British Biotechnology Journal
4(8): 932-945, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Relation of Sirtuin 1 Gene Polymorphisms with Lipid Profile in Hemodialysis Patients

Yasser Elghobashy[1], Safaa Tayel[1*], Abeer ALrefai[1], Said Khamis[2] and Hany Elbarbary[2]

[1]Medical Biochemistry Departments, Faculty of Medicine - Menoufia University, Egypt.
[2]Internal Medicine departments, Faculty of Medicine - Menoufia University, Egypt.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Original Research Article

Received 27th June 2014
Accepted 20th July 2014
Published 14th August 2014

ABSTRACT

Very little is known about the genetic variation of SIRT 1 and its effects on energy homeostasis in humans. Mammalian SIRT1deacetylates a host of target proteins that are important for apoptosis, the cell cycle, circadian rhythms, mitochondrial function and metabolism. In particular, much current research focuses on the impact of SIRT1 in glucose homeostasis, lipid metabolism and energy balance.

Objective: To study the relationship of sirtuin 1 gene polymorphisms with lipids profile in hemodialysis patients.

Subjects and Methods: This study included 70 Egyptian subjects (45 patients on hemodialysis and 25 age and gender matched healthy control group). The genotyping of SIRT1 rs7895833 in the promoter region, rs7069102 in intron 4, and rs2273773 in exon 5 was performed using polymerase chain reaction with confronting two-pair primers assay (CTPP). Serum TC, TG, HDLc, LDLc, fasting glucose, urea and creatinine were measured by standard colorimetric methods.

Results: The patients had higher diastolic and systolic BP (P<0.001), fasting blood glucose (P<0.001), TC (P<0.001), TG (P=0.006), LDLc (P=0.004), urea (P<0.001) and creatinine (P<0.001). Males and female patients differ according to cause of hemodialysis (P= 0.02) and serum creatinine (P=0.007). Control subjects of sirtuin1 rs7895833 showed significant A allele compared with patients (46% vs. 27.78% P= 0.04) while C allele of sirtuin1 rs7069102 not differ between groups (P>0.05). Sirtuin 1 rs2273773, patients

**Corresponding author: Email: drsafaa_tayel@yahoo.com;*

showed significant lower frequency of C allele compared with control (26.67% vs.44% P= 0.04,).
Conclusion: Significant association of SIRT1 rs7895833 and rs2273773 polymorphisms with dyslipidemia and blood pressure may modulate disease course in hemodialysis.

Keywords: Sirtuin 1; lipids; CTPP; hemodialysis.

1. INTRODUCTION

Patients on renal replacement therapy (RRT) are at increased risk of cardiovascular (CV) mortality and morbidity compared to the general population (Kundhal and Lok, [1]. Every year, between 10-20% of all patients on dialysis die, with about 45% of deaths attributed to CV causes (Collins et al. [2]). Established 'traditional' atherosclerosis risk factors, such as hypertension and dyslipidemia, have been recognized as independent predictors of cardiovascular disease (CVD) among chronic kidney disease (CKD) (Maheshwari et al. [3]). Thus it is important to search for a relationship of these risk factors and patients with end stage renal disease (ESRD) or undergoing hemodialysis.

Sirtuins, or silent information regulator 2 (Sir2) proteins are NAD-dependent protein deacetylases known to have effects against age-related diseases such as cancer, diabetes, cardiovascular, neurodegenerative and renal diseases (Gizem and Tiago, [4]). It was initially identified from studies of aging in yeast (Imai et al. [5] and Liang et al. [6]).

Sir2 protein is related to longevity in lower organisms such as yeast, flies, and worms (Blander and Guarente, [7]). Sir2 has also been concerned in life-span extension during caloric restriction in these organisms (Wang and Tissenbaum, [8]).

In mammals, there are seven sirtuins SIRT 1 - 7, all possessing a highly conserved central NAD^+- binding site and common catalytic domain (Haigis and Sinclair, [9]). Sirtuins are structurally different with respect to their N- and C-trmini (Frye, [10]), their subcellular localization and in that they utilize different substrates and protein binding partners (Guarente, [11]).

Mammalian SIRT1 is most homologous to yeast Sir2. With respect to sub-cellular distribution, SIRT1 is mainly nuclear, where it associates with euchromatin, although it can transiently be found in the cytoplasm (Tanno [12]). It can deacetylate a variety of substrates and is, therefore, involved in a broad range of physiologic functions. SIRT1 deacetylates forkhead transcription factor 1 (FOXO1) and promotes its activity (Nakae et al. [13]). FOXO1 may have protective or negative effects on insulin resistance and vascular function (Armoni et al. [14] and Rodgers et al. [15]). SIRT1 also deacetylates peroxisome proliferator-activated receptor gamma coactivator 1 (PGC-1) and increases its activity (Lagouge et al. [16]) PGC-1 is involved in a wide variety of biologic responses, including adaptive thermogenesis, mitochondrial biogenesis, glucose and lipid metabolism, fiber type switching in skeletal muscle, and heart development (Liang and Ward [17]).

The involvement of NAD^+ in the deacetylation reaction is thought to link sirtuin deacetylase activity to metabolism. SIRT1 regulates energy metabolism and mediates the longevity effect of calorie restriction (CR) by promoting gluconeogenesis and repressing glycolysis in the liver via deacetylation of PGC-1α (Rodgers et al. [18]). SIRT1 is associated with lipid metabolism through the activation of nuclear receptors, including PPAR-α (peroxisome proliferator

activated receptor) (Purushotham et al. [19]), LXR (liver X receptor), FXR (farnesoid X receptor) and negative regulation of SREBP (sterol-regulatory-element-binding protein) (Walker et al. [20]). Thus, variations of the SIRT1 gene might affect the determination of inter-individual variations of plasma lipid levels.

