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Sirtuin 1 Gene Expression in Non-alcoholic Fatty Liver Disease and Its Correlation with Biochemical and Clinical Features

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ABSTRACT

Background: Silent information regulator 1 [sirt1] is a protein that is widely expressed and has a crucial function in the prevention of oxidative stress, which is implicated in the development of a number of chronic disorders, such as fatty liver disease.

Objective: The aim of the current work was to investigate SIRT1 gene expression in non-alcoholic fatty liver disease and it is emerging role in pathogenesis of non-alcoholic fatty liver disease. **Subjects and Methods:** This case-control study included a total of twenty cases of Non-Alcoholic Fatty Liver Disease; NAFLD (**Group A)** and ten individuals as a control **(Group B)**, attending at Departments of Internal Medicine and Medical Biochemistry, Zagazig University Hospitals. They were assessed clinically by lab investigations and quantitative real time polymerase chain reaction (RT-PCR) to assess Sirt1 gene fold expression.

Results: There was a significant lowering of sirt1 gene expression p=0.0001 for patient with NAFLD compared to healthy controls. There was significant association between SIRT1 and BMI, as well as hyperlipidemia in NAFLD compared to healthy group. We found a significant association between SIRT1 and NAFLD patients with mild fibrosis and those without fibrosis. Cut off value ≤0.61 had 80.0% sensitivity and 80.0% specificity, positive predictive value 57.0%, negative predictive value 92.0% and accuracy was 80.0%.

Conclusion: It could be concluded that the significant correlation between SIRT1 gene expression and non-alcoholic fatty liver disease helps to differentiate patients with nonalcoholic fatty liver diseases and healthy ones.

Keywords: Sirtuin 1, Non-alcoholic Fatty Liver Disease.

INTRODUCTION

Chronic hepatic steatosis that is not caused by genetic, metabolic, infections, side effects of drugs, alcohol intake or malnutrition is described as nonalcoholic fatty liver disease (NAFLD) **(1).**

NAFLD is a condition in which the liver's hepatocytes accumulate too much lipid, which is linked to insulin resistance, obesity, and the metabolic syndrome. The prevalence of NAFLD in adults around the world ranges from 10% to 40%, with an estimated 24–25% of the global population suffering from the disease **(2).**

The Middle East has the highest incidence, followed by South America and Asia, while Africa has the lowest. Men are more likely than women to develop it between their fourth and sixth decade **(3).** In the majority of cases, NAFLD goes unnoticed until tests for liver enzymes reveal abnormally high levels. Other symptoms include bloating, right hypochondria pain, sleep difficulty, and/or apnea**.** Ultrasounds are commonly used to diagnose NAFLD, but a liver biopsy is the gold standard for determining the existence of NASH and the fibrosis stage of the liver **(3).**

A significant function for Silent information regulator 1[sirt1] in the pathophysiology, progression, and therapy of a number of diseases has been established by the discovery of this ubiquitously expressed protein. In order to control gene expression, Sirt1 functions as a NAD+-dependent deacetylase. When Sirt1 is knocked out in the liver, pancreas, and brain, the oxidative stress and inflammation that accompany these organ dysfunctions — such as fatty liver disease — lead to an increase in ROS and an inflammatory response. Sirt1 may play a role in preventing these processes, which are thought to be responsible for a variety of chronic diseases **(4)**.

It was shown that SIRT1 acted through regulating PPARa activity and the oxidation of fatty acids SIR-1's role in promoting insulin sensitivity and reducing inflammatory gene expressions was discovered to be elevated by an increase in SIRT1 activation **(5).**

It was the goal of this study to investigate SIRT1 gene expression in non-alcoholic fatty liver disease and it is emerging role in pathogenesis of non-alcoholic fatty liver disease.

SUBJECTS AND METHODS

This case-control study included a total of twenty cases of Non-Alcoholic Fatty Liver Disease; NAFLD (**Group A)** and ten individuals as a control **(Group B)**, attending at Departments of Internal Medicine and Medical Biochemistry, Zagazig University Hospitals.

