. Earlier of T2DM, resistance to insulin could be the prevalent feature, leading to elevated insulin levels (hyperinsulinemia). As the disease progresses, glucose uptake and administration become impaired, and both hyperglycemia and hyperinsulinemia help in the gradual destruction of pancreatic β-islet cells. Other salient sirtuin in glucose metabolism is SIRT4. Glutamate dehydrogenase (GDH) is one of the target enzymes of SIRT4 converting glutamate to α-ketoglutarate in the mitochondrion. SIRT4 thwarts amino-acid induced secretion of insulin by standing for GDH. During food-fasting, SIRT4 is inhibited in liver. This induces gluconeogenesis from amino acids and fats and the inhibition of SIRT4 allows secretion related to insulin out of β-cells [30]. SIRT4 is triggered and the reactions reported earlier are retreated in the feeding. Uncovered that SIRT3 promotes ketogenesis via triggering acetyl-CoA synthetase in mammalian cells. Therefore, SIRT3 is likely to play a key part in the augmented ketogenesis associated with diabetes mellitus. SIRT1, SIRT3, and SIRT4 are key contributors to the development of hepatic steatosis, a condition frequently observed in individuals with diabetes mellitus. Overall, the suppression of SIRT1 and SIRT3 and/or the activating SIRT4 may be responsible for the elevated risk of hepatic steatosis as diabetes progresses

Dong et al. found an association between genetic variations in SIRT5 and SIRT6 and the occurrence of atherosclerosis. They also identified significant links among gender and various risk factors: smoking was correlations with SIRT5 and UCP-4; hypertension was linked to SIRT3, SIRT5, and UCP-5; and diabetes showed correlations with SIRT5 and UCP-5. Such findings indicate that genetic varieties in sirtuins may contribute to developing the vascular aging traits independently of conventional risk factors. SIRT6 is a nuclear protein that functions as both an ADP-ribosyl transferase and a NAD+-dependent deacetylase. It's a key part in regulating lifespan. According to findings by Kanfi and colleagues, Kanfi et al, increased expression of SIRT6 led to an extended lifespan in male mice, which was associated with decreasing serum levels of insulin-like growth factor 1 (IGF-1) and elevated levels of IGF-1 binding protein. SIRT6 also contributes to DNA repair, maintaining telomere, genomic stability, and cellular aging. Moreover, it inhibits NF-κB signaling by deacetylating histone H3K9 at the chromatin level. Sirtuin 7 (SIRT7), an affiliate to mammalian sirtuin family and classified as a class III histone deacetylase (HDAC), has gathered significant attention recently ownig to its essential roles in maintaining genome stability, facilitating DNA repair, regulating stress responses, supporting metabolic balance, and influencing aging, inflammation, and cancer development .Interestingly, several key risk factors for cardiovascular and kidney diseases—such as inflammation and aging—are conditions in which SIRT7 was revealed to have a consistent role in experimental studies.

2. Materials and Methods

A total of 60 individuals took part in this experiment, and they were:

a. Diabetic Group: This group included 40 participants diagnosed with type 2 diabetes mellitus (T2DM), comprising 20 males and 20 femalesFor each subject, the following information was documented: full name, sex, age, presence of other medical conditions, smoking habits, type of treating, body how much weighted, how tall, body mass index (BMI), place of residence, occupation, personal medical history, family

history of diabetes, and the date the sample was collected .All patients were diagnosed based on the diagnostic guidelines provided by (ADA).

b. The controlling group has20 apparently healthy individuals (10 males and 10 females).

