Association between cluster of differentiation 24 (CD24) polymorphism, talin-1 gene expression and hepatocellular carcinoma prevalence in the Egyptian population

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Abstract

Introduction: Hepatitis C is considered one of the most common diseases in Egypt. We aim to clarify the association between cluster of differentiation 24 (CD24) polymorphism, talin-1 gene expression, and the prevalence of hepatocellular carcinoma (HCC) in Egyptian hepatitis C virus (HCV) patients.
Material and methods: The link between *CD24* polymorphism rs8734 and the prevalence of HCC was assessed between 200 control subjects and 400 HCV patients. Patients were classified as follows: 200 patients with HCV and 200 with HCV and HCC by histopathological assessment and PCR-restriction fragment length polymorphism (PCR-RFLP).
Results: The hepatitis C patients with HCC showed a significant increase in alpha-fetoprotein (AFP) and talin-1 gene expression compared to patients with HCV as well as healthy volunteers. Furthermore, the frequency of *CD24* 170 CT/TT genotype was significantly higher in HCV patients without complications (60%) compared to CC genotype (40%) (OR = 6 at $\chi^2 = 14.41$, $p = 0.0007$), and in HCV with HCC patients (90%) compared to CC genotype (10%) (OR = 36 at $\chi^2 = 14.41$, $p = 0.0007$).
Conclusions: These data suggest that *CD24* genetic polymorphism rs8734 and talin-1 gene expression may be a significant determinant for the prevalence of HCC in HCV patients.

Key words: hepatocellular carcinoma, SNP, HCV, CD24, talin-1, alpha-fetoprotein.

Introduction

Hepatocellular carcinoma (HCC) was recently reported to be the fifth most common type of malignancy, with a high mortality rate globally [1]. In Egypt, HCC is the fourth most common cancer [2]. Hepatocellular carcinoma is a complicated process that occurs due to multiple risks such as hepatitis C virus (HCV), hepatitis B virus (HBV), consumption of alcohol, and diabetes [3–5]. Hepatitis C virus causes acute and chronic hepatitis.

If the HCV is not treated, many pathways can lead to hepatocarcinogenesis [6]. The most frequent pathway is that HCV core protein regulates gene expression, which causes oxidative stress and leads to HCC [7].

Furthermore, recent sequencing studies have revealed that genetic variations are associated with well-established risk factors in certain ethnic populations [8, 9]. Genotype 4 is the most predominant genotype present in Egypt, with subtype 4a considered to be the dominant subtype [10].

Cluster of differentiation 24 (CD24) is a signal transducer or heat-stable antigen (HSA) that can be defined as a human protein encoded by the *CD24* gene. CD24 glycoprotein is present at the surfaces of neuroblasts and most B lymphocytes. It is a glycoprotein encoding gene expressed in B cells and on mature granulocytes. Glycosylphosphatidylinositol (GPI) anchors the encoded protein by cell surface links [11].

The *CD24* gene, found on chromosome 6 at position 21, is an alignment to genomic locations with similarity with that on chromosomes 1, 15, 20, and Y. Experimental determinations for corresponding translation and transcription of each genomic location are needed. *CD24* polymorphism can affect the risk of development of chronic HCV infection. The *CD24* P170T allele is associated with HCV infection at a higher level. Among the chronic HCV Egyptian patients, *CD24* P170T allele shows recessive associate [12]. The rapid progression of HCC and liver cirrhosis in the *CD24* P170T allele is significantly higher when compared with *CD24* P170C allele in HCV patients. Deletion of dinucleotide at position 1527 on *CD24* can reduce the risk of chronic HCV infection [13]. However, only a few studies have focused on the association between *CD24* and the progression of HCC.

Talin-1 is a probable indicator for early diagnosis of cancer since its elevated degree of expression in blood samples from people with cancer was adequate to differentiate them from normal human specimens [14]. Talin-1 is important for cell adhesion and motility, which is a very important factor in neoplasm metastasis and inflammation. Also it is responsible for the activation of integrins, which regulates cell apoptosis and growth of tumors [15].

We conducted this study to emphasize the relation between virus C and HCC and to clarify the association between cluster of differentiation 24 (CD24) polymorphism, talin-1 gene expression, and prevalence of HCC in Egyptian HCV patients.

Material and methods

Patient selection

A total of 600 Egyptian adults individuals were involved in this study.

The inclusion criteria included age ≥ 30 years and positive HCV RNA tests for HCV patients. A total of 200 healthy controls and 400 patients diagnosed with HCV were included in this study; they were selected from Theodor Bilharz Research Institute (TBRI). Patients were classified as follows: 200 patients diagnosed with chronic hepatitis C without liver carcinoma and 200 patients diagnosed with chronic hepatitis C with liver carcinoma. All cases were diagnosed according to histological assessment, and their clinical stage was determined according to the TNM staging system of the American Joint Committee on Cancer (AJCC) [16]. Liver cirrhosis was diagnosed according to abdominal sonography or liver biopsy. The evaluation of the participants for hepatic function was based on patient history, medical consultation, and serum hepatic function tests, and liver biopsies were done before any antiviral therapies were taken. The epidemiological factors included gender, body mass index (BMI), and age.

Exclusion criteria were defined as follows: patients infected with either hepatitis B virus (HBV) or HIV, patients having a background of any inflammatory diseases such as acute or chronic thyroid diseases, infections, and drug abuse were excluded. Regarding alcohol consumption, patients were excluded if they had up to an average of more than 2 drinks per day, and for persistent smoking habit, patients were excluded if they had smoked one cigarette per day in the latest three months.