This study aims to investigate the association of SIRT 1 gene single-nucleotide polymorphisms, namely, rs7895833, rs7069102, and rs2273773 with lipid profiles in hemodialysis (HD) patients.

2. SUBJECTS AND METHODS

2.1 Subjects

This study included 70 Egyptian subjects who were divided into two groups (1) 45 patients on hemodialysis, (23 hypertensive nephropathy (51.1%), 4 diabetic nephropathy (8.9%), 6 hypertensive and diabetic nephropathy (13.3%) and 12 patients with other causes like Focal segmental glomerulosclerosis (fsgs), amyloidosis and atrophic kidney (26.7%). All patients were receiving chronic hemodialysis therapy; they were selected from dialysis unit of internal medicine, Menoufiya University hospitals. The patients included 30 male and 15 female. Mean age was 46.69±15.30 years, and the average of dialysis duration were 3.44±1.9 years. (2) 25 sex and age matched healthy control group, (14 male and 11 females; mean age 46.50±14.83 years). All the participants in this study provided written informed consent, and the study protocol was approved by the ethics committees of Menoufiya University.

2.2 Methods

Blood samples were obtained after 12h of fasting. Blood was centrifuged and Serum was separated and used for routine laboratory measurements of the following biochemical parameter by standard coloremetric laboratory methods; serum total cholesterol, serum triglycerides, serum high-density lipoprotein cholesterol (HDLc), and serum low-density lipoprotein (LDLc) cholesterol, fasting serum glucose, serum urea and serum creatinine (kits provided by spinreact, spain).

Genotyping of SIRT1 Gene SNPs:- blood collected on EDTA was used for DNA extraction using Gene JET Whole Blood DNA Mini Kits (Thermo Scientific, Sigma), and extracted DNA was stored at – 20ºC for direct PCR amplification. The allele frequencies of rs7895833, rs7069102, and rs2273773 were compared with their respective values for general population, The genotyping of rs7895833 in the promoter region, rs7069102 in intron 4, and rs2273773 in exon 5 were performed using polymerase chain reaction with confronting two-pair primers assay (CTPP).

	(1) rs7895833	**(2) rs7069102**	**(3) rs2273773**
Forward primer 1	CCCAGGGTTCAACAA ATCTATGTTG	GTAGCAGGAACTACAG GCCTG	GTGTGTCGCATCCAT CTAGATAC
Reverse primer 1	GCTTCCTAATCTCCA TTACGTTGAC	CTATCTGCAGAAATAA TGGCTTTTCTC	GTAGTTTTCCTTCCT TATCTGACAG
Forward primer 2	GGTGGTAAAAGGCC TACAGGAAA	GAGAAGAAAGAAAGG CATAATCTCTGC	CTCTCTGTCACAAAT TCATAGCCT
Reverse primer 2	CCTCCCAGTCAACGA CTTTATC	GATCGAGACCATCCTG GCTAAG	CTGAAGTTTACTAAC CATGACACTG

The region containing this polymorphism was amplified by PCR with these primers in table (Invitrogen, USA) with initial denaturation at 95ºC for 10 min, followed by PCR using 25 pmole of each primer, 0.4 mmole of dNTPs, 1.5mmole of Mgcl2, 1.5 units of tag polymerase and 1x Tag buffer (New England Biolabs, Beverly, MA, USA). Thermal cycler conditions include the initial denaturation at 95ºC for 5 min, followed by 35 cycles (at 95ºC for 1 min, at 60ºC for 1 min, at 72ºC for 1 min), and additionally at 72ºC for 5 min using thermal cycler (thermal cycler Applied Biosystems 2720 (Singapore)). PCR products were visualized on a 2% agarose gel with ethidium bromide staining. Genotyping was performed as follows: for (rs7895833) 320, 241 bp for AA genotype; 320, 241, 136 bp for AG genotype; and 320, 136 bp for GG genotype, Fig. 1 for (rs7069102) 391, 277 bp for CC genotype; 391, 277, 167 bp for CG genotype; and 391, 167 bp for GG genotype Fig. 2 and for (rs2273773) 314, 228 bp for CC genotype; 314, 228, 135 bp for CT genotype; and 314, 135 bp for TT genotype Fig. 3.

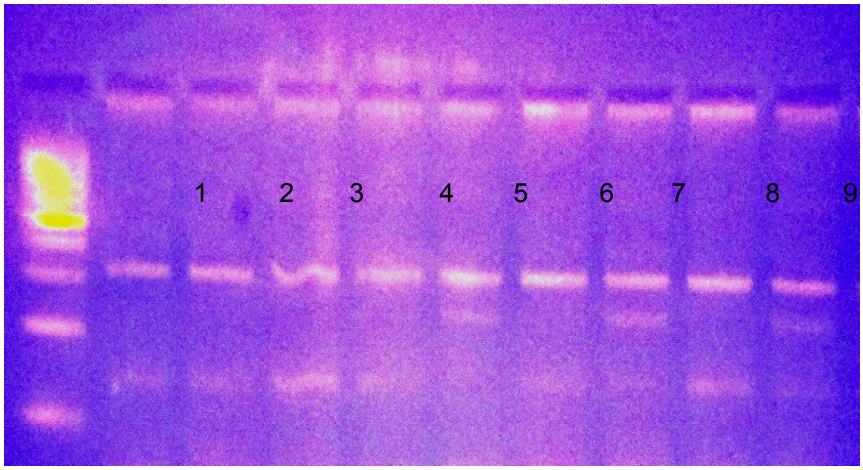

Fig. 1. For (rs7895833) lanes 1-4,6 and 8 shows GG genotypes, lane 5 shows AA genotype; lanes 7, 9 shows AG genotypes; using 100 bp ladder

