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Association between serum sirt 1 and advanced glycation end products levels in type 2 diabetic nephropathy patients

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Abstract

This study aimed to evaluate the association between serum sirtuin1 (Sirt1) and AGEs levels in type 2 diabetes patients with different stages of diabetic kidney disease. Eighty type 2 diabetes patients were divided into three groups: normoalbuminuric group, microalbuminuric group, and macroalbuminuric group, along with 20 age and sex-matched healthy individuals were included in the study. Fasting blood glucose, fasting insulin, Homeostasis model assessment for insulin resistance (HOMA-IR), renal function tests, lipid profile, serum Sirt1 (ELISA) and serum AGEs (ELISA) were evaluated. Comparing to the control group, serum Sirt1 level was significantly lowered in normoalbuminric patients ($p< 0.05$). While, serum level of AGEs was significantly increased in all diabetic patients ($p< 0.001$). Serum Sirt1 was negatively correlated with microalbumin in macroalbuminuric patients. Additionally, serum Sirt1 showed a positive correlation with insulin and AGEs levels in total diabetic patients ($p< 0.05$ and $p< 0.001$ respectively). Multiple linear regression analysis showed that serum AGEs level was the only independent factor for Sirt1 in diabetic groups. In conclusion**:** Serum Sirt1 may be associated with minimal renal impairment in type 2 diabetic nephropathy patients.

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Introduction

Diabetic nephropathy (DN) is one of the major microvascular complications of diabetes mellitus and occurs in approximately 30% of type 2 DM patients (Kumar *et al.,* 2013). Diabetic nephropathy is the leading cause of end-stage renal disease and a major risk factor for cardiovascular disease (Donate-Correa *et al.,* 2015). Patients with diabetic nephropathy are at a higher risk of morbidity and mortality than those without nephropathy (Afkarian *et al.,* 2013). In this case, early diagnosis and prevention of DN are crucial.

Sirtuin1 (Sirt1), the mammalian homolog of Silent information regulator 2 (Sir2), was originally identified as a NAD^+-dependent protein deacetylase. It is localized predominantly in the nucleus, and also can be found in the cytoplasm. Sirt1 is associated with the regulation of a wide variety of cellular processes via deacetylation of histone and non-histone proteins, including transcription factors and transcriptional coregulatory proteins (Guarente *et al.,* 2011). Sirt1 plays an important role in various physiological processes such as oxidative stress, glucose metabolism, lipid metabolism, insulin secretion, DNA stability, aging and tumor genesis (Haigis and Guarente, 2006). Several studies performed on rats showed that Sirt1 alleviated diabetic nephropathy by reducing renal cell apoptosis relieving renal inflammation and fibrosis (Makino *et al.,* 2010; Kitada *et al.,* 2011). Under hyperglycemic conditions, advanced glycation end products (AGEs) are produced by a nonenzymatic reactions between the hydroxyl group of a reducing sugars and free amines of proteins as well as of aminolipids and nucleic acids; it begins with the conversion of reversible Schiff base adducts, and then to more stable, covalently-bound Amadori rearrangement products. Over a period of weeks, these early glycation products undergo further reactions, such as rearrangements and condensation to become irreversibly cross-linked derivatives termed (AGEs) (Baynes and Thorpe, 1999). The formation and accumulation of advanced glycation end products (AGEs), has known to be related to the pathology of microvascular complications (Monnieret *et al.,* 2005).

Previous studies revealed that AGE-rich diet elevates AGE levels in non-diabetic subjects and increases biomarkers of inflammation and oxidative stress associated with insulin resistance (Monnier *et al.,* 2005; Cai *et al.,* 2012).

This study was performed in order to find out the correlation between serum Sirt1 and AGEs levels in diabetic patients with different urinary albumin excretion rate.