The study was approved by Ahram Canadian University (ACU) Human Ethics Committee (PBC-2020-04). The study was carried out following the recommendations and regulations of the Declaration of Helsinki. Before participation, all medical histories of all subjects were collected and written informed consent was taken from all participants.

Blood sampling and laboratory assays

Blood samples were divided into two parts under complete aseptic conditions. The first part was added to tubes containing EDTA (1 mg/ml) to isolate and extract DNA by spin column-based genomic DNA by removing polymerase chain reaction (PCR) inhibitors such as cations and proteins. The second part was taken into tubes, where serum was obtained by centrifugation at 4000 rpm for 15 min and serum kept frozen at –70°C for determination of aspartate transaminase (AST) [17], alkaline phosphate (ALP), alanine transaminase (ALT), γ-glutamyl-transferase (GGT), total cholesterol (TC), and high-density lipoprotein (HDL) [18–20]. Using standard laboratory spectrophotometric methods low-density lipoprotein cholesterol (LDL) and serum alfa-fetoprotein (AFP) levels were estimated using enzyme-linked immunoassay kits (commercial kit purchased from DRG, USA) [21].

Genotyping of cluster of differentiation 24

The cluster of differentiation 24 (*CD24*) gene amplification was performed using PCR-restriction fragment length polymorphism (PCR-RFLP). By measuring the concentration of each sample with a fluorometer device 1 µl of Qubit reagent was mixed with 199 µl of Qubit buffer to form Qubit working solution then 199 µl of that working solution mixed with 1 µl of the DNA sample "from the first step" in a PCR tube. After that the concentration was measured using a fluorometer device [22].

Accession number: rs8734

Quantitative real-time PCR assay of talin-1: talin-1 gene expression was detected in peripheral blood mononuclear cells (PBMCs). These cells were obtained from peripheral blood by the Ficoll density sedimentation process. A QIAamp viral RNA extraction kit was used for the extraction of total RNA. The quantification process was analyzed using TaqMan Gene Expression assay (Applied Biosystems Inc., Foster City, CA, USA). Levels of talin-1 expression were calculated using the threshold cycle method [23]. The following primers were used in the qRT-PCR [24]:
Talin-1 sense: 5′-TCTCCCAAA ATGCCAAGAAC-3′
Anti-sense: 5′-TGGCTATTGG GGTCAGAGAC-3′
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) sense: 5′-CCACTCC TCCACCTTTGAC-3′
Anti-sense: 5′-ACCCTGT TGCTGTAGCCA-3′.

Hepatic histopathological assessment by hematoxylin and eosin and Masson's trichrome staining

Liver specimens were taken by needle biopsy from all patients with hepatitis C upon signing a written consent form. Liver tissues were all treated with 10% neutral buffered formalin for 24 h and histo-processed. The blocks were then sliced into 3 µm thicknesses, using a rotary microtome. Parts were stained utilizing hematoxylin and eosin (H & E) stain [25].

Three colors can be used for muscle staining, collagen fibers, fibrin, and erythrocytes. The fundamental principle in trichrome staining is that less porous tissue is colored by the small dye molecule, whereas a higher molecular dye can infiltrate [26]. Hematoxylin and eosin and Masson's trichrome staining have been used for histopathological analysis. The level of steatosis, lobular inflammatory processes, and non-alcoholic steatohepatitis (NASH) was calculated using the SAF scoring system. The steatosis, activity and fibrosis score system (SAF) is a non-alcoholic fatty liver disease (NAFLD) score based on histological severity. All biopsies were classified according to the SAF system, and the severity of the disease was classified as mild, moderate or severe. The SAF activity score was measured by hepatocellular ballooning and lobular inflammation. Histopathological severe disease was described as SAF activity score > 3 for bridging fibrosis or cirrhosis. The regression formula for the fibrosis severity estimation includes 6 variables: age, years, BMI (kg/m^2), fasting glucose (FG)/diabetes, platelet count and albumin (g/dl) and AST/ALT ratio [27].

Statistical analysis

The differences in demographic characteristics between healthy controls and HCC patients were compared using Fisher's exact test and the Mann-Whitney *U* test. These data were expressed as mean ± SEM and compared between groups by Student's *t*-test. The data were analyzed using SPSS 25 software (IBM SPSS, USA). *P* values < 0.05 were regarded as statistically significant.

Results

Characteristics of subjects

Since various risk factors have been related to the pathogenesis and prevalence of liver cancer such as alcohol consumption, gender, and age, we first compared the mean and SEM for the clinical data of 400 HCV patients with those from 200 normal controls (Table I). There was no significant difference between the study groups in the sex distribution, BMI, or age. For the liver function tests, the level of ALT is significantly higher in hepatitis C patients (mean ± SEM = 125 ±20.53 IU/l) and hepatitis C with HCC patients (mean ± SEM = 135 ±11.69 IU/l) than in healthy control subjects (mean ± SEM = 27.8 ±0.61 IU/l). Meanwhile, there is no significance in it between the HCV group and the HCV with HCC group at $p < 0.05$. As regards GGT and ALP levels, both were significantly higher in all HCV patients than controls. Also, there is a significant difference in it between the HCV with HCC group and the HCV group at $p < 0.05$. Additionally, the serum AFP level in hepatitis C with HCC patients showed the highest significant increase to 4570 ±294 ng/ml compared to the healthy control group, 6.6 ±0.8 ng/ml ($p \leq 0.05$). Additionally, we observed that tobacco smoking and alcohol consumption are strongly associated with the prevalence of HCC (Table I). The power of the study was calculated as follows: total sample size: 600, number of groups: 3, effect size: 0.15, critical F: 3, and power: 0.9.