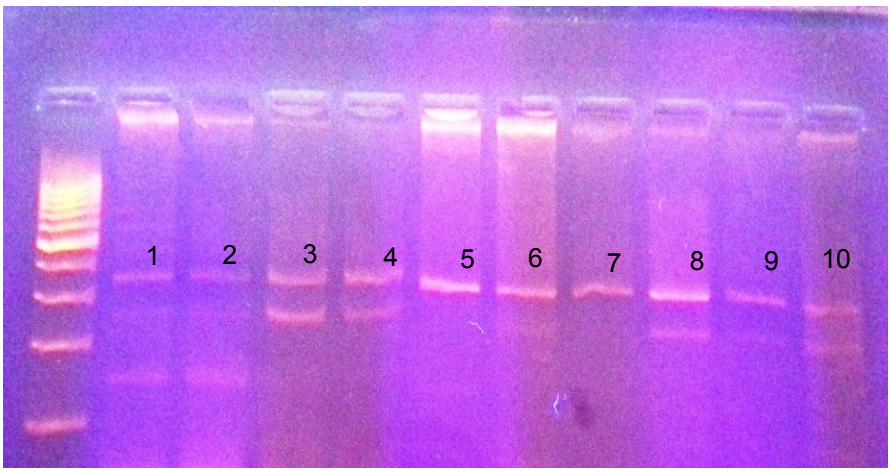

Fig. 2. For (rs7069102) lanes1-2 shows CG genotype; lanes 3,4, 6, 8 and 10 shows CC genotype; and lanes 5 and 7 for GG genotype using ladder 100bp

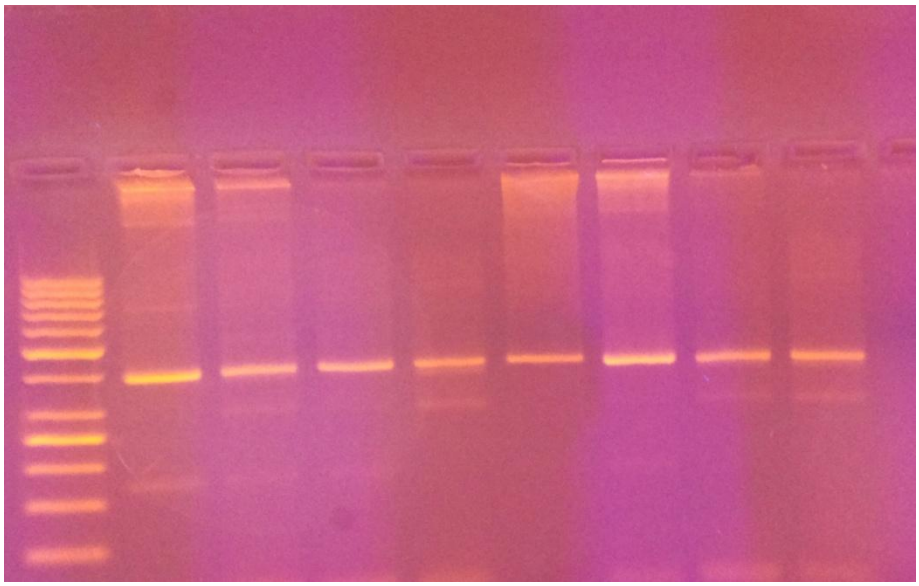

Fig. 3. For (rs2273773) lanes 1,5-6 for TT genotype, lanes 2,3 for CT genotype; and lanes 4, 7-8 for CC genotype using 50bp ladder

2.3 Statistical Analysis

Statistical analysis was done using SPSS software, version 16, Echosoft Corporation, USA. Categorical data are presented as percentages and continuous variables as means± standard deviation. Comparisons between groups were calculated by Chi-Square test with P for categorical variables and by t-test for continuous variables. One way ANOVA test was used when comparing more than 2 continuous variables. Linear regression analysis was used for independent relation of gene with other clinical variables. P values <0.05 were considered significant.

3. RESULTS

A total of 45 patients (30 male and 15 female) on chronic hemodialysis therapy were age (46.69±15.30 vs 46.50±14.83, P > 0.05) and sex matched (P > 0.05) with 25 healthy subjects (14 male and 11 females) were chosen as a control group. The patients had significantly higher diastolic (105.56±15.30 vs 79.60±8.88) and systolic BP (148.44±10.65 vs.121.60±14.34) (P<0.001), fasting blood glucose (128.87±23.79 vs 78.40±6.58) (P<0.001), TC (153.60±49.89 vs. 103.80±40.30) (P<0.001), TG (147.37±43.06vs. 92.40±40.34) (P=0.006), LDLc (93.92±16.38vs. 67.16±14.03) (P=0.004), urea (107.47±31.55 vs. 33.28±8.81) (P<0.001) and creatinine (5.68±1.82 vs. 0.86±0.23) (P<0.001) while HDLc (36.92±10.86 vs. 41.28±10.42) was not significant (P>0.05) (Table 1). In Table 2 males and females patients not differ in age, duration of hemodialysis diastolic and systolic BP, fasting blood glucose, TC, TG, LDLc, HDLc and urea (P > 0.05) however they differ according to cause of hemodialysis (P= 0.04) and serum creatinine (P=0.007). When comparing males and females patients with total patients only seum creatinine was significantly higher in males (P=0.02). Considering sirtuin1 rs7895833 genotypes were not significantly associated and healthy control subjects shows higher significant A allele compared with hemodialysis patients of (46% vs. 27.78% P= 0.04) with odds ratio (OR) = 2.2 (95%CI= 1.07-4.56) while