Material and methods

Subjects

This cross sectional study was conducted on 80 type 2 diabetic patients (58 male, 22 female) aged 58.4±7.9 years who attended outpatient clinic in the department of Endocrinology at Ain Shams University Hospitals, Cairo, Egypt, during the period from January 2016 to December 2016. Type 2 diabetes was diagnosed on the basis of the American Diabetes Association 2010 criteria. Patients with hepatic diseases; cardiac diseases; rheumatic diseases; neoplastic diseases; other kidney diseases or other endocrine diseases were excluded. Patients classified into three groups according to urinary microalbumin excretion: Normoalbuminuric group (microalbumin <30 mg/day, n = 30), Microalbuminuric group (microalbumin 30-300 mg/day, n= 30) and macroalbuminuric group (microalbumin> 300 mg/day, n= 20). Additionally, 20 age and sex-matched healthy volunteers were afford to routine medical check-up and were enrolled as the control group. Informed consent was obtained from all subjects. This study was carried out in accordance with The Declaration of Helsinki for experiments involving humans and it was conducted with the approval of the Hospital Ethical Committee.

Collection of serum and urine samples

Fasting blood and 24 hours urine samples were collected and separated by centrifugation at 3000 rpm for 20 min. then each sample was stored at -80°C until analysis.

Anthropometric and analytic determinations

All the patients and control subjects underwent complete medical examination, anthropometric measurements (body weight, height and measured

BMI), Fasting blood glucose (FBG) was measured by using hexokinase method, fasting insulin (FINS) was measured by ELISA kit (Bio Source International Inc., Europe S.A.), and homeostasis model assessment of insulin resistance (HOMA-IR) calculated according to Matthews *et al.,* 1985. Serum total cholesterol (TC), triacyglycerols (TG), low-density lipoprotein (LDL-C), high-density lipoprotein (HDL-C), were measured enzymaticaly (Stanbio, North Main Street, Boerne, Texas, USA) in all samples. Serum urea, creatinine (Scr) and blood urea nitrogen (BUN) were measured using commercial kits (STANBIO-USA).Urinary microalbumin was measured by sandwich ELISA kit (ORGENTEC Diagnostic GmbH- Germany).Serum Sirt1 and AGEs were respectively measured by commercial sandwich ELISA kits (Elabscience Co., USA and Glory Science Co., Ltd, USA).

Statistical Analysis

The IBM SPSS statistics (V17.0, Chicago, IL, USA) was used for all data analysis. Results were expressed as mean±SD. Differences between groups were analyzed using ANOVA, followed by bonferroni correction for continuous variables.

Pearson correlation analysis was applied and multiple linear regression analysis was performed with to clarify the association between serum Sirt1 and other factors. All *p* values were two-tailed; *p* value< 0.05 was considered statistically significant.

Results

General Clinical and Biochemical Parameters

The clinical and biochemical characteristics of the study population are shown in Table 1. Fasting blood glucose (FBG), TGs, TC and LDL-C of diabetic patients were significantly higher than those in control group ($p< 0.001$), while HDL-C and FINS levels (in normoalbuminria group only) were significantly lower than the control subjects ($p< 0.001$). Additionally, in diabetic patients, HOMA-IR, Urea, Scr and BUN in microalbuminuria and macroalbuminuria groups were significantly higher than those in normoalbuminuria group. Furthermore, the levels of Urea, S cr and BUN in macroalbuminuria group were significantly higher than those in microalbuminuria group ($p< 0.001$).

Table 1. Demographic and clinical data of the study groups.