Histopathological examination

Figure 1 shows a photomicrograph in of control human liver section showing normal hepatic lob-

Table I. Clinical and hemodynamic characteristics of participants

Variables	Control	HCV	HCV with HCC	*P*-value
Sex	200 (35 M/35 F)	200 (30 M/40 F)	200 (35 M/35 F)	0.01
Age [years]	40.51 ±0.66	41.38 ±0.55	40.38 ±0.55	0.05
BMI [kg/m^2]	23 ±0.22	24 ±0.26	24 ±0.12	0.01
Waist	0.75	0.82	0.83	0.01
Waist/hip ratio	0.83	0.85	0.84	0.01
Cigarette smoking:				0.01
No	170	55	45	
Yes	30	145	155	
Alcohol consumption:				0.001
No	55	26	20	
Yes	15	44	50	
Tumor status:				0.001
T1 + T2			128 (64%)	
T3 + T4			72 (36%)	
SBP [mm Hg]	120.3 ±8.44	140 ±7.32[a,b]	145 ±7.32[a,b]	0.01
DBP [mm Hg]	70.5 ±5.41	85 ±4.32[a,b]	95 ±4.32[a,b]	0.01
Serum TAG [mg/dl]	111.6 ±2.2	171.9 ±4.05[a]	175.9 ±4.05[a]	0.001
Serum TC [mg/dl]	150.3 ±1.70	230.7 ±3.09[a]	228.7 ±3.09[a]	0.001
Serum HDL-C [mg/dl]	56.53 ±0.57	29.71 ±0.92[a]	30.71 ±0.92[a]	0.01
Serum LDL-C [mg/dl]	95.37 ±1.81	150.6 ±3.4[a]	155.6 ±3.4[a]	0.01
GGT [IU/l]	21.5 ±0.77	85.3 ±0.66[a]	96.1 ±0.69[a,b]	0.01
ALP [IU/l]	60 ±2.78	192 ±1.56[a]	176.3 ±0.85[a,b]	0.001
AST [IU/l]	20.9 ±0.45	120.5 ±14.57[a]	131 ±10.77[a,b]	0.001
ALT [IU/l]	27.8 ±0.61	125 ±20.53[a]	135 ±11.69[a]	0.001
Serum AFP [ng/ml]	6.6 ±0.8	2800.9 ±110[a]	4570 ±294[a,b]	0.01

HCV – hepatitis C virus, BMI – body mass index, TAG – triacylglycerol, TC – total cholesterol, HDL-C – high-density lipoprotein cholesterol, LDL-C – low-density lipoprotein cholesterol, ALP – alkaline phosphatase test, AST – aspartate aminotransferase, ALT – alanine aminotransferase, GGT – γ-glutamyl-transferase, AFP – alpha-fetoprotein, SBP – systolic blood pressure, DBP – diastolic blood pressure. Data are given as mean + SEM. [a]Significantly different from control group at p < 0.05, [b]significantly different from HCV group at p < 0.05.

ules' architecture. In Figure 2 the histopathological sections reveal human liver sections showing (A and B): Mallory-Denk bodies and microvesicular steatosis (C): a human liver section with NASH was demonstrated showing peri-sinusoidal fibrosis (Masson's trichrome) with no CD4 expression. In contrast, histology analysis in Figure 3 indicates that the tumors in the group of hepatitis C patients with complications are malignant HCC, with the expression of CD4 on the inflammatory cells

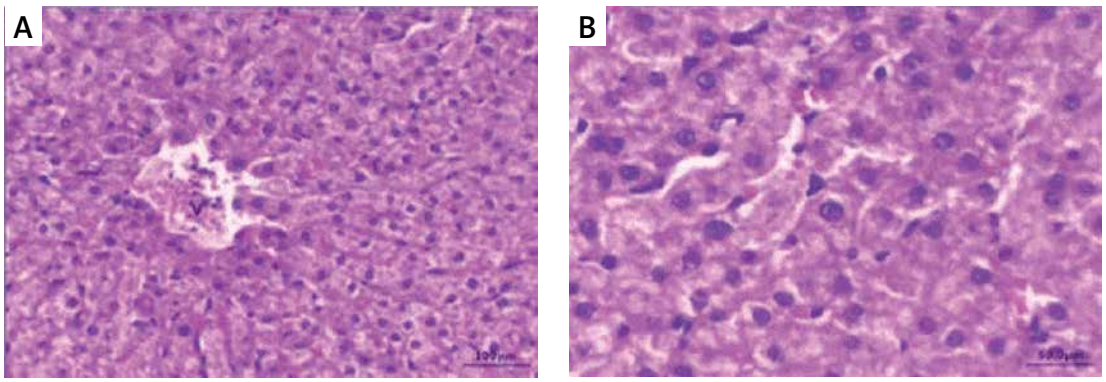

Figure 1. A photomicrograph in control human liver section showing normal architecture of hepatic lobules, in the form of hepatocytes as plates radiating from the central vein. The liver plates were separated from each other by irregular sinusoids. The hepatocytes appeared polyhedral in shape with acidophilic cytoplasm with large, rounded and vesicular nuclei (H & E, 100×)

