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Article

The impact of SIRT1 serum level and its gene single nucleotide polymorphism (rs7895833) on the prediction of hepatocellular carcinoma in a cohort of cirrhotic population

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Abstract. *Background*: SIRT1 role in cancer and specifically in HCC remains controversial. Depending on the tissue and the context, SIRT1 has so far been found to have oncogenic as well as tumor suppressive functions.

Objectives: The present work was designed to evaluate the role of serum SIRT1 as a possible biomarker for the diagnosis of HCC, the effect of SIRT1 single nucleotide polymorphism (SNP) on SIRT1 serum levels and its potential role in the development and progression of HCC.

Methods: Seventy patients with cirrhosis [40 with HCC and 30 without HCC] and 30 healthy controls were enrolled in the study. Serum level of SIRT1 was measured by ELISA and genotyping for SIRT1 SNP (rs7895833) was performed using TaqMan SNP Genotyping Assay.

Results: Serum SIRT1 levels in HCC patients were significantly lower than in cirrhotic patients without HCC and controls ($P<0.001$) and were inversely correlated with Child-Pugh score and HCC size and stage ($P<0.01$). ROC curve analysis revealed that serum SIRT1 was superior to serum alpha fetoprotein in the detection of HCC (AUC= 0.985 vs. 0.860). SIRT1 serum levels were significantly lower in subjects with heterozygous (A/G) variant than in those with homozygous (A/A) allele 1 ($P=0.002$). The mutant allele (G) was more prevalent among HCC patients than among controls.

Conclusion: Decreased SIRT1 level may play a role in the development and progression of HCC. Serum SIRT1 could be a promising biomarker for detection of HCC among cirrhotic patients and could be a potential therapeutic candidate.

Keywords: hepatocellular carcinoma; cirrhosis; SIRT1; gene polymorphism**.**

Introduction

Hepatocellular carcinoma (HCC) is a priority health problem worldwide; as it is listed as the most common primary hepatic malignancy of adults accounting for about 70–85% of all hepatic cancers.[1] It is considered as the fifth frequently diagnosed malignancy for males and the ninth for females worldwide and is categorized as the second most common cause of cancer related mortality.[2] In Egypt, the problem is even more evident due to doubling of its incidence rate in 10 years (2003-2013).[3] The observed growing incidence in Egypt could be a consequence of increasing hepatitis B virus (HBV) as well as HCV which are the primary risk factors,[4] improvements in the screening and diagnostic tools, as well as, the improved outcome and subsequently increased life span in cirrhotic patients who will have more time and more chance to develop the pathogenesis of HCC. Studies in Egypt have shown that most cases occurred in men who developed a cirrhotic liver due to HCV infection.[5]

HCC is defined as a heterogeneous malignancy which is a consequence of chronic infection causing oxidative stress and inflammation and it is found that a known risk factor in more than ninety percent of cases. However, there is a final common pathway which can explain its pathogenesis as repeated hepatocyte damage creates a pathological cycle of cell death and regeneration that eventually leads to cirrhosis and genomic instability which in turn causes the triggering of HCC.[6] Although surveillance programs of HCC have been improved, a significant percentage of patients have vascular invasion or extrahepatic metastasis (advanced stage) on diagnosis and HCC mortality rate worldwide is close to its incidence rate despite the current availability of several advanced therapies, as cases are mostly detected at an advanced, non-resectable stage. Therefore, more screening as well as surveillance strategies are needed to aid in the process of early diagnosis of HCC in the population at risk.[7] Ultrasound and serum alpha-fetoprotein (AFP) are the currently most commonly used tools for HCC screening.[8]

Although serum AFP is the most widely used tumor biomarker currently available for the detection of HCC, it had never been an ideal biomarker for early detection of HCC with its lower sensitivity and specificity levels than that required for optimum effective screening.[9] Moreover, despite the huge efforts done to find molecules as possible biomarkers for HCC, till now there is no single ideal marker for it.[9] Thus, there is an urgent need for improving the early detection and prognostication of patients with HCC using new relatively easy and noninvasive ways. In addition, new therapies which can target specific pathways incorporated in the pathogenesis and progress of HCC are needed.[10]