Sample collection

Five milliliters of blood were drawn from each participant, and the serum was separated from the samples. SIRT1 levels in the serum were meticulously measured by the enzyme-tied immunosorbent assay (ELISA) technique with the Human SIRTUIN 1 ELISA Kit (Elabscience, China)

Blood Collection

A total of 5 mL of venous blood was obtained out of each participant using a plastic syringe. The sample was then divided into two portions. The first portion was placed in an EDTA tube, and 100μL of this portion was transmitted into an Eppendorf tube The first tube contained 300μL of Shield's solution and was stored in a refrigerator until further use

The second portion of blood (2.5 mL) was transferred into a gel tube and allowed to clot at sorrounding temperature for a few seconds. The serum was separated from the clot by centrifugation at 2000g for 10 minutes. After separation, the serum was efficaciously aliquoted to several labeled Eppendorf tubes and stored at −20 °C for ELISA assay.

Total RNA

Total RNA, including small RNAs ranging from 17 to 200 nucleotides, was effectively isolated from various sources, including blood samples

Total RNA Extraction

Traditional RNA isolation using phase separation has been shown to selectively enrich certain miRNA species, which can introduce bias into downstream analyses. The method (Direct-zol™) ensures balanced recovery of minor RNAs, including miRNAs

Buffer Preparation

a. Add 10mL or 40mL of 95–100% ethanol to 40 mL or 160 mL of Direct-zol™ RNA PreWash concentrate, respectively

b. Add 48mL of 100% ethanol (or 52 mL of 95% ethanol) to 12 mL of RNA Wash Buffer concentrate, or 192 mL of 100% ethanol (or 208mL of 95% ethanol) to 48 mL of RNA Wash Buffer concentrate.

3. Results

They uncovered that the mean concentration of SIRT1 in the patients' serum was 1.26 ± 0.14 pg/ml, compared to 1.34 ± 0.20 pg/ml in healthy individuals, (Table 1) with no statistically significant difference (P = 0.85), as presented in Table 1.

Table 1. 7SIRT1concentrations in patients and control.

Group	Concentration pg\ml mean+SD
Control	1.34+ 0.20
Diabetic patients	1.26+0.14
p-value	0.85
significant	*Non-significant

Table 2 presents the serum concentrations of SIRT1 in both diabetic patients and healthy controls, categorized by gender. No significant differences statistically found in SIRT1 levels between males in those groups, with patients and controls both recording a mean of 1.30 ± 0.140 pg/mL (P = 0.400). Similarly, among females, no significant difference

was found: patients had a mean concentration of 1.20 ± 0.169 pg/mL, while controls had 1.25 ± 0.190 pg/mL (P = 0.550). Additionally, gender-based comparisons within each group revealed no significant variation.

Table 2. SIRT1 Concentrations by Gender in the Studied Groups.

Groups	Male	Female	P-value	LSD	significant*
Control	1.30 ±0.205	1.25±0.190	0.605	0.224	Non
Diabetic Patients	1.30±0.140	1.20±0.169	0.200	0.406	Non
P-value	0.400	0.550			

Non-significant P > 0.05*

4. Discussion

The findings uncovered no significant differences in SIRT1 serum concentrations among affected persons with T2DM and healthy controls. These results are ascribed to the small sample size or potential limitations of the ELISA technique used in measuring SIRT1 levels. Human serum proteins originate from multiple tissues and enter the bloodstream through secretion or leakage, making them useful indicators of physiological or pathological states, as described by Thadikkaran et al. In alignment with our findings, a study by Ozlem Gok et al recounted no statistically significant differences in the expression levels of miR-181a, miR-132, miR-9, and miR-34a between diabetic and control groups (P > 0.05). However, contrasting results were observed by Fathy et al., where serum SIRT1 levels were significantly lesser in the normal buminuric group (P < 0.05). in Conclusion Overall, the present study suggests that serum SIRT1 levels may not be directly associated with T2DM.

5. Conclusion

In light of findings of this study, the following can be drawn :

a. There are noticeable associations among (T2DM) and the manifestation of SIRT1
b. The expression of SIRT1 can be affected by the presence of T2DM
c. SIRT1 is upregulated in individuals with type 2 diabetes .
d. Between the ELISA and RT-PCR methods, RT-PCR proved to be more sensitive, accurate, and reliable in assessing SIRT1 expression in T2DM patients.

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