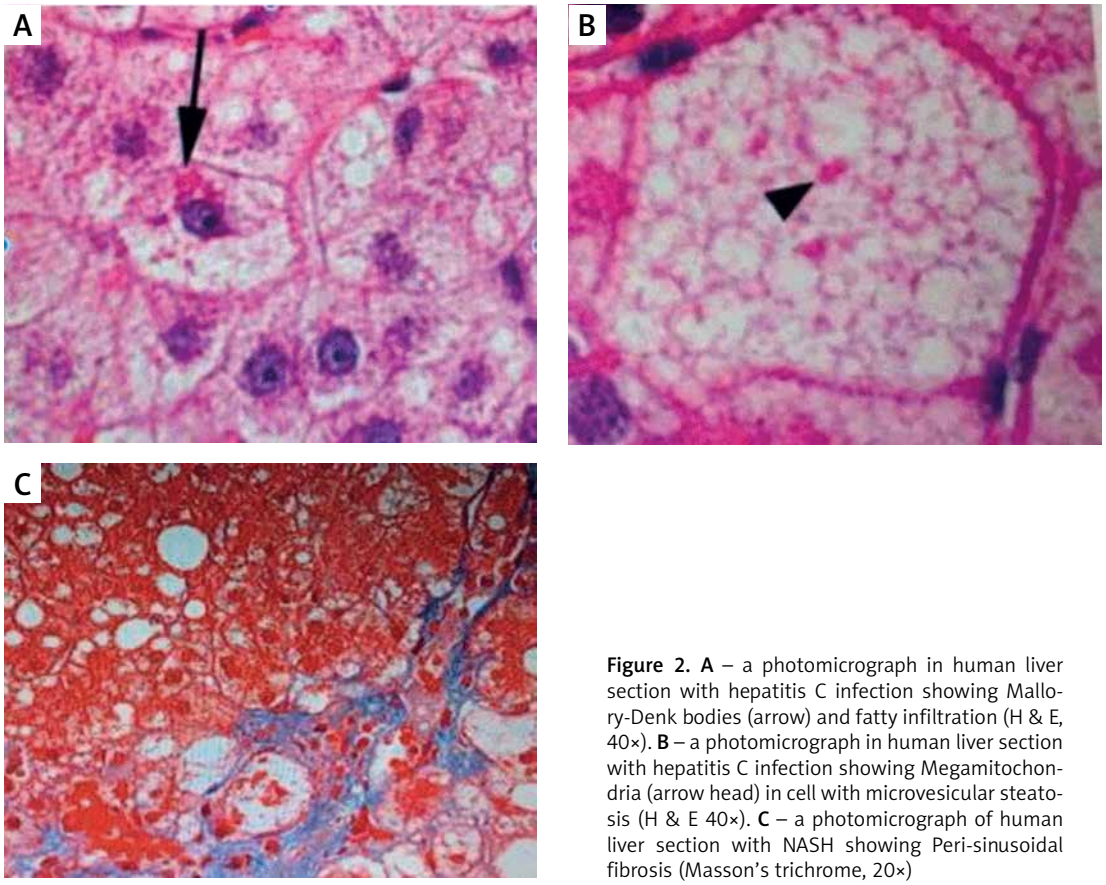

Figure 2. A – a photomicrograph in human liver section with hepatitis C infection showing Mallory-Denk bodies (arrow) and fatty infiltration (H & E, 40×). **B** – a photomicrograph in human liver section with hepatitis C infection showing Megamitochondria (arrow head) in cell with microvesicular steatosis (H & E 40×). **C** – a photomicrograph of human liver section with NASH showing Peri-sinusoidal fibrosis (Masson's trichrome, 20×)

which are determined by its heterogeneous and large nuclei; cancer cells are also characterized by double nuclei, showing: (A) masses of malignant cells with frequent mitosis, hyperchromatic nuclei and trabecular growth pattern (H & E 100×); (B) pseudoglandular growth pattern (H & E 100×); (C) loss of architecture, cellular degeneration and solid growth pattern (H & E 200×); (D) loss of architecture with dilated central vein and giant cell formation. These data focused on the correlation between *CD24* gene variation and the rapid development of HCC.

Genotype distribution and allele frequencies of CD24 170 C/T polymorphism in hepatitis C patient groups versus control group

The frequencies of *CD24* 170 CT/TT genotype were significantly higher in hepatitis C patients without complications (60%) compared to CC genotype (40%) with OR = 6 at χ^2= 14.41, p = 0.0007. Also the frequencies of *CD24* 170 CT/TT genotype were significantly higher in hepatitis C patients with HCC (90%) compared to CC genotype (10%) with OR = 36 at χ^2= 14.41, p = 0.0007 (Table II). No significant differences were found between the levels of AFP, AST, ALT, or GGT and the *CD24* 170 C/T polymorphisms in HCC patients (Table III). CD24 polymorphism correlated with prognosis in HCC patients which is estimated by its correlation with tumor status T1+T2 at $p < 0.0001$ in Figure 4. Additionally, Kaplan-Meier curve analysis was conducted to estimate the overall survival rate for the different genotypes of *CD24* polymorphism of the recipients at $p < 0.0001$ as presented in Figure 5.

Talin-1 gene expression

Table IV shows a 1.6-fold significant increase in talin-1 expression in HCV patients (mean ± SEM = 8.07 ±0.12 and 14.27 ±0.12 respectively) compared to the control group (mean ± SEM = 6.15 ±0.1) at $p < 0.0001$. Moreover, there was a 1.3-fold significant increase of talin-1 expression in HCV with HCC patients (mean ± SEM = 10.27 ±0.12) compared to HCV patients (mean ± SEM = 8.07 ±0.12) at $p < 0.0001$ as shown in Figure 6. A significant correlation between *CD24* polymorphism genotypes and talin-1 gene expression is clearly represented among our groups at $p < 0.0001$ in Figure 7. To evaluate the diagnostic value of talin-1 gene expression, we used ROC methods to calculate the sensitivity and specificity as shown in Figure 8. The AUC of talin-1 gene expression between the patients with HCV and HCC with those