The silent mating type information regulation homologs; Sirtuins (SIRT) are a highly conserved family of nicotinamide adenine dinucleotide (NAD)-dependent class III deacetylases which help in the determination of the lifespan of different organisms. SIRT-mediated protein deacetylation modifies the activity and/or intracellular localization of a wide variety of proteins.[11] There are 7 members of sirtuins in mammals (SIRT1–SIRT7); of them, SIRT1 is the best-characterized. SIRT1 is found to be localized in the nucleus, however, it also contributes to the regulation of cytosolic targets by nucleo-cytoplasmic shuttling.[11] SIRT1 possesses a big number of substrates as it targets histone in addition to non-histone proteins;[11] it acts through cleaving the nicotinamide ribosyl bond of NAD+ and transferring the acetyl group from the substrate's lysine side chain to NAD+, thus generating nicotinamide, 2'-O-acetyl-ADP-ribose and a deacetylated substrate. SIRT1 plays important roles in many cellular pathways, like cellular survival, the cellular stress response, energy metabolism and apoptosis. [12] In addition, SIRT1 is a modulator of epigenetics as it directly and indirectly affects histone acetylation besides chromatin compaction.[13] Although SIRT1 roles in mediating genomic stability normally protect cells from oncogenic transformation; its enzymatic activity may promote cancer growth through inactivating some proapoptotic factors.[14]

SIRT1 (Sirtuin 1) gene is an identical protein binding and transcription factor binding gene. It is located on chromosome 10. Its cytogenetic band is 10q21.3. Its Size is 33,729 bases with plus strand orientation.[15] Whether SIRT1 acts as a cancer promoter or tumor suppressor remains controversial, because of the temporal in addition to the characteristic distribution of SIRT1 up and downstream multiple targets and factors within different tissue contexts as well as the unclear explanations of the complex mechanisms underlying SIRT1 signaling during carcinogenesis.[16] Moreover, its action in liver tumors is still poorly understood [(17)] and its pleiotropic effect in epithelial hepatic malignancies and specifically in HCC remains a promising field for the aim of targeting of SIRT1 in therapy.[16,17] This completely unclear picture promoted us to conduct the present study to assess the potential role of *SIRT1* SNP in the development and progression of HCC through modulation of serum SIRT1 protein level and its use as a biomarker for early detection and prognostication of HCC.

Patients and Methods

The study included 40 cirrhotic patients with HCC before surgery and chemotherapy and 30 cirrhotic patients without HCC at the Hepatobiliary Unit, Department of Internal Medicine, Faculty of Medicine, University of Alexandria in the period from January 2017 to August 2018. The Main University hospital of Alexandria is considered as the specialist's referral center for the northern part of Egypt. It covers four governorates of Northern Egypt and serves approximately 14 million people. The present study was conducted in accordance with the ethical guidelines of the 1975 Declaration of Helsinki and Good Clinical Practice guidelines and was approved by the Local Ethics Committee of the Faculty of Medicine, University of Alexandria. An informed consent was obtained from all subjects included in the study. Abdominal ultrasonographic examination was used for initial diagnosis of HCC while triphasic CT or MRI examination were employed to assess tumor characteristics [maximum diameter, number of nodules, location, extension, presence of capsule, portal vein invasion and intrahepatic metastasis] and the existence

of cirrhosis, ascites and splenomegaly. In addition, blood samples were collected from 30 age and sex-matched healthy subjects.

Routine laboratory investigations were done for all the study subjects; they included complete blood picture using Advia 2120 hematology system (Siemens Health Care Diagnostics, USA), serum creatinine, liver test profile; serum albumin concentration, total and direct serum bilirubin concentration, serum alanine aminotransferase (ALT) activity, serum aspartate aminotransferase (AST) activity, serum gamma glutamyl transpeptidase (GGT) activity, prothrombin time (PT) in citrated blood sample and international normalized ratio (INR) and hepatitis virus markers as HCV antibody, hepatitis B surface antigen and hepatitis B core antibody using enzyme-linked immunosorbent assay (ELISA) and serum HCV RNA and HBV DNA levels using real time polymerase chain reaction. In the current study, we used Child-Pugh classification[18] and the Model for End Stage Liver Disease (MELD) score in order to assess the severity of liver disease among patients with cirrhosis with as well as without HCC.[19] The staging of HCC was determined according to Barcelona Clinic Liver Cancer (BCLC) staging system.[20]

Sampling

Five ml venous blood were collected by venipuncture from antecubital vein under complete aseptic technique from every subject. The first blood fraction (3ml) was collected in clean centrifuge tube without anticoagulant to separate serum for biochemical analysis; serum samples were separated by centrifugation for 15 minutes at 8000 rpm. Hemolyzed samples were discarded. The other blood fraction (2ml) was for DNA extraction; it was transferred into disposable plastic tubes containing ethylenediamine tetraacetic acid (EDTA). All samples were stored at -20°C.