CC genotype and C allele of sirtuin1rs7069102 not differ between hemodialysis patients and healthy control (P>0.05) with odds ratio (OR) = 1.6 (95%CI= 0.75-3.75). Sirtuin 1 rs2273773, genotypes were not significant while hemodialysis patients shows significantly lower frequency of C allele compared with healthy control (26.67% vs.44% P= 0.04), odds ratio (OR) = 2.28 (95%CI= 1.1- 4.76) Table 3. Relation of genotypes of sirtuin1 rs7895833 with measured clinical parameters revealed association of GG genotype with diastolic BP (P=0.004), TC (P=0.01) and LDLc (P= 0.001) while no significant association of GG genotype with age, duration of hemodialysis, systolic BP, blood glucose, TG, HDLc, blood urea and creatinine (P>0.05) in Table 4. Sirtuin 1 rs7069102 , CC genotype was associated with higher LDLc (P=0.01) while no significant association with age, duration of hemodialysis, diastolic and systolic BP, blood glucose,TC, TG, HDLc, blood urea and creatinine (P>0.05) Table 5. In Table 6 TT genotype was significantly associated with diastolic BP (P=0.01), TC (P=0.03) and LDLc (P=0.008) while no significant association of TT genotype with age, duration of hemodialysis, systolic BP, blood glucose, TG, HDLc, blood urea and creatinine (P>0.05). Table 7 showed linear regression analysis of different sirtuin 1 rs7895833, rs7069102 and rs2273773 alleles with clinical parameters. For rs7895833 G allele can be independently associated with diastolic (P=0.001) and systolic BP (P=0.04), TC (P=0.005), TG (P=0.01), LDL (P<0.001) and creatinine (P=0.04) while no significant association with blood glucose, HDLc and blood urea (P>0.05). For rs7069102 C allele can be independently associated with diastolic BP (P=0.04), LDL (P=0.04) and creatinine (P=0.04) while no significant association with systolic BP, blood glucose, TC, TG, HDLc and blood urea (P>0.05). For rs2273773 T allele can be independently associated with diastolic BP (P=0.009), TC (P=0.02) and LDL (P=0.007) while no significant association with systolic BP, blood glucose, TG, HDLc, blood urea and creatinine (P>0.05).

Table 1. Demographic and Clinical parameters in control subjects and hemodialysis patients

Age	**Control N= 25 46.50±14.83**	**Patients N= 45 46.69±15.30**	**P value 0.96**
Gender			
Males no (%)	14(56%)	30(66.67%)	0.53
Females no (%)	11(44%)	15(33.33%)	
Diastolic blood pressure	79.60±8.88	105.56±15.30	<0.001
Systolic blood pressure	121.60±14.34	148.44±10.65	<0.001
Fasting blood glucose mg/dl	78.40±6.58	128.87±23.79	<0.001
TC mg/dl	103.80±40.30	153.60±49.89	<0.001
TG mg/dl	92.40±40.34	147.37±43.06	0.006
HDLc mg/dl	41.28±10.42	36.92±10.86	0.108
LDLc mg/dl	67.16±14.03	93.92±16.38	0.004
Urea mg/dl	33.28±8.81	107.47±31.55	<0.001
Creatinine mg/dl	0.86±0.23	5.68±1.82	<0.001

Table 2. Demographic and clinical parameters in male and female patients

	All cases N= 45	Males N= 30	Females N=15	P 1 value	P2 value
Age (years)	46.69±15.30	46.10±14.70	49.80±17.98	0.74	0.46
Cause		20(66.67%)	3(20%)	0.14	0.02
HTN NEPH	23(51.1%)	1(3.33%)	3(20%)		
DM NEPH	4(8.9%)	3(10%)	3(20%)		
HTN&DM	6(13.3%)	6(20%)	6(40%)		
others	12(26.7%)				
Duration (years)	3.44±1.9	2.47±1.67	5.43±1.05	0.16	0.058
Diastolic blood pressure	105.56±15.30	104.0±15.88	108.67±14.07	0.63	0.34
Systolic blood pressure	148.44±10.65	149.33±10.80	147.33±10.99	0.83	0.564
Fasting blood glucose mg/dl	128.87±23.79	127.90±21.02	128.13±31.36	0.98	0.97
TC mg/dl	153.60±49.89	145.31±45.34	167.97±48.99	0.33	0.13
TG mg/dl	147.37±43.06	141.19±47.14	150.39±48.26	0.93	0.73
HDLc mg/dl	36.92±10.86	35.21±10.29	40.75±11.94	0.27	0.11
LDLc mg/dl	93.92±16.38	88.66±31.21	106.67±40.32	0.28	0.10
Urea mg/dl	107.47±31.55	105.90±37.31	109.53±17.74	0.93	0.72
Creatinine mg/dl	5.68±1.82	6.68±2.15	5.12±1.48	0.02	0.007

P1 value of ANOVA test;P2 value of t test between male and female subjects

Table 3. Distribution of sirtuin 1 genotypes and alleles in control subjects and hemodialysis patients

