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The relation between SNPs in the NME1 gene and response to sofosbuvir in Egyptian patients with chronic HCV

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Abstract

Background Hepatitis C virus (HCV) infection is considered one of the most urgent health problems in the world, with an incidence of approximately 71 million patients and 399,000 deaths per year from related liver diseases. In this study, we examined the association between 2 single nucleotide polymorphisms (SNPs) in the nucleoside diphosphate kinase 1 (NME1) gene (encoding one of the sofosbuvir metabolizing enzymes) and the response to the sofosbuvir plus daclatasvir regimen in Egyptian HCV-infected patients.

Results Our data showed a similarity in the distribution of the CC, CT, and TT genotypes of NME1 rs2302254 C/T ($p = 0.847$) and the CC, TC, and TT genotypes of NME1 rs16949649 T/C ($p = 0.937$) among patients who were either treatment responders or relapsers. Based on the univariate and multivariate logistic regression analyses of the significant predictors for sustained virological response (SVR), five factors showed a robust predictive potency for the treatment outcome: age, fasting blood glucose level, platelets, albumin, and alpha-fetoprotein. Strikingly, there was a significant correlation between the rs16949649T/C polymorphism and serum creatinine ($p = 0.023$). Higher creatinine levels were observed among the CC carriers than the TC or TT carriers.

Conclusions The 2 studied SNPs of NME1 had no significant association with SVR in Egyptian HCV-infected patients; however, the noticeable relation between rs16949649T/C and creatinine level might represent a foundation for future studies on the renal extra-hepatic manifestation of HCV and SNPs of NME1 gene.

Keywords HCV, DAAs, Sofosbuvir, SNPs, NME1

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1 Background

The hepatitis C virus (HCV) is classified, according to the World Health Organization (WHO), as a global health problem [1]; more than 71 million infected cases (~80–85% of those patients develop chronic hepatitis [2]. Chronic HCV infection causes severe liver damage due to the persistent induction of inflammation, with the highest incidence of cirrhosis and hepatocellular carcinoma (HCC) in many patients [3]. Compared to the other populations, Egyptians had the most elevated rate of HCV-infected cases within the world (almost 10 to 20% of the entire population), of which more than 90% were infected with HCV genotype (GT) 4a [4]. Recently, the efforts of screening national campaigns achieved remarkable progress in the control of HCV, and the percentage of infection in the total population fell from 10% in 2015 to 4.61% in 2020 [5].

HCV currently has no available vaccine, and current therapies depend only on antiviral drugs [6]. The standard regimen of anti-HCV therapy before 2011 was pegylated interferon-alpha (PEG-IFNα) plus ribavirin (RBV). This dual therapy formed cure rates between 45 and 60% for HCV genotypes 1 and 4 and 70–80% for GT2 and GT3 [7].

Direct-acting antivirals (DAAs), when introduced in the market as a firstline treatment for HCV, have made profound changes in the treatment plan for this disease for different reasons: (1) the increased treatment success rate [8], as measured by the high sustained virological response (SVR) following the treatment, (2) the attenuation of hepatic fibrosis progression with the consequent reduced risk of cirrhosis, and (3) reduced extra-hepatic manifestations, which improve to a high degree the quality of life of the treated patients [9, 10].

NS3/4 A protease inhibitors, i.e., telaprevir (TVR) and boceprevir (BOC), are the first-generation DAAs approved by the Food and Drug Administration (FDA) in 2011 as "triple therapy" together with PEG-IFN and ribavirin for HCV GT1, achieving a 65–75% SVR [11, 12]. After that, in 2013, another drug, simeprevir (SMV), a targeted enzyme in the HCV life cycle, was classified as an NS3/4A protease inhibitor and used once daily in combination with PEG-IFN and ribavirin for the treatment of GT1 and GT4 [13, 14]. Significant progress has been achieved by the discovery of sofosbuvir (SOF), that is, NS5B polymerase inhibitor, which binds to newly synthesized viral RNA causing chain termination [15].