Determination of serum level of alpha-fetoprotein

The kit for determination of serum alpha-fetoprotein was provided by Diagnostic Automation /Cortez Diagnostics, Inc. California, USA. The AFP Quantitative Test Kit is based on a solid phase enzyme-linked immunosorbent assay.[21]

Determination of serum level of SIRT1 [22]

Serum level of SIRT1 was detected by by Enzyme-Linked Immunosorbent Assay [(22)] kit provided by Bioassay Technology Laboratory, Shanghai, China) according to the manufacturer's directions. It is based on sandwich enzyme-linked immune-sorbent assay technology. The duplicate readings for each standard, control, and samples were averaged and the average zero standard optical density was subtracted. A standard curve was created and the mean absorbance value for each sample was used to determine the corresponding concentration of SIRT1 in ng/ml.

SIRT1 SNP Genotyping Assay [23]

Genotyping for *SIRT1* single nucleotide polymorphism (rs7895833) was done using TaqMan SNP Genotyping Assay. DNA was purified from whole blood samples using a spin column protocol [QIAamp DNA Blood Mini Kit] provided by Qiagen, Hilden, Germany.[24] NanoDrop 2000 (Thermoscientific; USA) was used to check DNA quality and quantity. *SIRT1* SNP (rs7895833) was genotyped using 40x TaqMan® Predesigned SNP Genotyping Assays (provided by Thermo Fisher Scientific, Waltham, Massachusetts, USA). The context sequence of *SIRT1* (rs7895833) SNP is: TGAGGTGGTAAAAGGCCTACAGGAA[A/G]TCAACGTAATGGAGATTAGGAAGCA

The A allele was detected with VIC® dye and the G allele with FAM™ dye.

The 40X Predesigned SNP Genotyping Assay was diluted to a 20X working solution with nuclease free water. The reaction mix was composed of 40X TaqMan® Genotyping Assay, TaqMan® Genotyping Master Mix and nuclease-free water. The recommended final reaction volume per well was 20 μL for a 48-well plate (17 μL reaction mix + 3 μL DNA sample. For reaction mix preparation, 10μL of 2X TaqMan® Genotyping Master Mix, 1μL of 20X Assay Working Solution (0.5μL 40X Taqman assay + 0.5μL Nuclease free water) and 6μL of nuclease-free water were added in each well. The total reaction volume uses 20ng of genomic DNA.Real time PCR was performed using Applied Biosystems StepOne™ Real-Time PCR System.In the real-time PCR system software, an experiment or plate document was using the following thermal Cycling Conditions; first AmpliTaq Gold® Enzyme Activation step at 95°C for 10 minutes then 40 cycles; each consist of 15 seconds at 95°C for denaturation and 1 minute at 60°C for Annealing/Extension.

Statistical Analysis

Data were analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp) The normality of distribution of the study sample was verified using the Kolmogorov-Smirnov test. Significance of the obtained results was judged at the 5% level. The used tests were Chi-square test, Fisher's Exact or Monte Carlo correction, F-test (ANOVA), Post Hoc test (Tukey), Kruskal Wallis test and Post Hoc (Dunn's multiple comparisons test). Spearman coefficient was used for correlations. The sensitivity and specificity of serum SIRT1 were assessed by plotting a receiver-operating characteristic (ROC) curve and determining its cut-off value. For SNP, Hardy-Weinberg equation was used to explore the equilibrium of the sample population using the $\chi 2$ test. Odd ratio (OR) was used to calculate the ratio of the odds and 95% Confidence Interval.

Results

Characteristics of cirrhotic patients with and without HCC and healthy controls

Demographic analysis of the study revealed no statistically significant difference between the groups by gender or sex. (Table 1) Routine lab investigations for all the study subjects are shown in (**Table 1**). In addition, table 1 shows that serum AFP levels were significantly higher in HCC group and cirrhotic group than in healthy controls ($p<0.001$) as well as in HCC group than in

cirrhotic group (p<0.001). No statistically significant difference between cirrhotic patients with and without HCC regarding MELD score. (Table 1)

Table 1: Characteristics of cirrhotic patients with HCC (Group I), cirrhotic patients without HCC (Group II) and healthy controls (Group III).