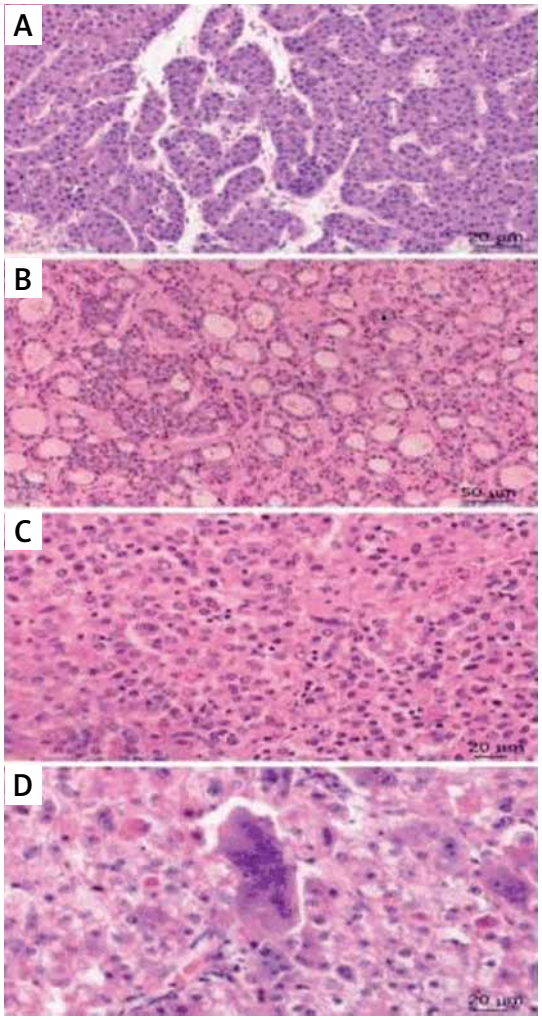

Figure 3. A photomicrograph in human liver section with hepatitis C infection and hepatocellular carcinoma showing: **A** – masses of malignant cells with frequent mitosis, hyper chromatic nuclei and trabecular growth pattern (H & E, 100×); **B** – pseudo glandular growth pattern (H & E, 100×); **C** – loss of architecture, cellular degeneration and Solid growth pattern (H & E, 200×). **D** – loss of architecture with dilated central vein and giant cell formation (H & E, 200×)

with HCV was 0.9 (95% CI: 0.52–0.69; p = 0.009) for predicting the risk of developing cancer, indicating that talin-1 gene expression had significant accuracy as a predictor for cancer prevalence risk.

SAF scores for biopsies of HCV patients

Tables V and VI show the most frequent diagnosis of hepatitis C patients. The baseline diagnosis with NASH was concordant with the reference classification which states that less than –1.455 indicates the absence of fibrosis (F0-F2), between –1.455 and 0.675 is an indeterminate score while more than 0.675 indicates the presence of fibrosis (F3–F4). Table V shows the most frequent diagnosis among the two pathologists in HCV patients with HCC. 40 out of 70 (57%) diagnosed with NASH were concordant with the reference classification. In Table VI, 34 out of 70 (49%) HCV patients without HCC were diagnosed with NASH with full agreement between reference and baseline classification.

Table VII shows the distribution frequency of *CD24* gene variation among the 400 patients with HCV with their clinical status and histological examinations. The patients with HCV were evaluated to understand the effect of *CD24* 170 CT/TT genotypes on the clinical stage, lymph node involvement, distant metastasis, vascular invasion, and liver cirrhosis. There was a significant difference in the effect of *CD24* 170 CT/TT genotype on lymph node involvement, distant metastasis and liver cirrhosis as well.

Discussion

In our study, we investigated the association between *CD24* polymorphisms and the prevalence of HCC in HCV patients. Hepatocellular carcinoma is the third most common cause of death due to cancer worldwide [28]. In Egypt, HCC represents around 1.68% of the total malignancies and 11.75% of all digestive organs' malignancies and metabolic syndrome diseases [29]. The distribution analysis of the *CD24* genotypes involved 400 HCV patients and 200 normal controls indicating that the frequencies of *CD24* 170 CT/TT genotype were significantly higher in HCV patients without complications (60%) compared to CC genotype (40%) with OR = 6 at $\chi^2 = 14.41$, $p = 0.0007$, and the frequencies of *CD24* 170 CT/TT genotype were significantly higher in HCV with HCC (90%) compared to CC genotype (10%) with OR = 36 at $\chi^2 = 14.41$, $p = 0.0007$.

Moreover, among the chronic HCV Egyptian patients, the *CD24* P170T allele shows a stronger association with the rapid development of HCC and liver cirrhosis compared with the *CD24* P170 C allele. In contrast, deletion of the dinucleotide at position 1527 on *CD24* can reduce the development of chronic HCV infection [30]. In addition, *CD24* polymorphisms may increase the genetic susceptibility factor for HBV infection. This was investigated by a recent study which included 609 HBV patients and 383 healthy controls; the study showed an increased risk of prevalence of HBV infection in patients with the P170T/T genotype compared with P170C/T and P170C/C genotypes using the logistic regression model [31].

An important explanation for how the *CD24* SNP affects the risk of development of chronic HCV infection. Our previous results have suggested that the P170T allele, which is expressed at a higher level than P170C, encodes a certain protein, which is responsible for the progression

Table II. Difference in genotype frequency of *CD24* SNP 170 between all studied groups

Groups	*N*	Genotype frequency		OR	95% CI
		CC	CT + TT		
Control	200	160 (80%)	40 (20%)		
HCV	200	80 (40%)	120 (60%)	6	0.88–13.83
HCV with HCC	200	20 (10%)	180 (90%)	36	2.07–30.89
$\chi^2 = 14.41$, $p = 0.0007^*$					

Statistically significant difference at $p < 0.05$ using χ^2 test.
HCC – hepatocellular carcinoma, HCV – hepatitis C virus.