GS-461203 is the active metabolite of sofosbuvir (SOF [GS-7977]), and after being taken up by hepatocytes, GS-461203 is converted by cellular enzymes to GS-331007, which causes chain termination [16, 17]. The metabolic pathway of sofosbuvir includes three enzymes that work consecutively: cathepsin A (CatA), which performs hydrolysis; histidine triad nucleotide-binding protein 1 (HINT1), which performs phosphoramidate cleavage; and nucleoside diphosphate (NDP), which performs phosphorylation [18].

The nucleoside di-phosphate kinase family includes different members; one of the most important members is NME1. Several studies have discussed the role of the NME1 gene in aggressive cancers, such as ovarian cancer, melanoma, gastric cancer, breast cancer, and HCC [19, 20]. However, our previous study is the sole one investigating the relationship between this gene and the response to pharmaceutical drugs (i.e., SOF/DCV) in HCV patients [21].

Several reports have explored the role of viral factors in the context of resistance to DAA therapy. These studies have focused on analyzing newly developed drug-resistance mutations, which result in high sequence diversity and the formation of numerous quasispecies in HCV-infected patients [22, 23]. However, the possible contribution of host factors to this context is negligible in most studies. In the current study, we examined the association between single nucleotide polymorphisms (rs16949649 and rs2302254) in the NME1 gene, a vital member of the SOF metabolic pathway, and the response rate to SOF/DCV combinatory drugs.

2 Patients, materials and methods

2.1 Ethics and consent to participate

All experiments were approved by the medical research ethics committee of the National Research Centre in Cairo under number 16198 on 25 May 2018, Egypt, in accordance with the Helsinki Declaration of 1975, as revised in 2008. Before collecting blood samples, each patient signed a written informed consent form.

2.2 Subjects

This cross-sectional study involved 136 Egyptian patients who received DAAs for 12 weeks (SOF 400 mg/DCV 60 mg) aged between 31 and 74 years and 48 subjects who served as a control group. Between October 2018 and December 2019, all patients were recruited from the Endemic Medicine Department, Faculty of Medicine, Cairo University, and Kafr El-Sheikh Cardiac and Liver Centre.

Patients ($n=136$) were separated into two groups depending on whether or not they attained SVR12 after treatment: responders ($n=71$), who did, and nonresponders ($n=65$), who did not.

The patients in the study were all naive HCV patients (those who had not previously received any treatment, including peg IFN), with albumin > 3.5 g/dl, an international normalized ratio (INR) of < 1.2, a platelet count of > 150 000/mm, and total serum bilirubin of < 1.2 mg/

dl at baseline. The patients were all free of the human immunodeficiency virus (HIV), and the hepatitis B virus (HBV) and, as well as non-diabetic, non-alcoholic, drug-addicted free, and free of nonviral liver disorders. All the healthy control volunteers in the study were negative HCV and HBV virological indicators, non-obese, F0 on Fibro Scan, and normal abdominal ultrasound. The fibrosis-4 index (FIB-4) was developed to assess all people noninvasively to measure the degree of hepatic fibrosis.

2.3 Genomic DNA extraction

Following the instructions on the datasheet, DNA was isolated from peripheral blood using a Qiagen DNA extraction kit (Qiagen, Santa Clarita, CA).

2.4 Genotyping by real-time PCR

Real-time PCR was utilized to genotype the NME1 SNPs rs16949649 and rs2302254 using the allelic discrimination assay methodology (Applied Biosystems, USA).

The following are the stages involved in performing an allelic discrimination assay:

One µL of extracted human DNA was taken, then1.25 µL of a 20X primer/probe was added, then 12.5 µL of 2X TaqMan Universal PCR master mix was added, and. finally, this volume was mixed with nuclease-free water to a final volume of 25 µL.

The assay was run at three-time intervals at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min by using a Rotor-Gene real-time PCR system (Qiagen, Santa Clarita, CA).

2.5 Statistical analysis

The data were coded and entered using the statistical software for the social sciences (SPSS) version 26 from IBM Corp. in Armonk, New York, United States of America. For quantitative data, the mean and standard deviation were utilized as measuring tools, while the frequencies (number of cases) and relative frequencies (percentages) were used for categorical variables.