Variables	Control	Normoalbuminuria	Microalbuminuria	Macroalbuminuria
Gender (M/F)	20 (12/8)	30 (20/10)	30 (22/8)	20 (16/4)
Age(years)	57.3±6.7	58.3±7.4	58.6±8.8	58.4±7.6
BMI (Kg/m²)	32.4± 3.4	30.7±5.4	30.2±5.4	29.7±3.6
DM duration(years)	----	4.5±1.1	$6.4±1.8^{d}$	$8±1.1^{e,f}$
FBG (mg %)	91.5±9.1	$214.7±74.5^{a**}$	$237.2±134.9^{b**}$	$224± 82.6^{c**}$
FINS (µIU/L)	16.3±3.8	$9.2±4.4^{a*}$	14.7±11.6	14.6±11.5
HOMA-IR	3.6±0.9	4.6±2	$8.1±6.16^{b,d*}$	$8.1±6.8^{c*}$
TC (mg %)	159.5±7.2	$214±58.5^{a**}$	$207±50.1^{b**}$	$227±56.4^{c**}$
TGs (mg %)	54.2±4.5	$130.6±76.9^{a**}$	$112.2±57.6^{b**}$	$118.6±57.7^{c**}$
HDL-C (mg %)	49.3±7.5	$38.9±2.2^{a**}$	$38.2±4^{b**}$	$36.4±5.4^{c**}$
LDL-C mg %)	99.4±9.6	$155.4±46.1^{a**}$	$151.2±43.1^{b**}$	$167.8±50^{c**}$
S cr (mg %)	0.8±0.15	0.9±0.3	$1.7±0.5^{b,d**}$	$4.5±1.4^{c,e,f**}$
Urea (mg %)	31.1± 9	42.8±14.2	$70.6±30.3^{b,d**}$	$134.2±34.3^{c,e,f**}$
BUN (mg %)	15± 4.2	19.9±6.6	$32.9±14.2^{b,d**}$	$62.7±16^{c,e,f**}$
Microalbumin (mg/day)	12.4±4.8	15.9±7.1	$105±69.01^{b,d**}$	$396±14.4^{c,e,f**}$
AGEs(ng/L)	31.4 ±3.9	$49.7±13.3^{a**}$	$51.2±15.1^{b**}$	$56.3±24.5^{c**}$
SIRT-1(ng/ml)	3.1± 2.04	$2.2± 0.6^{a*}$	2.4±0.8	2.6±1.03

Data are means±SD for Gaussian variables and results at *: P <0.05, **: P <0.001.

a, b, c: normoalbuminuria, microalbuminuria and macroalbuminuria groups vs. control group, d and e: microalbuminuria and macroalbuminuria groups vs. normoalbuminuria group and f: macroalbuminuria group vs. microalbuminuria group. BMI: body mass index, FBG: fasting blood glucose, FINS: fasting insulin, HOMA-IR: homeostasis model assessment of insulin resistance, TC: total cholesterol, TGs: triglycerides, HDL: high-density lipoprotein, LDL low-density lipoprotein, Scr: serum creatinine, BUN blood urea nitrogen, AGEs: advanced glycation end products, Sirt1: sirtuin1.

Circulation Level of Sirt1and AGEs

Differences in the level of serum Sirt1 and AGEs according to albuminuria are also shown in Table 1. Serum level of Sirt1 in normoalbuminuric patients was significantly lower than the control subjects ($p< 0.05$). Otherwise, serum level of AGEs in all diabetic groups was significantly higher than the control group ($p< 0.001$).

Correlation Analysis

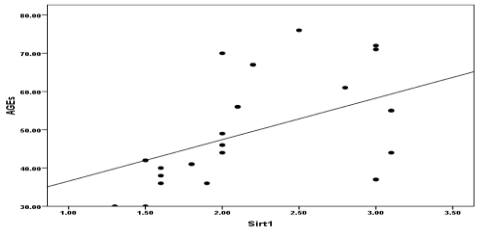
Pearson's correlation analysis between serum Sirt1 and various factors in patients with type 2 diabetes is summarized in Fig. (1). Serum Sirt1 was positively correlated with fasting blood glucose in normoalbuminuric patients (r= 0.377, $p< 0.05$) and with AGEs in all diabetics, normo- and microalbuminuric groups (r= 0.451, $p< 0.001$, r= 0.488, $p< 0.01$ and r= 0.578, $p< 0.01$ respectively). Also, Sirt1 was positively correlated with fasting insulin in both all diabetics and macroalbuminuria group (r= 0.224, $p< 0.05$ and r= 0.439, $p< 0.001$ respectively) while negatively with microalbumin in macroalbuminuric patients (r= -0.511, $p< 0.05$).