	Control N= 25	Patients N= 45	P value
1- rs7895833			
GG	7(28%)	24(53.3%)	X2= 4.6
GA	13(52%)	17(%37.8)	P= 0.09
AA	5 (20%)	4 (8.9%)	
G allele	27 (54%)	65(72.22%)	X2= 3.9
A allele	23 (46%)	25(27.78%)	P= 0.04
OR (95% CI)	OR= 2.2 (95%CI= 1.07-4.56)		
2- rs7069102			
CC	17(68%)	22(48.89%)	X2= 2.6
CG	5(20%)	17(37.78%)	P= 0.26
GG	3(12%)	6(13.33%)	
C allele	39(78%)	61(67.78%)	X2= 1.18
G allele	11(22%)	29(32.22%)	P= 0.27
OR (95% CI)	OR= 1.6 (95%CI= 0.75-3.75)		
3- rs2273773	7(28%)	26(57.78%)	X2= 5.8
TT	14(56%)	14(31.11%)	P= 0.054
TC	4(16%)	5(11.11%)	
CC			
T allele	28(56%)	66(73.33%)	X2= 4.2
C allele	22(44%)	24(26.67%)	P= 0.04
OR (95% CI)	OR= 2.28 (95%CI= 1.1- 4.76)		

Table 4. Relation of different sirtuin 1 rs7895833 genotypes with demographic and clinical parameters

	rs7895833			P value
	GG	GA	AA	
Age (years)	48.21±15.83	46.23±14.14	44.32±16.64	0.90
Duration (years)	3.59±1.14	4.46±1.39	1.95±0.71	0.35
Diastolic blood pressure	113.81±15.32	99.23±11.87	97.27±11.03	0.004
Systolic blood pressure	151.90±11.23	146.15±9.60	144.559.34	0.125
Fasting blood glucose mg/dl	125.57±26.09	132.38±22.61	131.0±21.74	0.57
TC mg/dl	176.0±48.66	138.46±49.37	128.70±35.44	0.013
TG mg/dl	184.37±35.58	118.31±49.10	111.08±38.17	0.21
HDLc mg/dl	35.31±10.13	38.29±11.85	38.40±11.62	0.6
LDLc mg/dl	113.78±30.38	82.83±36.94	69.13±15.97	0.001
Urea mg/dl	109.62±26.6	109.77±31.80	100.64±41.03	0.52
Creatinine mg/dl	6.11±1.92	5.85±1.95	4.66±1.04	0.08

Table 5. Relation of different sirtuin 1 rs7069102 genotypes with clinical parameters

	rs7069102			P value
	CC	CG	GG	
Age (years)	50.68±15.67	44.03±13.99	40.21±15.51	0.25
Duration (years)	3.36±0.11	4.01±0.83	2.39±0.59	0.81
Diastolic blood pressure	111.36±16.41	98.75±11.47	102.86± 13.8	0.056
Systolic blood pressure	150.91±11.50	145.63±8.92	147.14±11.12	0.35
Fasting blood glucose mg/dl	124.59±23.13	133.56±23.55	131.57±24.2	0.49
TC mg/dl	168.15±49.59	138.56±46.39	142.20±52.28	0.14
TG mg/dl	173.02±40.74	114.56±45.04	141.76±45.06	0.27
HDLc mg/dl	36.259±10.73	38.51±12.0	35.40±9.54	0.71
LDLc mg/dl	107.90±33.94	86.74±34.48	77.85±34.74	0.01
Urea mg/dl	109.27±28.59	107.38±30.23	102.0±35.90	0.35
Creatinine mg/dl	6.07±1.91	5.73±1.80	4.35±0.92	0.056

Table 6. Relation of different sirtuin 1 rs2273773 genotypes with demographic and clinical parameters

	rs2273773			P value
	TT	TC	CC	
Age (years)	51.10±15.15	43.06±15.58	43.43±13.58	0.30
Duration (years)	3.48±1.32	3.84±1.1	2.28±1.9	0.81
Diastolic blood pressure	113.50±15.65	98.33±12.0	101.43±12.15	0.01
Systolic blood pressure	152.0±11.51	145.56±8.55	145.71±11.33	0.13
Fasting blood glucose mg/dl	125.55±22.95	134.06±26.84	125.0±17.55	0.5
TC mg/dl	174.97±46.36	136.13±49.2	137.43±42.94	0.03
TG mg/dl	177.72±45.03	125.91±44.98	115.86±40.67	0.37
HDLc mg/dl	35.68±10.26	37.18±11.62	39.82±11.5	0.59
LDLc mg/dl	110.86±33.52	81.94±34.85	76.34±30.9	0.008
Urea mg/dl	109.15±29.52	104.0±30.61	111.57±34.6	0.64
Creatinine mg/dl	6.22±1.91	5.33±1.95	5.057±0.59	0.14

Table 7. Linear regression analysis of different sirtuin 1 rs7895833, rs7069102 and rs2273773 alleles with clinical parameters

	rs7895833		rs7069102		rs2273773	
	β	P	β	P	β	P
Diastolic blood pressure	-.477	0.001	-.295	0.04	-.384	0.009
Systolic blood pressure	-.300	0.04	-.183	0.22	-.265	0.07
Fasting blood glucose mg/dl	.108	0.48	.146	0.33	.052	0.73
TC mg/dl	-.412	0.005	-.247	0.10	-.339	0.02
TG mg/dl	-.357	0.01	-.204	0.17	-.284	0.059
HDLc mg/dl	.128	0.40	.011	0.94	.130	0.39
LDLc mg/dl	-.524	<0.001	-.303	0.04	-.397	0.007
Urea mg/dl	-.105	0.49	-.500	0.61	-.032	0.97
Creatinine mg/dl	-.306	0.04	-.300	0.04	-.258	0.08

4. DISCUSSION

Cardiovascular disease is a major cause of morbidity and mortality among patients with chronic kidney disease, and accounts for 50% of all deaths in them. The high risk of cardiovascular morbidity and mortality in ESRD patients is associated with a high prevalence of classic cardiovascular risk factors (hypertension, diabetes mellitus, dyslipidemia, smoking, and advanced age) (Maria et al. [21]). Dyslipidaemia does not only accelerate atherosclerosis in these patients but also progresses the renal disease (Mshelia et al. [22]).