Variables	Group I HCC (n = 40)	Group II Cirrhosis (n = 30)	Group III Control (n = 30)	*P* value
Age (years)	55.13 ± 5.18	54.0 ± 7.36	52.07 ± 8.4	0.193[a]
Sex				
Male, *n* (%)	33 (82.5)	20 (66.7)	18 (60.0)	1.000[d]
Female, *n* (%)	7 (17.5)	10 (33.3)	12 (40.0)	
Hemoglobin (g/dI)	10.12 ± 1.92*	10.44 ± 1.73*	13.81 ± 1.21	<0.001[a]
Platelet count (x10^3/cmm)	135.20 ± 54.42*	121.30 ± 62.00*	294.4 ± 103.2	<0.001[b]
Serum creatinine (mg/dl)	1.12 ± 0.29*	1.08 ± 0.30*	0.93 ± 0.19	0.019[a]
Serum albumin (g/dl)	2.51 ± 0.70*	2.52 ± 0.60*	4.36 ± 0.57	<0.001[a]
Total serum bilirubin (mg/dl)	2.39 ± 0.95*	3.19 ± 1.55*	0.45 ± 0.20	<0.001[b]
Serum AST (U/L)	118.8 ± 75.59*	87.87 ± 52.68*	21.13 ± 4.62	<0.001[b]
Serum ALT (U/L)	84.23 ± 60.95*	51.50 ± 23.69*	21.10 ± 4.69	<0.001[b]
Serum GGT (U/L)	101.95 ± 39.62*	84.63 ± 36.48*	23.63 ± 9.02	<0.001[a]
PT (seconds)	15.03 ± 1.89*	15.62 ± 2.28*	11.56 ± 0.31	<0.001[a]
INR	1.36 ± 0.18*	1.46 ± 0.27*	1.06 ± 0.08	<0.001[b]
Serum AFP (ng/ml)	348.0 ± 344.4*+	15.42 ± 10.83*	3.55 ± 1.46	<0.001[b]
Child-Pugh				
Score	7.88 ± 1.73	9.03 ± 2.25	-	0.017[c]
Class A, *n* (%)	13 (32.5)	6 (20.0)	-	
Class B, *n* (%)	21 (52.5)	15 (50.0)	-	
Class C, *n* (%)	6 (15.0)	9 (30.0)	-	
MELD score	13.60 ± 3.23	14.80 ± 3.65	-	0.151[c]
HCC diameter (cm)				
BCLC stage				
Stage 0, *n* (%)	5 (12.5)	-	-	
Stage A, *n* (%)	13 (32.5)	-	-	
Stage B, *n* (%)	12 (30.0)	-	-	
Stage C, *n* (%)	4 (10.0)	-	-	
Stage D, *n* (%)	6 (15.0)	-	-	

AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; GGT, Gamma glutamyl transferase; PT, Prothrombin time; INR, International normalized ratio; MELD, Model for End Stage Liver Disease; BCLC, Barcelona Clinic Liver Cancer.

Continuous data are represented as mean ± SD and categorical data are represented as number and percentages.

[a]One-way ANOVA test with pairwise comparison using *post Hoc* test (Tukey)[b]Kruskal Wallis test with pairwise comparison done using *Post Hoc* test (Dunn's for multiple comparisons test), [c]Student's *t* test, [d]Chi square test.

***Significant difference from healthy controls (*P* < 0.05).**

+Significant difference from cirrhotic patients without HCC (*P* < 0.05).

Serum SIRT1 and SIRT1 SNP in cirrhotic patients with and without HCC and healthy controls

Assessment of serum SIRT1 level revealed that there was a significant decrease in the level of serum SIRT1 in HCC group compared to the cirrhotic group ($p<0.001$) and healthy controls ($p<0.001$) whereas there was no statistically significant difference between cirrhotic group and healthy controls ($p=0.584$) as shown in (**Table 2**).