When comparing two groups, unpaired t-tests were used, and analysis of variance (ANOVA) with multiple comparisons post hoc tests were utilized whenever more than two groups were being compared [24].

In order to compare categorical data, the chi-square ($\triangle 2$) test was carried out. When it was anticipated that the frequency would be fewer than 5, an exact test was used instead of the chi-square ($\triangle 2$) test [25]. Odds ratios (ORs) with 95% confidence intervals were calculated.

To find independent predictors of response, logistic regression was used. [26]. A value of P that was lower than 0.05 was considered to indicate statistical significance.

Table 1 The relation between clinical characteristics and treatment outcome in hepatitis C patients treated with SOF + DCV and healthy control

	Healthy control	HCV patients		
		Responder N = 71	Non-responder N = 65	*P* value
Age (year)	35.8 ± 8.3	50.62 ± 8.47	59.73 ± 5.70	**< 0.001**
Fasting blood glucose (mg/dL)	87.8 ± 6.6	99.97 ± 25.06	93.00 ± 10.44	**0.034**
Serum Creatinine (mg/dL)	0.74 ± 0.11	0.85 ± 0.20	0.94 ± 0.18	**0.009**
White blood cell ($\chi 10^3$/µl)	5.36 ± 0.79	5.48 ± 1.73	4.57 ± 1.22	**0.001**
Hemoglobin (g/L)	14.36 ± 1.05	13.50 ± 1.59	11.75 ± 1.88	**< 0.001**
Platelets ($\chi 10^3$/µl)	308.04 ± 62.6	158.82 ± 57.64	177.86 ± 32.17	**0.018**
AST (IU/L)	26.92 ± 5.2	66.74 ± 39.12	69.42 ± 32.45	0.662
ALT (IU/L)	37.16 ± 13.25	65.32 ± 40.38	69.12 ± 30.90	0.536
Prothrombin concentration%	90.04 ± 3.5	88.89 ± 8.85	83.44 ± 22.11	0.219
Bilirubin (umol/L)	0.27 ± 0.08	0.91 ± 0.37	1.12 ± 0.29	**< 0.001**
Albumin (g/L)	4.09 ± 0.5	3.93 ± 0.42	4.17 ± 0.29	**< 0.001**
Alpha-fetoprotein (Log)	–	0.82 ± 0.49	0.54 ± 0.20	**< 0.001**
Baseline HCV RNA (log IU/mL)	–	5.00 ± 0.99	5.23 ± 0.88	0.161
Fibrosis-n (%)	–			
FIB-4 < 1.45		41 (57.7%)	25 (37.9%)	
FIB-4 = 1.45–3.25		10 (14.1%)	22 (33.3%)	**0.016**
FIB-4 > 3.25		20 (28.2%)	19 (28.8%)	

ALT alanine aminotransferase, *HCV* hepatitis C virus, *AST* aspartate aminotransferase, *N* denotes to the number of patients or healthy controls. *P* values were calculated by Student's *t* test (data are expressed as the mean and SD) and the chi-square test (The data are presented in the form of frequencies and percentages). Significant *p* values are highlighted in bold, and $p \leq 0.05$ was considered significant

3 Results

3.1 The research subjects' baseline clinical and demographic characteristics

The study included 136 patients with HCV infection: 71 patients achieved SVR12 (responders), and 65 patients failed to achieve SVR12 (nonresponders) for the SOF + DCV regimen, in addition to 48 healthy controls. For all healthy control, all the clinical parameters are within the normal range. For HCV patients, all the following parameters showed significant differences between responders and nonresponders: age, fasting blood glucose, serum creatinine level, white blood cell counts, hemoglobin level, platelet counts, bilirubin level, albumin level, fibrosis stage and alpha-fetoprotein (Table 1, $p < 0.05$). However, aspartate aminotransferase (AST), alanine aminotransferase (ALT), prothrombin concentration, and viral load showed nonsignificant differences (Table 1, $p > 0.05$) between the two groups.