They were assessed clinically by lab investigations and Quantitative real time polymerase chain reaction (RT-PCR) to assess Sirt1 gene fold expression.

Ethical Consideration:

This study was ethically approved by Zagazig University's Research Ethics Committee. Written informed consent of all the participants was

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obtained and submitted them to Zagazig University (ZU-IRB#6872). The study protocol conformed to the Helsinki Declaration, the ethical norm of the World Medical Association for human testing.

Inclusion criteria: Age 18-70 years, gender: both male and female, and patients with nonalcoholic fatty liver diseases (NAFLD) based on clinical, laboratory and radiological evidence.

Exclusion criteria: Chronic viral infections (HBV, HCV), autoimmune liver diseases (Primary Biliary Cirrhosis, Primary Sclerosing Cholangitis, and Autoimmune Hepatitis), metabolic liver diseases (Wilson Disease, Hereditary Hemochromatosis), hepatocellular carcinoma, chronic alcoholism, and patients taking medications whish affect inflammations (Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

All patients were subjected to:

- Full history taking including: Age, sex, education status, age at onset of Diabetes, and duration of disease, family history; daily activity, and hyperlipidemia (use of medication, serum cholesterol concentration >220 mg/dL or serum triglycerides concentration >150 mg/dl).
- Thorough clinical examination: with special emphasis on vital data, and all body systems examination, to assess the blood pressure and anthropometric measurements.
- Complete blood count and biochemical assessment including, random blood sugar, glycosylated hemoglobin, fasting lipid profile, vitamin D, Hemoglobin A1c, Liver function tests, kidney function tests.
- **Special laboratory test:** Quantitative real time polymerase chain reaction (RT-PCR) to assess Sirt1 gene fold expression relative to the housekeeping gene
- **Other testes to exclude chronic inflammatory conditions:**

Imaging:

Ultrasonography: In the identification of non-alcoholic fatty liver disease NAFLD (increased echogenicity, brightly colored fatty liver), abdominal ultrasound can be a noninvasive, widely available, and accurate method.)

Transient elastography (fibroscan): an improved ultrasonography technique for assessing hepatic stiffness. Stiffness of the liver is an indication of fibrosis or scarring in the liver.

Electrocardiography [ECG].

Statistical analysis:

In order to analyze the data acquired, Statistical Package of Social Services version 20 was used to execute it on a computer (SPSS). In order to convey the findings, tables and graphs were employed. The quantitative data was presented in the form of the mean, median, standard deviation, and confidence intervals. The information was presented using qualitative statistics such as frequency and percentage. The student's t test (T) is used to assess the data while dealing with quantitative independent variables. Pearson Chi-Square and Chi-Square for Linear Trend (X^2) were used to assess qualitatively independent data. The significance of a P value of 0.05 or less was determined.

RESULTS

Table 1 shows that there was no statistically significant difference between patients with nonalcoholic fatty liver diseases and healthy control: regarding to sex a ($p>0.05$). There was significant difference between Patients with nonalcoholic fatty liver diseases and healthy control: regarding to age and body mass index ($p=0.0001$), four fifths (80.0%) of cases obese.

Table (1): Characteristics of studied groups; patients with nonalcoholic fatty liver diseases and healthy control

Variables	(NAFLD) No. 20	Healthy control No. 10	Test of sig	p
Sex No. (%) Males Females	 14(70.0%) 6(30.0%)	 8(80%) 2(20%)	**f**	0.68
Age (years) Mean± SD (range)	 49.4±8.8 32-63	 29.1±7.5 21-41	t 6.2	0.0001
BMI (kg/m^2) Normal Over weight Obese	36.4±7.4 0 4(20.0%) 16(80.0%)	22.1±1.3 10(100.0%) 0(0.0) 0(0.0)	**χ 2** 30	0.0001