Results of *SIRT1* SNP (rs7895833) A/G genotype distribution among control and patient (HCC and cirrhosis) groups is presented in Table 2. The three genotypes frequencies didn't violate the HWE. [p=0.088, 0.524 and 0.543 for Group I (HCC patients), Group II (cirrhotic group without HCC) and Group III (Healthy control) respectively] *SIRT1* SNP (rs7895833) A/G genotype distribution showed that there was no significant difference between the three groups. (p=0.073). Nevertheless, in pairwise comparisons using chi square test, statistically significant difference was observed between HCC and control groups (p=0.047). The wild type; Homozygous (A/A) was significantly more prevalent among the control group than among the HCC group while the heterozygous mutation (A/G) was significantly more prevalent among the HCC group than among the control group. Regarding allele frequency in the whole sample (n=200), 164 (82.0%) were allele A and 36 (18.0%) were allele G. Allelic distribution revealed that neither of the two alleles (A or G) had significantly higher frequency among any of the three groups. (p= 0.156). However, when we tried pairwise comparisons between HCC and control groups, the mutant allele was found to be more prevalent among the HCC group than within the control group (p=0.047). When comparing between Group I and Group II for (A/G) vs (A/A), the odds ratio (OR) was 1.56 and (A/G+G/G) vs (A/A) OR was 1.28. Similarly, when comparing between Group I and Group III for (A/G) vs (A/A), the OR was 2.96 and (A/G+G/G) vs (A/A) OR was 2.96. This means that the risk of HCC seems to be increased when mutant allele (G) is present, mainly in the form of the heterozygous variant (A/G).

Table 2: Statistical comparison between the three studied groups as regards serum SIRT1 and *SIRT1* SNP.

	Group I HCC (n = 40)	Group II Cirrhosis (n = 30)	Group III Control (n = 30)	P
SIRT1 level (ng/ml)	7.3[b](4.8 – 20.1)	33.7[a](8.8 – 59.7)	36.4[a](6.5 – 59.2)	<0.001*
SIRT1- SNP:				
Homozygous (A/A) allele 1	23[a](57.5%)	19[ab](63.3%)	24[b](80.0%)	
Heterozygous (A/G)	17[a](42.5%)	9[ab](30.0%)	6[b](20.0%)	0.073
Homozygous (G/G) allele 2	0[a](0.0%)	2[a](6.7%)	0[a](0.0%)	
Allele frequency (n=200):				
Allele 1 (A)	63[a](78.8%)	47[a](78.3%)	54[a](90.0%)	0.156
Allele 2 (G)	17[a](21.3%)	13[a](21.7%)	6[a](10.0%)	
Presence of the polymorphic allele (G):				
Homozygous (A/A) allele 1	23[a](57.5%)	19[ab](63.3%)	24[b](80.0%)	
Heterozygous (A/G) + Homozygous (G/G) allele 2	17[a](42.5%)	11[ab](36.7%)	6[b](20.0%)	0.135

p: p value for comparison between the three studied groups

Common letters are not significant (i.e. Different letters are significant)

***: Statistically significant at p ≤ 0.05**

Statistical correlations between serum SIRT1 levels and other parameters in cirrhotic patients with and without HCC

Significant negative correlation was found between serum SIRT1 levels and Child-Pugh score in Group I and Group II (r= -0.650 and r= -0.441 respectively and p <0.001 and p =0.015 respectively) as well as between serum SIRT1 levels and HCC diameter and BCLC stage in Group I (r= -0.512 and r= -0.488 respectively and p=0.001* and p=0.001* respectively) while the negative correlation between serum SIRT1 levels and MELD score in both groups (r= -0.050 and r= -0.165 respectively) and between serum SIRT1 levels and AFP in Group I (r=-0.169) didn't reach the statistically significant level (p=0.761, p=0.383 and p=0.297 respectively). (**Table 3**)

Table 3: Statistical correlations between serum SIRT1 levels and other parameters in cirrhotic patients with and without HCC.