The present study showed that the HD patients had significantly higher diastolic BP, systolic BP, fasting blood glucose, TC, TG and LDLc than control subjects.

This in agreement with Herspink et al. who stated that the blood pressure is commonly high in HD patients and this phenomenon has been, attributed to several causes, among them the chronic volume overload in HD patients, due to impaired blood pressure homoeostasis function (Herspink et al. [23]). As regards dyslipidemia associated with HD patients in the present study elevated serum concentrations of total and LDL cholesterol as well as increased serum triglycerides concentrations are typical lipid abnormalities in patients with nephrotic syndrome (Joven et al. [24]). Both proteinuria and hypoalbuminemia stimulate the activity of 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase and ACAT (Vaziri et al. [25]), as well as may decrease the expression of the LDL receptor in the liver (Vaziri [26]). Impaired clearance of TG-rich lipoproteins Kashyap et al. [27]) and elevated hepatic synthesis of VLDL Vaziri et al. [28]) seem to be the main causes of hypertriglyceridemia in patients with massive proteinuria. Other studies showed that HD patients usually display elevated triglycerides (TG), reduced high density lipoprotein (HDL) cholesterol and elevated concentration of lipoprotein-a (Pichard [29]), while total and low density lipoprotein (LDL) cholesterol usually remain within normal limits (Deighan et al. [30]). Vaziri et al. stated also that in CKD patients there are deficiencies of lipoprotein lipase and VLDL receptor in skeletal muscles and adipose tissues (Vaziri et al. [31]).

SIRT1, the human homolog of Sir2, that controls numerous physiological processes, is considered a candidate gene for predicting variation between humans (Olechnowicz-Tietz et al. [32]). In the current study we investigate the association between variation in single nucleotide polymorphisms (SNPs) rs7895833, rs7069102, and rs2273773 in the SIRT1 gene with lipid profiles in hemodialysis (HD) patients.

As regards the SIRT1 gene polymorphisms, the GG genotype of the rs7895833 has a significant higher diastolic blood pressure, TC and LDLc than the GA and AA genotypes and that the CC genotype of the rs7069102 has a higher LDLc than the CG and GG genotypes while the TT genotype of the rs2273773 was significantly associated with diastolic BP, TC and LDLc than the TC and CC genotypes. Beside that the G allele of the rs7895833 and the T allele of the rs2273773 are significantly higher among HD patients than control.

Shimoyama et al. [33] stated that HD patients showed significantly low frequencies of the A allele of rs7895833 and the G allele of rs7069102 compared with age-matched general population and that the serum total cholesterol and LDL cholesterol were significantly higher in G allele carriers of the rs7069102.

In a study carried by Kilic et al. [34] the frequencies of mutant GG genotype and mutant G allele for rs7069102 C>G in intron 4 and the frequencies of mutant TT genotype and mutant T allele for rs2273773 C>T in exon 5, were significantly higher in CVD patients as compared to controls. The risk for CVD was increased by 2.4 times in carriers of mutant G allele compared with carriers of wild-type C allele for rs7069102 C.G and 1.9 times in carriers of mutant T allele compared with carriers of wild-type C allele for rs2273773 C>T while no association between for rs7895833 A>G SNP and the risk of CVD. According to these results, heterozygote CG genotype for rs7069102 C.G and heterozygote CT genotype for rs2273773 C.T may be protective against to CVD.

In contrast to these findings Zillikens et al. [35] reported that the A allele carriers of the rs7895833 showed an increase in body mass index (Zillikens et al.). And Armand et al. reported that there was a significantly difference in the G allele of rs7069102 between obese subjects and controls, and thus G allele carriers of rs7069102 are at a higher risk of obesity than non carriers. Based on these reports, A allele carriers in rs7895833 and G allele carriers in rs7069102 tend to be obese, and thereby at a high risk for cardiovascular disease (Armand et al.) [36].

Aging is a universal process that affects all organs including the kidney. CR promotes longevity and slows aging (Fontana et al.) [37]. One possible mechanism by which CR exerts such beneficial effects involves the actions of sirtuins, especially SIRT1. In adipose tissue, SIRT1 interacts with peroxisome proliferator–activated receptor (PPAR)-γ to repress its transcriptional activity, leading to inhibition of adipogenesis during fasting and activation of lipolysis (Picard et al.) [38]. This results in fat loss, which is an important component of the effect of caloric restriction on longevity in mammals. Therefore SIRT1 may improve or retard age related disease processes (Guarente and Franklin) [39].

SIRT1 activates LXR that operates as cholesterol sensor to protect the organism from cholesterol overload, and reduces cholesterol loading in macrophages, consequently protecting against atherosclerosis (Nomiyama et al.) [40]. Thus the G allele carriers of rs7895833 and T allele carriers' of the rs2273773 might have reduced activities of SIRT1 and LXR, thereby leading to hypercholesterolemia.

Kilic et al. reported that SIRT1 protein levels for all studied SNPs were significantly increased in the patients carrying heterozygote mutant genotypes as compared to controls. The heterozygote CG genotype (rs7069102) and heterozygote CT genotype (rs2273773) may be protective against to CVD. Therefore, the increase in the SIRT1 protein expression may suggest a compensatory mechanism to protect the people from the detrimental effects of CVD. In addition, for rs7069102, the mutant genotype (GG) caused a significant increase in

the SIRT1 expression level suggesting that this SNP in the SIRT1 gene is related with the oxidative stress, thereby, CVD development. Also, increase in SIRT1 protein level of patients carrying wild type genotype of rs7895833 may suggest the deteriorating effects of CVD-induced oxidative stress. An overall decrease in the protein levels of eNOS for all three SNPs was observed (Kilic et al.) [34].