Table 2 displays the multiple regression analysis of Sirt1 with the other parameters. Serum AGEs was the only factor predicting serum Sirt1 level in all diabetic patients ($p< 0.001$).

Table 2. Multiple linear regression of Sirt1 and related factors.

	B	Std. Error	Standardized Coefficients	t	*P*
DM duration	0.007	0.047	0.019	0.051	0.880
FBG	0.001	0.002	0.165	0.815	0.434
FINS	0.027	0.022	0.325	1.219	0.225
TC	0.001	0.006	0.085	0.196	0.849
TGs	0.001	0.003	0.063	0.226	0.769
HDL-C	0.025	0.035	0.122	0.699	0.454
LDL-C	-0.002	0.006	-0.108	-0.329	0.754
Scr	0.103	0.127	0.209	0.811	0.371
AGEs	0.018	0.005	0.397	3.455	0.001
Microalbumin	-0.001	0.001	-0.213	-0.976	0.311
HOMA-IR	-0.040	0.043	-0.269	-0.919	0.372
BUN	0.007	0.007	0.169	0.887	0.412

FBG: fasting blood glucose, FINS: fasting insulin, HOMA-IR: homeostasis model assessment of insulin resistance, TC: total cholesterol, TGs: triglycerides, HDL: high-density lipoprotein, LDL low-density lipoprotein, Scr: serum creatinine, BUN blood urea nitrogen, AGEs: advanced glycation end products, Sirt1: sirtuin1



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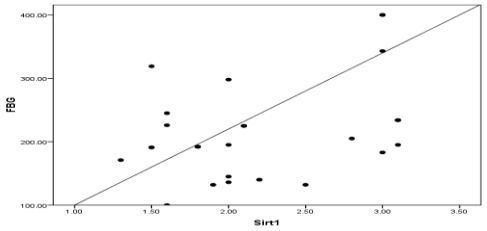

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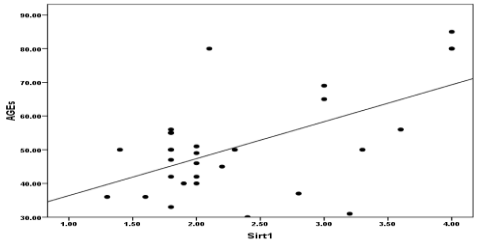

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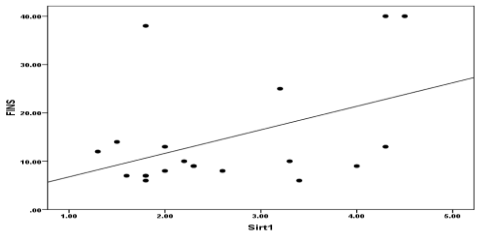

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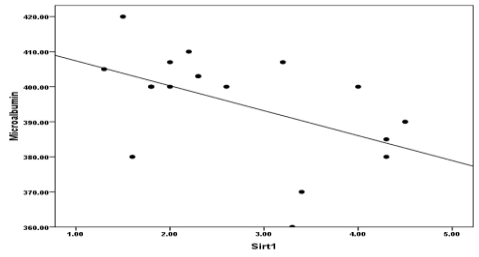

E

Fig. 1. Correlation between serum Sirt1 and other parameters in all diabetic patients.

A & B: represents normoalbuminuria group, C: represents microalbuminuria group and D & E: represent macroalbuminuria group.