Parameters	Serum SIRT1 (ng/ml)			
	Group I HCC (n = 40)		Group II Cirrhosis (n = 30)	
	r_s	P	r_s	P
Chilr-Pugh score	-0.650*	<0.001*	-0.441*	0.015*
MELD score	-0.050	0.761	-0.165	0.383
Serum AFP (ng/ml)	-0.169	0.297	0.370*	0.044*
HCC diameter (cm)	-0.512*	0.001*	-	-
BCLC stage	-0.488*	0.001*	-	-

r_s: Spearman coefficient

***: Statistically significant at p ≤ 0.05**

Serum SIRT1 as a biomarker for the diagnosis of HCC

ROC curve analysis revealed that serum SIRT1 was superior to serum AFP as a predictor for the diagnosis of HCC in cirrhotic patients. When both were combined, the sensitivity and specificity were increased to 97.50 % and 93.33% respectively (AUC = 0.995, 95% CI = 0.986-1.00, p<0.001). (**Figure 1**)

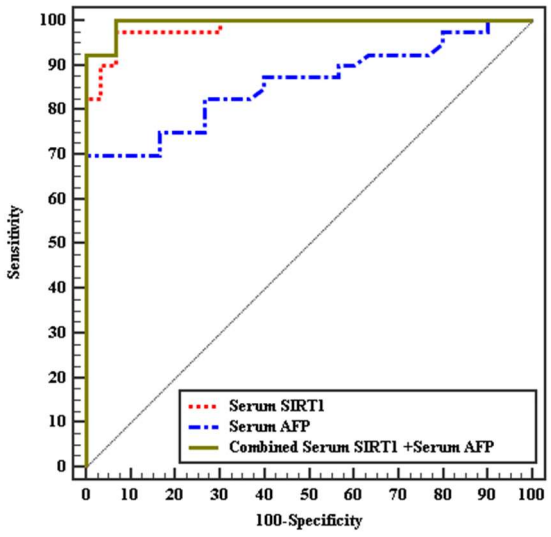

Figure (1): ROC curve for serum SIRT1 (ng/ml), serum AFP (ng/ml) and combined serum SIRT1 (ng/ml) and serum AFP (ng/ml) in discriminating cirrhotic patients with and without HCC.

Association of SIRT1 rs7895833 A/G genotypes with serum SIRT1 levels and other clinical and biochemical parameters

Table 4 shows a significant association between rs7895833 A/G genotypes and serum SIRT1 levels (P = 0.566); as serum SIRT1 level was significantly higher in study subjects with the homozygous (A/A) allele 1 than in those with heterozygous (A/G) variant (p=0.002) while no significant association was observed between rs7895833 A/G genotypes and serum AFP level (p=0.673). Studying SNP rs7895833 A/G genotypes with demographic variables and biochemical parameters among the three groups revealed no significant association.

Table 4: Relation between SIRT1-SNP with serum SIRT1 level, serum alpha-fetoprotein level, sex, age, liver test profile for total sample (n = 100).

Parameters	SIRT1-SNP			P
	Homozygous (A/A) (allele 1) (n = 66)	Heterozygous (A/G) (n = 32)	Homozygous (G/G) (allele 2) (n = 2)	
Serum SIRT1 (ng/ml)	25.5[a](4.8–59.7)	9.4[b](4.8–46.5)	12.6[b](8.8–16.3)	0.008*
Serum AFP (ng/ml)	10.8(1.2–1500.0)	14.2(2.0–970.0)	9.6(6.0–13.2)	0.697
Sex				
Male, n (%)	44(66.7%)	5(78.1%)	2(100.0%)	0.404
Female, n (%)	22(33.3%)	27(21.9%)	0(0.0%)	
Age (years)	52.8±7.4	56.1±5.7	52.0±0.0	0.084
GGT (U/L)	66.0(11.0–180.0)	82.5(11.0–220.0)	55.0(27.0–83.0)	0.351
ALT (U/L)	38.5(15.0–258.0)	39.0(17.0–230.0)	44.5(19.0–70.0)	0.701
AST (U/L)	55.5(13.0–324.0)	57.5(15.0–280.0)	60.0(30.0–90.0)	0.867
PT (seconds)	13.9±2.3	14.8±2.6	13.7±1.2	0.168
Total bilirubin (mg/dl)	1.8(0.1–6.5)	1.9(0.2–5.1)	2.2(1.8–2.6)	0.481
Serum albumin (g/dl)	3.2±1.0	2.77±1.05	2.20±0.0	0.058

p: p value for comparison between the three studied groups

Common letters are not significant (i.e. Different letters are significant)