55.0% of patients had type 2 diabetes, duration of diabetes ranged from 2-26 years with mean 13.9±7.9 years as well as 20.0% of patients had hypertension, with duration ranged from 3-20 years with mean 14.5±8.02years. 45.0% of type2 diabetic treatment with insulin and 25% of patients had mid fibrosis **(Table 2).**

Table (2): Basic clinical characteristics of patients with nonalcoholic fatty liver diseases:

Variables	(NAFLD) No.20	
	No.	**%**
Type2 Diabetes	11	(55.0%)
DuratioNo.DM mean± SD median (range)	13.9±7.9 12(2-26)	
Hypertension	4	(20.0%)
Duration HTN mean± SD median(range)	14.5±8.02 17.5(3-20)	
Intake drugs	13	(65.0%)
Drug types Amlodipine Atenolol Insulin Insulin, amlor Insulin, zestril10mg	1 1 9 1 1	(5.0%) (5.0%) (45.0%) (5.0%) (5.0%)
Fibroscan grade No fibrosis Mild fibrosis	 15 5	 75.0% 25.0%

Table 3, shows; there was significant higher value of PLT, in patients with nonalcoholic fatty liver diseases compared to healthy control p=0.0001. There is significant higher value of, liver enzymes (AST, ALT) p<0.05. in patients with nonalcoholic fatty liver diseases compared to healthy. But there is significant lower value of, serum albumin p<0.0001. in patients with nonalcoholic fatty liver diseases compared to healthy.

Table (3): CBC, Liver and serum creatinine of studied groups; Patients with nonalcoholic fatty liver diseases and healthy control

CBC	**(NAFLD) No.20**	**Healthy control No.10**	**t**	**p**
WBCs (× 10^9/L)	7.26±1.6	8±1.3	1.305	.202
RBCs (× 10^{12}/L)	4.9±0.29	4.84±0.23	.565	.576
HB (g/dl)	14.3±0.71	13.95±0.68	1.291	.207
PLT (× 10^9/L)	334.2±78.9	211.9±51.7	4.33	.0001*
Liver function tests	**(NAFLD) No.20**	**Healthy control No.10**	**t**	**p**
AST (U/L)	25.3±3.8	15.4±1.9	U 2.6	.009*
ALT (U/L)	36.7±8.4	15.7±1.2	U 3.112	.002*
T Bilirubin (mg/dl)	0.52±0.16	0.39±0.1	U 0.928	.354
D. Bilirubin (mg/dl)	0.21±.06	0.19±0.05	1.085	.287
Serum protein (g/dl)	6.8±0.24	6.96±0.12	1.31	0.2
Serum Albumin (g/dl)	4.2±0.43	4.76±0.21	4.42	.0001*
Serum Creatinine (mg/dl)	0.831±0.12	0.83±0.08	.001	0.99

There was significant higher value of, lipid profile (Triglycerides, Cholesterol) p<0.0001. in patients with nonalcoholic fatty liver diseases compared to healthy control, there is significant higher value of blood sugar, HBA1C in patients with nonalcoholic fatty liver diseases compared to healthy control=0.0001, p=0.009 respectively **(Table 4).**

Table (4): Lipid profile and blood glucose profile of studied groups; Patients with nonalcoholic fatty liver diseases and healthy control

	(NAFLD) No.20	**healthy control No.10**	**u**	**p**
Triglycerides (mg/dl)	227±23	88.±6.5	3.66	.0001*
Cholesterol (mg/dl)	208.5±7.6	86.1±3.7	4.4	.0001*
FBS (mg/dl)	148.5±6.4	89.9±8.8	t 3.82	.0001*
HBA1C (%)	7.1±1.6	4.6±0.27	U 2.63	.009*

There was significant lower value of sirt1, for patients with nonalcoholic fatty liver diseases compared to healthy control **(Table 5).**