3.2 Distribution of rs2302254 C/T and rs16949649 T/C in healthy controls and HCV patients

The distribution of NME1 rs2302254 genotypes in healthy control was 54.2% CC, 41.7% CT, and 4.2% TT, and the distribution of genotypes for HCV patients CC was more prevalent (56.6%), CT(36%), and TT (7.4%) and for NME1 rs16949649 T/C the distribution of genotypes as follow for healthy control TC more prevalent (37.5%), TT(35.4%) and CC (27.1%) and HCV patients TC more prevalent (54.4%) while CC (24.3%) and for TT (21.3%). The distribution of the C allele versus T allele for rs2302254 C/T is (74.6%, 75%). For the T allele (25%, 25.4%), respectively for healthy control and HCV patients and rs16949649 T/C, the distribution of the C allele was (45.8%, 51.5%) and for T allele (54.2%, 48.5%), respectively for both groups healthy control and HCV patients. There were no significant differences (p value > 0.05) for the two groups (healthy control and HCV patients) for genotypes or alleles for the two SNPs (rs2302254 C/T and rs16949649 T/C, as shown in Table 2).

3.3 Association of two SNPs in NME1 with treatment outcome of the SOF + DCV regimen

Association between NME1 polymorphisms and response to the SOF + DCV regimen. The frequency distribution of genotypes and alleles of the rs2302254C/T and rs16949649T/C polymorphisms is shown in Table 3 ($p = 0.937$, 0.847, 0.792 and 0.581). We did not find a

Table 2 Distribution of SNPs in the NME1 gene for the healthy controls and HCV patients

Groups	rs2302254 C/T						
	CC	CT	TT	*P* value	C	T	*P* value
Control N = 48	26 (54.2%)	20 (41.7%)	2 (4.2%)	0.587	72 (75%)	24 (25%)	0.943
Patients N = 136	77 (56.6%)	49 (36.0%)	10 (7.4%)		203 (74.6%)	69 (25.4%)	
Groups	**rs16949649 T/C**						
	CC	TC	TT	*P* value	C	T	*P* value
Control N = 48	13 (27.1%)	18 (37.5%)	17 (35.4%)	0.251	44 (45.8%)	52 (54.2%)	0.343
Patients N = 136	33 (24.3%)	74 (54.4%)	29 (21.3%)		140 (51.5%)	132 (48%)	

N = sample size, $p \geq 0.05$ is considered nonsignificant

Table 3 Distribution of SNPs in the ***NME1*** gene for the two groups of patient responders and nonresponders

Groups	rs2302254 C/T						
	CC	CT	TT	*P* value	C	T	*P* value
Responders N = 71	39 (54.9%)	26 (36.6%)	6 (8.5%)	0.847	104 (73.2%)	38 (26.8%)	0.581
Non-responders N = 65	38 (58.4%)	23 (35.4%)	4 (6.2%)		99 (76.2%)	31 (23.8%)	
Groups	**rs16949649 T/C**						
	CC	TC	TT	*P* value	C	T	*P* value
Responders N = 71	17 (23.9%)	38 (53.6%)	16 (22.5%)	0.937	72 (50.7%)	70 (49.3%)	0.792
Non-responders N = 65	16 (24.6%)	36 (55.4%)	13 (20.0%)		68 (52.3%)	62 (47.7%)	

N = number of patients, $p \geq 0.05$ was considered nonsignificant

Table 4 Characteristics of subjects by NME1 rs16949649 T/C and rs2302254 C/T genotype in hepatitis C virus patients