***: Statistically significant at p ≤ 0.05**

Discussion

As in all forms of human malignancy, HCC is multifactorial in origin and results from a complex number of genetic and other events occurring against a background of host factors. [25] SIRT1 can play a role as tumor suppressor or as tumor promoter depending on the targets, its cellular location or specificity of the cancer pathology and type. [26] We found that the mean serum SIRT1 levels were significantly lower in Group I (HCC patients) than in the liver cirrhosis and control groups suggesting its role as a predictive marker of HCC among cirrhotic patients. In general, it was revealed it is very difficult to specify the action of SIRT1 in tumorigenesis.[16,27] Consistent with our results, Wang et al. revealed significantly decreased expression of SIRT1 in HCC[28] which suggests SIRT1 role as a tumor suppressor rather than a promoter in the liver tissues. [27,28] Moreover, Song et al. showed that SIRT1 level in the cytoplasm was considered as an independent tumor suppressor in HCC and survival analysis revealed that its overexpression was found in those with longer overall survival.[29] It is hypothesized that SIRT1 localizes in the cytoplasm where it increases the cell sensitivity to apoptosis. [30]

Many factors explain SIRT1 role as a tumor suppressor. SIRT1 causes deacetylation of autophagy (ATG) regulators (e.g., ATG5, ATG7, and ATG8) thus they promote mitophagy.[31] Also, it deacetylates Forkhead box protein O1 (FoxO1) as well as Forkhead box protein O3a (FoxO3a), inducing the expression of many elements in the autophagy machinery and upregulate the gene expression of manganese superoxide dismutase (MnSOD) and catalase, that play an important role in protecting the cell from reactive oxygen species induced oxidative damage. SIRT1 deacetylates thereby promotes the transcriptional activity of Nuclear factor (erythroid-derived 2)-like 2 (NRF2) besides upregulating the expression of antioxidant genes targeted by NRF2, including mitochondrial MnSOD, heme oxygenase-1 (HO-1), and glutathione.[32] More typically, SIRT1 was revealed to protect hepatic cells from canceration by suppressing the nuclear factor-kappa B signaling pathway, which constitutive activation was detected in HCC tissues. [33] Interestingly; Pinkston JM et al [(34)]revealed that factors which activate SIRT1 can both increase the lifespan in animals and significantly protect against cancer. [34] Besides, lower circulating SIRT1 is suggested to be a distinctive marker of frailty, diabetes mellitus, hypertension, cognitive status and a number of comorbidities. [35] These findings when put together, with the fact that aging is considered as the most potent carcinogen, [36] make SIRT1 to be a suggested as antiaging and cancer protecting candidate.

As regards the suggestion of SIRT1 as prognostic marker, our findings suggest that serum level of SIRT1 may indicate the severity of liver disease. We found a significant negative correlation between serum SIRT1 levels and Child-Pugh score and BCLC stage. Consistent with our results, a multivariate analysis performed by Zhang et al [37], found that activated SIRT1 was considered as a significant predictor of longer recurrence-free-*survival* (RFS) in HCC. They found a significant correlation between activated SIRT1 and activated AMPK in HCC tissues which harbour mutant p53. They suggested that combining these 2 markers can powerfully predict for good prognosis in

these patients.[37] In addition, lower SIRT1 levels were similarly a predictor of increased cancer aggression and bad prognosis in other cancers as in breast cancer patients where it was suggested that BRCA1 decreases cancer cell proliferation by a pathway which is mediated by SIRT1.[38] Moreover, it was found that SIRT1 negatively regulates transcription of miR-15b-5p thus suppresses colorectal cancer metastasis [39] and that SIRT1 expression was positively correlated to the overall survival in malignant ovarian serous tumors.[40]

On the other aspect, Wang et al suggested that SIRT1 overexpression increases uncontrolled tumor growth in HCC and decreases survival rate. [41] Furthermore, Luo et al found that SIRT1 mRNA expression was at high level in HCC [42] and Portmann et al hypothesized that SIRT1 expression protects the tumor cells favoring their survival. [43]Also, Chen et al reported higher level of SIRT1 expression in HCC tissues compared to the normal tissue. [44] Most of the studies, which suggest that SIRT1 is oncogenic, explain that SIRT1 inhibit p53 as well as several other tumor suppressor genes. [45] Thus, SIRT1 up-regulation induces deacetylated inactivation of p53, that in turn allows cell proliferation even with damaged DNA. [46] However, through a negative feedback loop, inactivating p53 may induce the reduction in SIRT1 expression level, thereby increasing p53 activity. [27] On the other hand, there is still a possibility that SIRT1 increases as a consequence of the process of tumorigenesis not as a risk factor. There are many explanations for the discrepancy between studies demonstrating SIRT1 role in cancer; in some studies, SIRT1 expression was assessed only at transcriptional level,[47] or using only cancer cell lines, and/or using human tissue samples but without considering the different molecular subtypes or without having the proper sample size statistically. [48]