Table (5): Sirt1 of studied groups; Patients with nonalcoholic fatty liver diseases and healthy control

	(NAFLD) No.20	healthy control No.10	u	p
Sirt1 mean± SD median (range)	0.756±0.18 0.744(0.42-1.17)	1.165±0.1 1.17(.98-1.28)	4. 09	0.0001*

Table 6, shows; there is significant difference of sirt1 for patients with no fibrosis, mild fibrosis of nonalcoholic fatty liver diseases and healthy control p=0.0001. There was significant lower value of sirt1 for patients with mild fibrosis of nonalcoholic fatty liver diseases compared to, no fibrosis of nonalcoholic fatty liver diseases p=0.01. There was significant lower value of sirt1 for patients with, no fibrosis of nonalcoholic fatty liver diseases compared to healthy control p=0.0001 **(Table 6).**

Table (6): Comparison sirt1 value according to Fibro scan finding among studied groups

	Fibro scan finding			KW	p	LSD
	(NAFLD) No.20		Healthy control No.10			
	No fibrosis No.15	Mild fibrosis No.5				
sirt1 mean± SD median (range)	0.832±0.193 0.85 (0.55-1.17)	0.528±0.0.097 0.57 (0.42- 0.64)	1.165±0.1 1.17 (0.98-1.28)	21.5	0.0001*	(.01)* (.0001)** (.0001)** *

There was significant and direct relation between sirt1and Foxo, p=0. 0001. But there was significant and inverse relation between sirt1, and fibroscan value p=0.002, p=.0001 respectively. Otherwise there was no significant relation between, sirt1, and age years, BMI, WBCs, RBCs, Hemoglobin, PLT, AST, ALT total bilirubin, direct bilirubin, serum protein, serum Albumin triglyceride, cholesterol, CRP, FBS, HBA1C, serum creatinine P>0.05 **(Table 7).**

Table (7): Correlation between gene expression, sirt1 and age years, BMI, DM , HTN Duration ,WBCs, RBCs, Hemoglobin, PLT,AST, ALT total bilirubin, direct bilirubin, serum protein, serum Albumin triglyceride, cholesterol, CRP ,FBS, HBA1C, serum creatinine for Patients with nonalcoholic fatty liver diseases (No.20)

Variables	Sirt1	
	r	p
Age (years)	0.023	0.924
BMI **(kg/m^2)**	-.084-	0.724
Duration DM	0.233	0.491
Duration HTN	0.105	0.895
WBCs (× 10^9/L)	-.082-	0.73
RBCs (× 10^{12}/L)	0.176	0.457
HB (g/dL)	0.074	0.757
PLT (× 10^9/L)	0.25	0.287
AST (U/L)	0.042	0.861
ALT (U/L)	0.223	0.346
T Bilirubin (µmol/L)	0.046	0.846
D. Bilirubin (µmol/L)	0.038	0.875
Serum protein (g/dL)	-.107-	0.652
Serum albumin (g/dL)	0.025	0.918
Triglycerides (mg/dL)	-.056-	0.816
Cholesterol (mg/dL)	-.043-	0.859
CRP (mg/L)	0.072	0.762
FBS (mg/dl)	-.065-	0.785
HBA1C (mg/dl)	0.208	0.379
Serum creatinine (mg/dl)	0.052	0.828
Fibroscan value (dB/m)	**-.654-****	**0.002**

Table 8, figure 1 show the sensitivity and specificity obtained to sirt1 value to differentiate patients with nonalcoholic fatty liver diseases from healthy control. Cut off value ≤1.067 had 90.0% sensitivity and 80.0% specificity, positive predictive value 90.0%, negative predictive value 80.0% and accuracy was 93.3%.