	rs16949649 T/C			
	TT N (29)	**TC N (74)**	**CC N (33)**	***P* value**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	
Age (year)	53.90 ± 9.74	55.73 ± 7.86	54.33 ± 9.21	0.549
Fasting blood glucose (mg/dL)	93.55 ± 14.99	98.01 ± 22.56	95.97 ± 16.59	0.580
Serum creatinine (mg/dL)	0.90 ± 0.18	0.86 ± 0.20	0.97 ± 0.18	**0.023**
White blood cell ($\chi 10^3$/μl)	5.30 ± 1.98	4.84 ± 1.32	5.30 ± 1.68	0.241
Hemoglobin (g/L)	12.32 ± 2.35	12.67 ± 1.75	12.98 ± 1.96	0.408
Platelets ($\chi 10^3$/μl)	164.90 ± 54.13	172.20 ± 47.76	161.18 ± 43.49	0.513
AST (IU/L)	66.33 ± 47.59	70.14 ± 32.50	64.29 ± 32.67	0.718
ALT (IU/L)	65.01 ± 43.08	69.52 ± 35.31	63.30 ± 31.87	0.676
Prothrombin concentration%	83.33 ± 7.87	86.23 ± 25.56	84.27 ± 10.00	0.833
Bilirubin (umol/L)	0.98 ± 0.36	1.00 ± 0.37	1.06 ± 0.30	0.623
Albumin (g/L)	4.01 ± 0.45	4.05 ± 0.34	4.07 ± 0.41	0.861
	rs2302254 C/T			
	TT N (10)	**CT N (49)**	**CC N (77)**	***P* value**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	
Age (year)	54.18 ± 9.03	53.31 ± 6.43	55.52 ± 8.2	0.661
Fasting blood glucose (mg/dL)	96.12 ± 19.34	99.01 ± 21.26	97.01 ± 20.19	0.965
Serum creatinine (mg/dL)	0.87 ± 0.21	0.88 ± 0.21	0.9 ± 0.175	0.476
White blood cell ($\chi 10^3$/μl)	5.22 ± 1.52	4.7 ± 1.41	4.91 ± 1.61	0.104
Hemoglobin (g/L)	12.99 ± 1.76	12.1 ± 1.89	12.43 ± 2.04	0.250
Platelets ($\chi 10^3$/μl)	168.57 ± 46.5	169.20 ± 46.62	167.7 ± 49.5	0.902
AST (IU/L)	69.31 ± 33.6	68.14 ± 34.41	66.89 ± 38.0	0.788
ALT (IU/L)	69.6 ± 38.97	67.13 ± 31.22	65.07 ± 33.96	0.771
Prothrombin concentration%	85.1 ± 11.13	87.3 ± 24.69	85.21 ± 24.17	0.986
Bilirubin (umol/L)	0.98 ± 0.32	1.1 ± 0.29	1.02 ± 0.37	0.714
Albumin (g/L)	4.06 ± 0.36	3.98 ± 0.29	4.03 ± 0.39	0.793

ALT alanine aminotransferase, *AST* aspartate aminotransferase; N = number of patients

Significant *p* values are highlighted in bold, and $p \leq 0.05$ is considered significant

significant difference between responders and nonresponders on the spot of genotype or allele (Table 4).

3.4 Association of SNPs in *NME1* rs16949649 T/C and rs2302254 C/T with clinical parameters in HCV patients

There were no statistically significant differences in clinical parameters between the three genotypes (TT, TC and CC) of NME1 rs16949649 T/C. Only one biochemical parameter, serum creatinine level, among the 11 parameters inspected was significantly higher for the CC genotype (0.97 ± 0.18) than for the TC genotype (0.86 ± 0.20) and TT genotype (0.90 ± 0.18). There were no variations in clinical parameters that could be considered statistically significant between the three

Table 5 Stepwise binary logistic regression for clinical data to detect independent predictors of response

	***P* value**	**OR**	**95% CI**	
			Lower	**Upper**
Responder versus non-responder				
Age	< 0.001	0.695	0.605	0.797
Fasting blood glucose	0.001	1.088	1.036	1.142
platelets counts	0.002	0.978	0.964	0.992
Albumin concentration	0.020	0.053	0.004	0.629
Log alpha-fetoprotein	0.007	15.343	2.131	110.483

$p \leq 0.05$ is considered significant

genotypes (TT, CT, and CC) of the NME1 rs2302254 C/T gene. The difference between the three genotypes (TT, CT, and CC) among the 11 parameters that were examined was insignificant.