Discussion

Hyperglycemia is a major contributing factor to the pathogenesis of multiple organs and tissues damage

caused by complex inflammatory and oxidative stress reactions (Nakagawa *et al.,* 2011). One of the most devastating microvascular complications is diabetic nephropathy. In the current study, Serum Sirt1 level was significantly lowered in normoalbuminuria group than that in the control group. This is supported by Chuang *et al.,* 2011 who found that increased level of AGEs was associated with decreased Sirt1 expression in tissue kidney specimens. While Sirt1 level was observed to increase gradually but not significantly with increasing the severity of disease. The increase in serum Sirt1 level may be due to its secretion from apoptotic cells that increased through different stages of diabetic nephropathy. Verzola *et al.,* 2007 proved that apoptosis of both glomerular and tubular cells is enhanced and contributed to decreased nephron remodeling and it was considered as a sensitive index for disease progression in patients with type 2 diabetic nephropathy. Conversely, Kume *et al.,* 2010; Kitada *et al.,* 2011 showed that Sirt1 expression level decreased in the kidney tissue of type 2 diabetic rats. Further, Shao *et al.,* 2017 afforded that serum Sirt1 level was lower in all stages of type 2 diabetic nephropathy compared to each other and compared to the control group.

In β-cell-specific Sirt1-overexpressing transgenic mice, Sirt1 improves glucose tolerance and enhances insulin secretion in response to glucose Moynihan *et al.,* 2005. Further, Bordone *et al.,* 2006 reported that a low serum Sirt 1 level resulted in disturbed insulin response to glucose stimulation in diabetic mice. Also, Lee *et al.,* 2009 demonstrated that Sirt1 protects β-cells against various toxic stresses such as oxidative stress and cytokines by suppressing NF-κB signaling. This is supported by our results that serum Sirt1 level was positively correlated with insulin in all diabetics ($p< 0.05$).

In the present study, serum Sirt1 was negatively correlated with urinary microalbumin in macroalbuminuric group only ($p< 0.05$). This result is in consistent with Wang *et al.,* 2016 who found that Sirt1 activation can reduce urinary albumin excretion and proximal tubule epithelial apoptosis both in human kidney proximal tubule epithelial (HK-2) cells and in diabetic rats.

This study revealed that serum Sirt1 was positively correlated with serum AGEs in all diabetics. However, it did not correlate with AGEs in macroalbuminuria group alone. Furthermore, multiple linear regression analysis showed that serum AGEs was an independent factor that significantly influenced serum Sirt1. Conversely, Chuang *et al.,* 2011 reported that hyperglycemia aggravated podocyte apoptosis via increasing the production of advanced glycation end products (AGEs), which in turn increases FOXO4 acetylation and suppresses Sirt1 expression in glomeruli of diabetic mice. Additionally, Yubero-Serrano *et al.,* 2015 reported that reduction of AGEs was accompanied with increase in Sirt1 levels in patients with diabetic kidney disease treated with sevelamer carbonate.

Our results afforded that a highly significant elevation in serum AGEs level in all stages of nephropathy compared to the control group. This result in accordance with Wu *et al.,* 2010 who revealed that AGEs were increased in type 2 diabetes patients, suggesting that AGEs could be a significant contributor to the progression of DKD in patients with either type 1 or 2 diabetes via enhancement of the expression of TNF receptors. Further, Yubero-Serrano *et al.,* 2015 reported that the levels of systemic and cellular AGEs were decreased in patients with diabetic kidney disease treated with sevelamer carbonate while the patients restored the levels of several innate defenses and had improved inflammation. On the other hand, Shimoike *et al.,* 2000 reported that serum AGE levels in only diabetic patients with overt proteinuria and hemodialysis were significantly higher than those in the normal subjects. However, there were no significant differences in normo- and microalbuminuric patients. They suggested that increased serum AGEs level may be mainly due to decreased removal in the kidney rather than increased production by high glucose levels or oxidative stress.

This study has certain limitations: 1) Small number of patients in groups so we think that our results should be confirmed in larger cohorts. (2) Patients under medications for diabetes mellitus and renal diseases precluded us from providing a comprehensive estimation of serum Sirt1 level with diabetic nephropathy.

Conclusion

serum Sirt1 level may be changed during the minimal renal impairment in the course of DM. Also, serum AGEs level is the only predictor for serum Sirt1 in diabetic nephropathy patients.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper. This research did not receive any specific grant from funding agencies in the public, commercial, or not –for-profit sectors.

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