A recent study carried by Tarantino et al. lower serum levels of SIRT4 were present in obese subjects with hepatic steatosis (HS) and intramuscular TG (IMTG), independent of the severity of obesity. SIRT4 levels showed a strict relationship to some parameters reckoned as CAD risk factors, that is, low HDL and visceral obesity expressed as high waist-to-hip (W/H) ratio. In other words, obese individuals with moderately low SIRT4 levels, due to disturbed muscle fat β-oxidation—a primary event in the etiology of obesity—were still able to provide a sufficient β-oxidation of FFAs that leads to less organ fat storage without forming excess ROS (Tarantino et al.) [41].

Sirtuin 1 (SIRT1) depletion in vascular endothelial cells mediates endothelial dysfunction and premature senescence in diverse cardiovascular and renal diseases. Vasko et al. established a novel mechanistic molecular link between endothelial SIRT1 depletion, downregulation of matrix metalloproteinase-14 (MMP-14), and the development of nephrosclerosis (Vako et al. [42].

Current understanding of the role of SIRT1 in renal physiology and pathogenesis of renal diseases is limited, (Hao et al.) [43]. SIRT1 is highly expressed in endothelial cells, where it regulates numerous functions, including nitric oxide synthase, cell senescence, and autophagy (Borradaile and Pickering) [44]. This spectrum of functions would explain the association of endothelial SIRT1 deletion with impaired vasoreactivity and increased numbers of senescent endothelial cells.

5. LIMITATIONS

Limitation of this study was lack of measurement of sirtuin 4 in patients and correlation with sirtuin 1 SNPs especially that evidenced by recent studies its association with lipid meatabolism and oxidative stress in obese patients and CAD risk that attributes to end stage renal diseases. Further studies are needed to point out relation of sirtuin 1and sirtuin 4 with CVD in end stage renal diseases.

6. CONCLUSION

From the previous data the present study demonstrates that SIRT1 polymorphisms are associated with cholesterol metabolism and hypertension in Egyptian HD patients.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kundhal K, Lok C. Clinical epidemiology of cardiovascular disease in chronic kidney disease. Nephron Clin Pract. 2005;101:c47–c52.

2. Collins A, Foley R, Herzog C, et al. Excerpts from the United State renal data system 2007 annual data report. Am J Kidney Dis. 2008;51:S1–320.
3. Maheshwari N, Ansari M, Darshana M, Lal K, Ahmed K. Pattern of lipid profile in patients on maintenance hemodialysis. Saudi J Kidney Dis Transplant. 2010;21:565–570.
4. Gizem D, Tiago FO. SIRT1 and SIRT2: emerging targets in neurodegenration. EMBO Mol Med. 2013;5:344 – 352.
5. Imai S, Armstrong CM, Kaeberlein M, Guarente L. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. Nature. 2000; 403: 795–800.
6. Liang F, Kume S, Koya D. SIRT1 and insulin resistance. Nat. Rev. Endocrinol. 2009;5:367–373.
7. Blander G, Guarente L. The Sir2 family of protein deacetylases. Annu Rev Biochem. 2004;73:417–35.
8. Wang Y, Tissenbaum HA. Overlapping and distinct functions for a Caenorhabditis elegans SIR2 and DAF-16/FOXO.Mech Ageing Dev. 2006;127:48–56.
9. Haigis MC, Sinclair DA. Mammalian sirtuins: biological insights and disease relevance. Annu Rev Pathol. 2010;5:253–295.
10. Frye RA. Phylogenetic classification of prokaryotic and eukaryotic Sir2-like proteins. Biochems Biophys Res Commun. 2000;273:793–798.
11. Guarente L, Franklin H. Epstein Lecture: Sirtuins, aging, and medicine. N. Engl. J. Med. 2011;364:2235–2244.
12. Tanno M, Sakamoto J, Miura T, Shimamoto K, Horio Y. Nucleocytoplasmic shuttling of the NAD-dependent histone deacetylase SIRT1. J Biol Chem. 2007;282:6823-6832.
13. Nakae J, Cao Y, Daitoku H, et al. The LXXLL motif of murine forkhead transcription factor FOXO1 mediates Sirt1-dependent transcriptional activity. J Clin Invest. 2006;116:2473–83.
14. Armoni M, Harel C, Karni S, et al. FOXO1 represses peroxisome proliferator-activated receptor-gammal and –gamma2 gene promoters in primary adipocytes. A novel paradigm to increase insulin sensitivity. J Biol Chem. 2006;281:19881–91.
15. Rodgers JT, Puigserver P. Fasting-dependent glucose and lipid metabolic response through hepatic sirtuin 1. Proc Natl Acad Sci U S A. 2007;104:12861–6.
16. Lagouge M, Argmann C, Gerhart-Hines Z, et al. Resveratrol improves mitochondrial function and protects against metabolic disease by activating Sirt1 and PGC-1alpha. Cell. 2006;127:1109–22.
17. Liang H, Ward WF: PGC-1alpha: A key regulator of energy metabolism. Adv Physiol Educ. 2006;30:145–51.
18. Rodgers JT, Lerin C, Gerhart-Hines Z, Puigserver P. Metabolic adaptations through the PGC-1 α and SIRT1 pathways. FEBS Lett. 2008;582:46–53.
19. Purushotham A, Schug T, Xu Q, Surapureddi S, Guo X, Li X: Hepatocyte-specific deletion of SIRT1 alters fatty acid metabolism and results in hepatic steatosis and inflammation. Cell Metab. 2009;9:327–338.
20. Walker AK, Yang F, Jiang K, Ji JY, Watts JL, Purushotham A, et al. Conserved role of SIRT1 orthologs in fasting-dependent inhibition of the lipid/cholesterol regulator SREBP. Genes Dev. 2010;24:1403–1417.
21. Maria SF, Sandra R, Elísio C, Denisa M, Laetitia T, et al.,: Risk Factors for Mortality in Hemodialysis Patients:Two-Year Follow-Up Study. Disease Markers. 2013;35(6)791–798.
22. Mshelia DS, Buratai LB, Mamza YP. Lipid profile in pre-dialysis chronic kidney disease patients attending University of Maiduguri Teaching Hospital, Maiduguri-Nigeria. Niger J Clin Pract. 2009;12(2):173-8.