Table III. Association of *CD24* C/T SNP 170 genotypic frequencies with HCC laboratory status

Characteristic	α-Fetoprotein [ng/ml]	AST [IU/l]	ALT [IU/l]	GGT
CD24 C/T SNP 170				
CC	2800.2 ±110	125.1 ±20.6	120.2 ±14	85 ±0.66
CT + TT	4570 ±294	135.2 ±11.6	131.3 ±10	96 ±0.69
P-value	0.448	0.537	0.501	0.545

*Mann-Whitney U test was used between two groups.*P-value < 0.05 indicates statistical significance.*
HCC – hepatocellular carcinoma, AST – aspartate aminotransferase, ALT – alanine aminotransferase, GGT – γ-glutamyl-transferase.

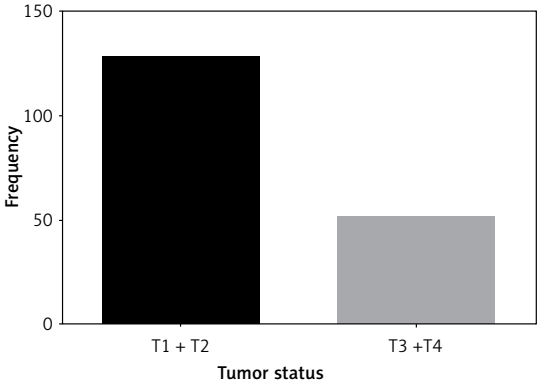

Figure 4. Correlation between frequency of CD24 polymorphism and tumor status in hepatocellular carcinoma patients, $p < 0.0001$

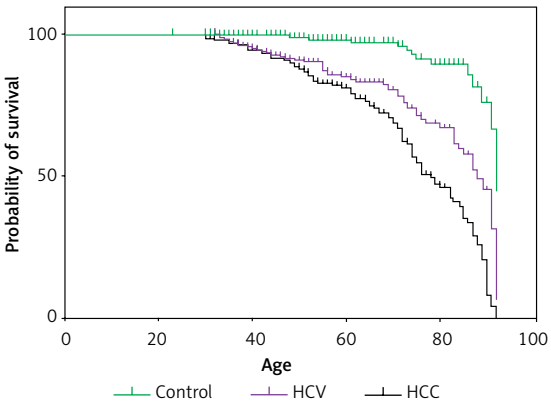

Figure 5. Kaplan-Meier curves for probability of survival by CD24 polymorphism among groups at $p < 0.0001$

Table IV. Gene expression of talin-1 in hepatitis C virus patients (HCV patients) and hepatitis C virus with hepatocellular carcinoma patients (HCV with HCC patients) compared to control group at $p < 0.05$

Group	Talin-1 gene expression
Control	6.15 ±0.1
HCV	8.07 ±0.12[a]
HCV with HCC	10.27 ±0.12[a,b]

[a]Significant compared to control group at P < 0.05, [b]significant compared to control group at p < 0.05.

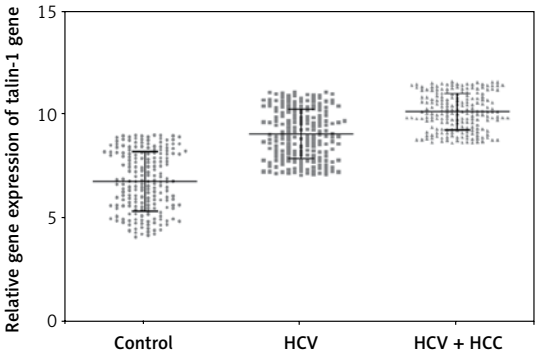

Figure 6. Column scatter dot plot showing talin-1 gene expression among control, HCV, and HCV + HCC groups, $p < 0.0001$

of chronic HCV infection by affecting the efficiency of cleavage of posttranslational GPI. These results agree with another study [32] which supports the idea that the P170T allele affects the progression of chronic HCV infection through posttranslational mechanisms. Given the fact that CD24 is expressed mainly in the neuronal and hematopoietic cells, Huang and Hsu31 stated that there are many other tumor cells that have been shown to increase the expression of CD24 mainly in liver tumors [21]. Another study [33] also suggested that *CD24* SNPs are a prognostic marker for hepatic carcinoma. In this regard, another study confirmed that during liver

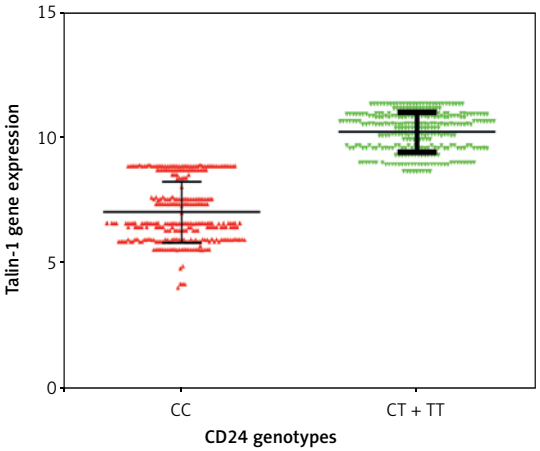

Figure 7. Correlation of CD24 170 SNP genotypes with talin-1 gene expression in hepatitis C virus patients, $p < 0.0001$