3.5 Stepwise logistic regression analysis was performed to determine the predictive factors for the SOF + DCV regimen in HCV-infected patients

Table 5 showed that 5 independent factors had a significant association with SVR, namely, age (odds ratio 0.695, 95% CI 0.605–0.797, $p = < 0.001$), fasting blood glucose level (odds ratio 1.088, 95% CI 1.036–1.142, $p = 0.001$), platelet count (odds ratio 0.978, 95% CI 0.964–0.992, $p = 0.002$), albumin concentration (odds ratio 0.053, 95% CI 0.004–0.629, $p = 0.020$) and alpha-fetoprotein (odds ratio 15.343, 95% CI 2.131–110.483, $p = 0.007$). These results indicate that these clinical parameters have significant predictive value for response to treatment.

4 Discussion

The main goal is to discover new biomarkers for treatment outcomes, which can be achieved by defining the host genetic factors that control the response to drugs. Our previous study showed a strong association between SNPs as crucial mediators in the metabolic pathway of SOF and the response to the SOF/DCV regimen in Egyptian patients infected with HCV [21]. To expand our prior findings, we continued investigating whether there is a correlation between the response rate and polymorphisms in the other genes of the same pathway. For example, Rs2302254 and rs16949649 are 2 SNPs in the NME1 gene that encode a major kinase enzyme in the SOF biochemical pathway. We found no association between SNPs and response, but we found an association between genotype CC rs16949649 and high serum creatinine levels.

SOF is a nucleotide polymerase inhibitor, which places it in the direct-acting antiviral drug category. A SOF metabolic cascade contains the sequential activation of several enzymes. [27], namely, uridine monophosphate-cytidine monophosphate kinase and NDP (nucleotide phosphorylation), HINT1 (cleavage of the phosphoramidite), and CatA (hydrolysis of the carboxyl ester group). These enzymes work together to produce the active form, denoted GS-461203 (triphosphate metabolite of SOF), which is incorporated into HCV RNA by NS5B, finally leading to chain termination [28]. Here, we focused on the pharmacogenetics of SOF-metabolizing enzymes due to our belief in the vital function executed by SOF in the clearance of HCV infection, which makes it commonly shared by different HCV treatment protocols; therefore, the applicability of our finding is likely to extend to other therapeutic regimens.

Even though three-quarters of people with acute hepatitis C virus (HCV) infections will eventually develop chronic liver disease, one-fourth of those patients will eliminate the virus on their own. Polymorphisms in the gene that encodes IFN3 (IFNL3; formerly known as IL28B) have been connected with the resolution of HCV infection in people of both European and African descent. These polymorphisms have also been associated with variances in responsiveness to medication treatment for HCV. In addition, genome-wide association studies (GWAS) have reported HLA class II associations with spontaneous HCV clearance [29, 30]

The NME1 gene is also known as NM23-H1 and NDPK-A. NME1 is found on chromosome 17q21 and encodes the NME1 protein. This is a metastasis suppressor gene that helps prevent cancer metastasis [31, 32]. In highly metastatic cells, NME1 mRNA expression is decreased. Impaired expression of NME1 has been observed in several cancers with an inverse relationship with metastasis; these tumors include breast cancer, melanoma, ovarian cancer, cervical cancer, colorectal cancer, gastric cancer, and HCC [20]. Jie Yang et al. showed a correlation between higher recurrence and poor prognosis of HCC and upregulated expression of NME1 and considered it a future biomarker for targeted therapy of HCC [33]. The SNPs rs16949649 and rs2302254 are found in the 5′ promoter region of the NME1 gene. SNPs in promoter regions significantly impact the related transcriptional activity [34]. Rajagopal et al. found associations between the CC genotype NME1 rs16949649 and an increased risk of breast cancer in Indian women [35], while Qu et al. found correlations between the TT genotype in rs16949649, the CC genotype in rs2302254 and lymph node metastasis risk for gastric cancer in a northern Chinese population [36]. Huang et al. found that the CC genotype rs16949649 and the TT genotype rs2302254 did not increase the risk of endometriosis [37]. This is the first study to examine the relationship between SNPs rs16949649 and the rs2302254 NME1 gene in HCV-infected patients. However, these SNPs did not seem to play a role in susceptibility to HCV infection, hepatic fibrosis, or responsiveness to DAA. The C alleles rs16949649 and rs2302254 were the most predominant in all the studied groups.