Table (8): Performance of sirt1 as marker to discriminate patients with nonalcoholic fatty liver diseases

	Patients with nonalcoholic fatty liver diseases (20)	**Healthy control (No.10)**
Cut off sirt1 ≤1.067 >1.067	18 2	2 8
Sensitivity	90.0%	
Specificity	80.0%	
Positive predictive value	90.0%	
Negative predictive value	80.0%	
Accuracy	86.7%	

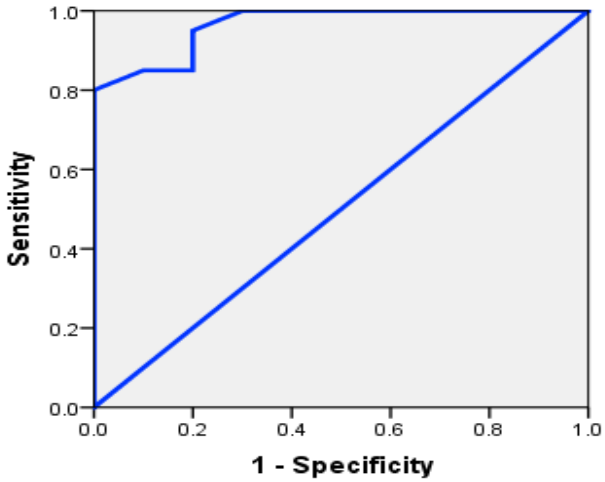

Figure 1: ROC Curve to detect the best cut-off value of sirt to patients with nonalcoholic fatty liver diseases. Area under curve (AUC) 0.965. So sirt1excellent marker to discriminate patients with nonalcoholic fatty liver diseases

Table 9, shows the sensitivity and specificity obtained to sirt1 value to differentiate fibrosis patients with nonalcoholic fatty liver diseases. Cut off value ≤0.61 had 80.0% sensitivity and 80.0% specificity, positive predictive value 57.0%, negative predictive value 92.0% and accuracy was 80.0%.

Table (9): Performance of sirt1 as marker to discriminate patients with nonalcoholic fatty liver diseases with mild liver fibrosis

	Patients with nonalcoholic fatty liver diseases with mild fibrosis (No. 5)	**patients with nonalcoholic fatty liver diseases no fibrosis (No.15)**
Cut off sirt1 ≤0.61 >0.61	4 1	3 12
Sensitivity	80.0%	
Specificity	80.0%	
Positive predictive value	57.0%	
Negative predictive value	92.0%	
Accuracy	80.0%	

DISCUSSION

As a generic phrase, NAFLD refers to a wide range of liver damage that is produced by hepatocyte injury and inflammation, as well as fibrosis of the liver. Hepatocellular carcinoma (HCC) can be minor or severe, and it is commonly found on a liver biopsy with steatosis, ballooning, and lobular inflammation with or without fibrosis being the most common histological findings [(6)].

In addition to regulating cellular senescence and ageing, SIRT1 is critical in regulating gene expression; proliferation; differentiation; metabolism; carcinogenesis; and the immune response[(7)].

SIRT1 has been shown in the liver to play a role in regulating hepatic glucose and lipid metabolism, insulin signaling, inflammation, and liver protection against nutritional overload-induced NAFLD [(8)].

In our study, NAFLD more in men (70.0%) than women (30.0%), This observation went in agreement with **Lonardo *et al.* [(9)],** who found that men were more likely than women to have NAFLD, NAFLD is more common in women after menopause, suggesting that estrogen is protective.

In our study the prevalence of NAFLD higher in obese (80.0%) and overweight (20.0%), This was nearly agreed with **Divella *et al.* [(10)]** who found that obesity and metabolic syndrome were associated with an increased risk of nonalcoholic fatty liver disease (NAFLD) because of insulin resistance.

In our study, 55.0% of patients had type2diabetes, duration of diabetes ranged from 2-26 years with mean 13.9±7.9 years, this went in agreement with **Kosmalski *et al.* [(11)]** where insulin resistance plays a significant role in the pathophysiology of NAFLD and is associated with an increased frequency of NAFLD in people with type 2 diabetes.