In the current study, regarding *SIRT1* SNP (rs7895833), we found no statistically significant difference between the three groups. When the odds ratio (OR) was calculated between group I and group III; (A/G) vs (A/A) OR =2.96. Similarly, when comparing between group I and group II; (A/G) vs (A/A) OR =1.56 which means that the risk of HCC was found to be increased when the heterozygous mutant variant (A/G) is present nevertheless this association didn't reach the statistical significance level in cirrhotic patients.

Regarding allele frequency, it was found that neither of the two alleles (A or G) was significantly more frequent among any of the three groups of the study. However, pairwise comparisons showed that the mutant allele seems to be more prevalent among the HCC group than among the control group (p=0.047). Serum SIRT1 level was only significantly higher in study subjects with the homozygous (A/A) allele 1 than in those with heterozygous (A/G) variant (p=0.002). As regards serum AFP level, no statistically significant difference was observed between study subjects with the homozygous (A/A) allele 1 of SIRT1-SNP, those with heterozygous (A/G) variant and those with homozygous (G/G) allele 2 variant (p=0.673).

Hou et al found that *SIRT1* SNPs (rs33957861-rs11599176-rs12413112-rs35689145) as well as their expression were associated with the occurrence of alcoholic fatty liver disease (AFLD), and there was an association between these four SNPs and body mass index (BMI) in AFLD patients, but none of them was related to *SIRT1* expression. [49] Although many studies had been carried out on the relation between the SNPs of SIRT1 and human diseases, very little is known about the effect of polymorphism of *SIRT1* gene on cancer in general until now. Moreover, to our knowledge, nothing is known about the association of *SIRT1* polymorphism and liver cancer

development and this is the first study to try to investigate such association in Egyptians. Rizk et al [50] were the first to study the relation between *SIRT1* SNP and susceptibility as well as prognosis of breast cancer in Egyptians. They reported an association between *SIRT1* SNP, namely rs3758391 (C/T), rs12778366 (C/T), and ,to a lesser extent, rs3740051 (A/G) and breast cancer risk showing that the G allele (for rs3740051) in addition to the T allele (for rs3758391 and rs12778366) were found as potential risk factors. [50]

The non-significant association between SIRT1 gene polymorphism and HCC presented here may be due to the relatively small number of the cases and controls, which is the main limitation of our study, as well as due to the complex multifactorial genetic contribution to liver cancer. Expanding the sample size might be able to find a more meaningful result as regards gene polymorphism.

Conclusion

Decreased production of SIRT1 and its gene polymorphism can play a role in the development and progression of HCC. Serum SIRT1 is a promising candidate to be used as a biomarker to detect HCC in cirrhosis.

Recommendations

The current study underlies the need of thorough understanding of the physiology and the pathophysiology of *SIRT1* gene and SIRT1 protein as promising biomarkers in HCC. Their evaluation side by side with AFP may improve the sensitivity and specificity that may help better early detection of HCC. Furthermore, we suggest implementation of the study in large population-based studies in order to derive normal reference and to explore the associated mechanisms. Studies specifying the localization of SIRT1 and the effects of cytotoxic drugs on SIRT1 levels are required as this may explain the pathological consequences of the variations of its level.

Ethics approval and informed consent

The study was approved by the Ethics Review Board of the Faculty of Medicine, Alexandria University (IRB NO: 00007555). Informed consent was obtained from all patients.

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Competing interests

The authors declare they have neither financial nor non-financial competing interests.

Authors' contribution

S.M., S.H.M., H.A., F.D., H.Y., Study conception and design; S.M., H.Y., Data acquisition, laboratory investigations, data analysis and interpretation; H.A., Clinical assessments and data acquisition; S.M., H.Y., Writing the article; S.M., S.H.M., H.A., F.D., H.Y., Revision of the article and final approval of the version to be published; S.M., S.H.M., H.A., F.D., H.Y., Agreement to be accountable for all the different aspects of the work in ensuring that all the questions related to the accuracy, specificity and integrity of the work are properly investigated and resolved.

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