23. Herspink H, Ninomiya T, Zoungas S, de- Zeeuw D, Grobbee D, Jardine M, et al.: Effect of lowering blood pressure on cardiovascular events and mortality in patients on dialysis: a systematic review and meta-analysis of randomised controlled trials. Lancet. 2009;373:1009–1015.
24. Joven J, Villabona C, Vilella E, et al: Abnormalities of lipoprotein metabolism in patients with the nephritic syndrome. N Engl J Med. 1990;323:579–584.
25. Vaziri ND, Sato T, Liang K. Molecular mechanisms of altered cholesterol metabolism in rats with spontaneous focal glomerulosclerosis. Kidney Int. 2003;63:1756–1763.
26. Vaziri ND. Molecular mechanisms of lipid disorders in nephrotic syndrome. Kidney Int. 2003;63:1964–1976.
27. Kashyap ML, Srivastava LS, Hynd BA et al. Apolipoprotein CII and lipoprotein lipase in human nephritic syndrome. Atherosclerosis. 1980;35:29–40.
28. Vaziri ND, Kim CH, Phan D, et al.: Up-regulation of hepatic Acyl CoA: diacylglycerol acyltransferase-1 (DGAT-1) expression in nephrotic syndrome. Kidney Int. 2004;66:262–267.
29. Prichard S. Impact of dyslipidemia in end-stage renal disease: J Am Soc Nephrol. 2003;14:S315–S320.
30. Deighan C, Caslake M, McConnell M, Boulton-Jones J, Packard C. Atherogenic lipoprotein phenotype in end-stage renal failure: origin and extent of small dense low density lipoprotein formation. Am J Kidney Dis. 2000;35:852–862.
31. Vaziri ND, Yuan J, Ni Z, Nicholas SB, Norris KC Lipoprotein lipase deficiency in chronic kidney disease is compounded by downregulation of endothelial GPIHBP1 expression. Clin Exp Nephrol. 2012;16(2):238–243. [PubMed: 22009636].
32. Olechnowicz-Tietz S, Gluba A, Paradowska A, Banach M, Rysz J. The risk of atherosclerosis in patients with chronic kidney disease. Int Urol Nephrol. 2013;45(6):1605-12.
33. Shimoyama Y, Mitsuda Y, Tsuruta Y, Suzuki K, Hamajima N, Niwa T. SIRTUIN 1 gene polymorphisms are associated with cholesterol metabolism and coronary artery calcification in Japanese hemodialysis patients. J Ren Nutr. 2012;22(1):114-9. doi: 10.1053/j.jrn.10.025.
34. Kilic U, Gok O, Bacaksiz A, Izmirli M, Elibol-Can B and Uysal O.: SIRT1Gene Polymorphisms Affect the Protein Expression in Cardiovascular Diseases. PLoS ONE. 2014; 9(2): e90428.
35. Zillikens MC, van Meurs JB, Rivadeneira E, et al.: SIRT1 genetic variations is related to BNI and risk of obesity. Diabetes. 2009;58:2828-2834.
36. Armand VP, Sigri B, An V, et al. Association of SIRT1 gene variation with visceral obesity. Hum Gene. 2008;124:431-436.
37. Fontana L, Partridge L, Longo VD. Extending healthy life span–from yeast to humans. Science. 2010;328: 321–326.
38. Picard F, Kurtev M, Chung N, Topark-Ngarm A, Senawong T, Machado De O, et al. Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR-gamma. Nature. 2004;429:771–776.
39. Guarente L, Franklin H: Epstein Lecture: Sirtuins, aging, and medicine. N. Engl. J. Med. 2011;364:2235–2244.
40. Nomiyama T, Bruemmer D. Liver X receptors as therapueutic targets in metabolim and atherosclerosis. Curr Atheroscler Rep. 2008;10:88-95.
41. Tarantino G, Finelli C, Scopacasa F, Pasanisi F, Contaldo F, Capone D, Savastano S. Circulating Levels of Sirtuin 4, a Potential Marker of Oxidative Metabolism, Related to Coronary Artery Disease in Obese Patients Suffering from NAFLD, with Normal or Slightly Increased Liver Enzymes. Oxidative Medicine and Cellular Longevity; 2014. Article ID 920676. 10 pages.

42. Vasko R, Xavier S, Chen J, Lin CHS, Ratliff B, Rabadi M, et al. Endothelial Sirtuin 1Deficiency Perpetrates Nephrosclerosis through Downregulation of Matrix Metalloproteinase-14: Relevance to Fibrosis of Vascular Senescence. J Am Soc Nephrol. 2014;25:276–291.
43. Hao CM, Haase VH. Sirtuins and their relevance to the kidney.JAmSocNephro. 2010;l21:1620–1627.
44. Borradaile NM, Pickering JG: NAD(+), sirtuins, and cardiovascular disease.Curr Pharm Des. 2009;15:110–117.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=606&id=11&aid=5736