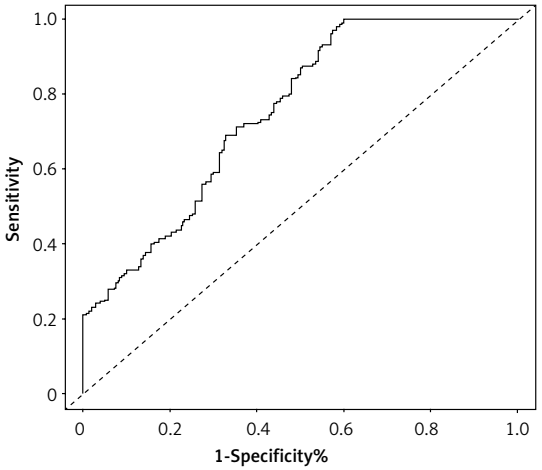

Figure 8. AUC curve analysis of talin-1 gene expression, $p < 0.0001$, AUC = 0.9

Table V. Evaluation of liver biopsies by the liver pathologists in hepatitis C virus patients with hepatocellular carcinoma

Case	Reference classification	Algorithmic classification	SAF	Disease severity
1	NASH	NASH	S2A4F1	significant
2	steatosis	steatosis	S1A1F1	mild
3	NASH	NASH	S1A4F3	significant
4	NASH	NASH	S3A1F2	significant
5	steatosis	steatosis	S3A1F0	mild
6	NASH	NASH	S3A3F2	significant
7	NASH	NASH	S2A4F1	significant
8	steatosis	steatosis	S1A1F1	mild
9	NASH	NASH	S3A1F2	significant
10	NASH	NASH	S2A4F1	significant
11	NASH	NASH	S3A1F2	significant
12	NASH	NASH	S2A4F1	significant
13	steatosis	steatosis	S1A1F1	mild
14	NASH	NASH	S3A3F2	significant
15	NASH	NASH	S2A4F1	significant
16	steatosis	steatosis	S1A1F1	mild
17	steatosis	steatosis	S1A1F1	mild
18	NASH	NASH	S3A4F3	significant
19	NASH	NASH	S3A3F2	significant
20	NASH	NASH	S2A4F1	significant
21	NASH	NASH	S2A4F1	significant
22	steatosis	steatosis	S1A1F1	mild
23	steatosis	steatosis	S1A0F0	mild
24	steatosis	steatosis	S1A1F1	mild
25	NASH	NASH	S3A3F2	significant
26	NASH	NASH	S3A1F2	significant
27	NASH	NASH	S3A1F2	significant
28	NASH	NASH	S2A4F1	significant
29	steatosis	steatosis	S1A0F0	mild
30	steatosis	steatosis	S1A1F1	mild

Table V. Cont.

Case	Reference classification	Algorithmic classification	SAF	Disease severity
31	steatosis	steatosis	S1A0F0	mild
32	NASH	NASH	S3A3F2	significant
33	NASH	NASH	S3A1F2	significant
34	NASH	NASH	S3A1F2	significant
35	NASH	NASH	S2A4F1	significant
36	NASH	NASH	S3A1F2	significant
37	steatosis	steatosis	S1A1F1	mild
38	NASH	NASH	S3A1F2	significant
39	NASH	NASH	S3A1F2	significant
40	NASH	NASH	S2A4F1	significant
41	steatosis	steatosis	S3A1F0	mild
42	steatosis	steatosis	S1A1F1	mild
43	steatosis	steatosis	S1A0F0	mild
44	NASH	NASH	S3A1F2	significant
45	steatosis	steatosis	S3A1F0	mild
46	steatosis	steatosis	S1A1F1	mild
47	NASH	NASH	S3A3F2	significant
48	NASH	NASH	S3A3F2	significant
49	NASH	NASH	S2A4F1	significant
50	steatosis	steatosis	S3A1F0	mild
51	NASH	NASH	S3A3F2	significant
52	steatosis	steatosis	S1A0F0	mild
53	steatosis	steatosis	S1A1F1	mild
54	NASH	NASH	S3A3F2	
55	NASH	NASH	S2A4F1	significant
56	NASH	NASH	S3A3F2	significant
57	steatosis	steatosis	S2A1F1	mild
58	steatosis	steatosis	S1A1F1	mild
59	NASH	NASH	S3A1F2	significant
60	steatosis	steatosis	S2A1F1	mild
61	steatosis	steatosis	S2A1F1	mild
62	NASH	NASH	S2A4F1	significant
63	NASH	NASH	S3A3F2	significant
64	NASH	NASH	S3A1F2	significant
65	NASH	NASH	S2A4F1	significant
66	steatosis	steatosis	S1A1F1	mild
67	steatosis	steatosis	S2A1F1	mild
68	steatosis	steatosis	S1A1F1	mild
69	NASH	NASH	S3A1F2	significant
70	NASH	NASH	S2A4F1	significant
71	NASH	NASH	S3A1F2	significant
72	NASH	NASH	S2A4F1	significant
73	steatosis	steatosis	S1A0F0	mild
74	steatosis	steatosis	S1A1F1	mild
75	steatosis	steatosis	S1A1F1	mild
76	steatosis	steatosis	S1A1F1	mild
77	NASH	NASH	S3A1F2	significant

Table V. Cont.