Several previous studies have demonstrated a relationship between baseline clinical parameters, such as pretreatment HCV RNA levels, age, liver fibrosis status, insulin resistance, higher pretreatment AST levels, and response to the standard treatment of HCV (PEG-IFNα) plus (RBV) [38] and El-Garawani et al. explain the relation between clinical parameters, e.g., age, diabetes

mellitus, AST and albumin and response to DAA and found these clinical parameters had prediction value on achievement of SVR[39]. Our study demonstrated the relationship between basal clinical parameters and response to (SOF + DCV) parameters, including age, fasting blood glucose level, platelet count, albumin concentration, and log alpha-fetoprotein level, and found a significant association with SVR that was considered a great predictor of response.

After achieving SVR and viral clearance, liver fibrosis is strongly related to the progression to HCC in HCV patients. Several previous studies demonstrated this finding after the previous regimen for the treatment of HCV (PEG-IFNα) plus (RBV) [40, 41]. Shiha et al. scored for predicting HCC in HCV patients with liver cirrhosis and severe fibrosis and achieved SVR following the DAA regimen[42]. Our study showed differences in fibrosis stage when comparing responders and nonresponders. Nonresponders had fibrosis scores higher than responders. Creatinine is the end product of protein and muscle metabolism and is excreted in urine through glomerular filtration at a relatively constant rate [43]. Serum creatinine is an essential indicator of kidney function. One of HCV's most well-known extrahepatic symptoms is chronic kidney disease (CKD). Kaartinen et al. showed that infection with HCV appears to influence both glomerular and renal tubular cells, representing involvement in HCV-induced renal manifestations even in their early phase [44]. This was verified by Ferri et al., who showed that nephropathy might develop at any time during infection with HCV [45]. In our study, the CC genotype of rs16949649 T/C was associated with elevated basal levels of serum creatinine. This is considered an exciting finding referring to the implication of NME1 rs16949649 T/C could be related to renal extrahepatic manifestation in HCV patients.

5 Conclusion

To this end, our data indicated that neither the polymorphism of the NME1 gene rs2302254 C/T nor rs16949649 T/C was associated with the response to SOF/DCV in the Egyptian population. However, our finding on the association between the polymorphism of the rs16949649 T/C and the creatinine level in serum opens the door for future studies regarding the impact of this SNP on the renal extra-hepatic manifestation associated with HCV disease. A study that includes a larger sample size is warranted to confirm the latter hypothesis.

Abbreviations

ALT	Alanine aminotransferase
AFP	Alpha-fetoprotein
AST	Aspartate aminotransferase
BOC	Boceprevir
CatA	Cathepsin A
DAAs	Direct-acting antivirals
FBG	Fasting blood glucose level
FDA	Food and Drug Administration
GT1	Genotypes 1
GT4	Genotypes 4
HCV	Hepatitis C Virus
HCC	Hepatocellular carcinoma
HINT1	Histidine triad nucleotide-binding protein
NDP	Nucleoside diphosphate
PEG-IFNα	Pegylated interferon-alpha
PLT	Platelets
RBV	Ribavirin
SMV	Simeprevir
SNPs	Single nucleotide polymorphisms
SVR	Sustained virological response
TVR	Telaprevir

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Author contributions

MA: Conceptualization, methodology, writing—original draft, review and editing. MK: Conceptualization, methodology, project administration, resources, supervision, writing—review and editing. ST: Methodology, investigation. DO: Methodology. MM: Methodology. SO: Methodology. HE: Methodology, supervision. All authors read and approved the final manuscript.

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Availability of data and materials

The authors confirm that the data supporting the findings of this study are available within the article.

Declarations

Ethics approval and consent to participate

All experiments were approved by the medical research ethics committee of the National Research Centre in Cairo under number 16198 on 25 May 2018, Egypt, in accordance with the Helsinki Declaration of 1975, as revised in 2008. Before collecting blood samples, each patient signed a written informed consent form. All eligible individuals agreed to voluntary participation and signed an informed consent form.

Consent for publication
All participants in this study give consent to publish the data.

Competing interests
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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