In the present study, there was no significant difference among groups regarding liver enzymes [normal in both NAFLD and healthy group], and this was in agreement with study done by **Portillo-Sanchez *et al.* [(12)],** who found that Type 2 diabetes patients with normal aminotransferase (50 percent) and obese patients (36 percent) had a very high prevalence of NAFLD.

In our study there was significant higher of glycosylated hemoglobin HBA1c in non-alcoholic fatty liver disease NAFLD in compare to healthy group and this went in agreement with study done by **Chen *et al.* [(13)]** according to a study, NAFLD is thought to be exacerbated by elevated HbA1c levels. In the development of NAFLD, altered hemoglobin may play a role, either directly or indirectly, in the activation of the receptor for advanced glycation end products [RAGE].

In our study 20.0% of patients had hypertension, with duration ranged from 3-20 years with mean 14.5±8.02years, This was in agreement with **Zhao *et al.* [(14)]** who reported that, NAFLD may serve as a new trigger for hypertension development and progression, independent of obesity, diabetes, and other risk factors.

In our study there was significant hyperlipidemia [triglycerides and cholesterol] in NAFLD p<0.0001compared to control group, this went in agreement with **Shahab *et al.* [(15)]** study in united states, who found a substantial link between NAFLD and hyperlipidemia, with hyperlipidemia being the greatest risk factor for NAFLD and being linked to cardiovascular mortality CV, the primary cause of death in NAFLD patients.

In our study There was significant lower value of sirt1 for patients with mild fibrosis of nonalcoholic fatty liver diseases compared to non-fibrosis NAFLD p=0.0001 and this goes in agreement with study by **Sun *et al.* [(16)]**, they found that a decrease in SIRT1 resulted in an increase in hepatic fibrogenesis in the MCD diet [a classical dietary model of NASH]-induced mouse model of NASH, which is a major source of liver fibrogenesis, as activation of HSCs, which are activated by SIRT1 deficiency, increased fibrogenesis in the MCD diet model.

In our study there was significant lower of sirt1 gene expression p=0.0001in NAFLD compared to healthy, this goes in agreement with **Ding *et al.* [(17)]** the study done on genetic modulating mice in which The expression level of sirt1 can be altered, providing more direct evidence for sirt1's role in oxidative stress Regulation.

In our study there was significant and direct relation between SIRT1 gene expression and body mass index BMI compared to healthy, where SIRT1 gene expression is low in overweight and obese in comparing to non-obese healthy group and this was agreement with study done by **Clark *et al.* [(18)]** that, SIRT1 expression levels are significantly more in non-obese people than in obese individuals, which validates a Danish study that suggested SIRT1 transcription is significantly higher in lean people than obese individuals.

In our study, we found a significant association and agreement between cut off SIRT1 and NAFLD patients with mild fibrosis and without fibrosis.

Significant area under curve with cutoff value to differentiate fibrosis and non-fibrosis patients with nonalcoholic fatty liver diseases. Cut off value ≤0.61 had 80.0% sensitivity and 80.0% specificity, positive predictive value 57.0%, negative predictive value 92.0% and accuracy was 80.0%.

In our study, we found a significant association and agreement between cut off SIRT1 and non-alcoholic fatty liver disease compared to controlled group. Significant area under curve with cutoff value for differentiate patients with nonalcoholic fatty liver diseases from healthy control ≤1.1067, with 90.0%

sensitivity and 80.0% specificity, positive predictive value 90.0%, negative predictive value 80.0% and accuracy was 86.7%.

CONCLUSION

It could be concluded that the significant correlation between SIRT1 gene expression and non-alcoholic fatty liver disease helps to differentiate patients with nonalcoholic fatty liver diseases and healthy ones.

SIRT1 gene expression is low in non-alcoholic fatty liver disease NAFLD, so activation of SIRT1 and it is downstream signaling is very likely to serve as promising therapeutic approaches for treatment of fatty liver disease.

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