Case	Reference classification	Algorithmic classification	SAF	Disease severity
78	steatosis	steatosis	S1A1F1	mild
79	steatosis	steatosis	S1A1F1	mild
80	steatosis	steatosis	S1A1F1	mild
81	steatosis	steatosis	S1A0F0	mild
82	NASH	NASH	S3A1F2	significant
83	steatosis	steatosis	S3A1F0	mild
84	steatosis	steatosis	S1A1F1	mild
85	NASH	NASH	S3A3F2	significant
86	NASH	NASH	S3A3F2	significant
87	NASH	NASH	S2A4F1	significant
88	steatosis	steatosis	S3A1F0	mild
89	NASH	NASH	S3A3F2	significant
90	steatosis	steatosis	S1A0F0	mild
91	steatosis	steatosis	S1A1F1	mild
92	NASH	NASH	S3A3F2	
93	NASH	NASH	S2A4F1	significant
94	NASH	NASH	S3A3F2	significant
95	steatosis	steatosis	S2A1F1	mild
96	steatosis	steatosis	S1A1F1	mild
97	NASH	NASH	S3A1F2	significant
98	steatosis	steatosis	S2A1F1	mild
99	steatosis	steatosis	S2A1F1	mild
100	NASH	NASH	S2A4F1	significant
101	NASH	NASH	S3A3F2	significant
102	NASH	NASH	S3A1F2	significant
103	NASH	NASH	S2A4F1	significant
104	NASH	NASH	S3A1F2	significant
105	steatosis	steatosis	S1A1F1	mild
106	steatosis	steatosis	S1A1F1	mild
107	NASH	NASH	S3A4F3	significant
108	NASH	NASH	S3A3F2	significant
109	NASH	NASH	S2A4F1	significant
110	NASH	NASH	S3A1F2	significant
111	NASH	NASH	S2A4F1	significant
112	steatosis	steatosis	S1A0F0	mild
113	NASH	NASH	S2A4F1	significant
114	steatosis	steatosis	S1A0F0	mild
115	NASH	NASH	S3A1F2	significant
116	NASH	NASH	S3A1F2	significant
117	steatosis	steatosis	S2A1F1	mild
118	steatosis	steatosis	S2A1F1	mild
119	NASH	NASH	S2A4F1	significant
120	NASH	NASH	S3A3F2	significant
121	NASH	NASH	S3A1F2	significant
122	NASH	NASH	S2A4F1	significant
123	NASH	NASH	S3A1F2	significant

Table V. Cont.

Case	Reference classification	Algorithmic classification	SAF	Disease severity
124	steatosis	steatosis	S1A1F1	mild
125	steatosis	steatosis	S1A1F1	mild
126	NASH	NASH	S3A4F3	significant
127	NASH	NASH	S3A3F2	significant
128	NASH	NASH	S2A4F1	significant
129	NASH	NASH	S3A1F2	significant
130	NASH	NASH	S2A4F1	significant
131	steatosis	steatosis	S1A0F0	mild
132	NASH	NASH	S2A4F1	significant
133	steatosis	steatosis	S1A0F0	mild
134	steatosis	steatosis	S1A0F0	mild
135	steatosis	steatosis	S1A0F0	mild
136	NASH	NASH	S3A1F2	significant
137	NASH	NASH	S2A4F1	significant
138	steatosis	steatosis	S1A0F0	mild
139	steatosis	steatosis	S1A0F0	mild
140	steatosis	steatosis	S1A0F0	mild
141	NASH	NASH	S3A1F2	significant
142	NASH	NASH	S2A4F1	significant
143	steatosis	steatosis	S1A0F0	mild
144	steatosis	steatosis	S1A0F0	mild
145	steatosis	steatosis	S1A1F1	mild
146	steatosis	steatosis	S1A0F0	mild
147	NASH	NASH	S3A3F2	significant
148	NASH	NASH	S2A4F1	significant
149	NASH	NASH	S3A1F2	significant
150	NASH	NASH	S2A4F1	significant
151	steatosis	steatosis	S1A0F0	mild
152	NASH	NASH	S2A4F1	significant
153	steatosis	steatosis	S1A0F0	mild
154	steatosis	steatosis	S1A0F0	mild
155	steatosis	steatosis	S1A1F1	mild
156	steatosis	steatosis	S1A0F0	mild
157	NASH	NASH	S3A3F2	significant
158	NASH	NASH	S2A4F1	significant
159	NASH	NASH	S3A1F2	significant
160	NASH	NASH	S2A4F1	significant
161	steatosis	steatosis	S1A0F0	mild
162	NASH	NASH	S2A4F1	significant
163	steatosis	steatosis	S1A0F0	mild
164	steatosis	steatosis	S1A0F0	mild
165	steatosis	steatosis	S1A1F1	mild
166	steatosis	steatosis	S1A0F0	mild
167	steatosis	steatosis	S1A0F0	mild
168	steatosis	steatosis	S1A1F1	mild
169	steatosis	steatosis	S1A0F0	mild

Table V. Cont.

Case	Reference classification	Algorithmic classification	SAF	Disease severity
170	NASH	NASH	S3A3F2	significant
171	NASH	NASH	S2A4F1	significant
172	NASH	NASH	S3A1F2	significant
173	NASH	NASH	S2A4F1	significant
174	steatosis	steatosis	S1A0F0	mild
175	NASH	NASH	S2A4F1	significant
176	steatosis	steatosis	S1A0F0	mild
177	NASH	NASH	S3A1F2	significant
178	steatosis	steatosis	S2A1F1	mild
179	steatosis	steatosis	S2A1F1	mild
180	NASH	NASH	S2A4F1	significant
181	NASH	NASH	S3A3F2	significant
182	NASH	NASH	S3A1F2	significant
183	NASH	NASH	S2A4F1	significant
184	NASH	NASH	S3A1F2	significant
185	steatosis	steatosis	S1A1F1	mild
186	steatosis	steatosis	S1A1F1	mild
187	NASH	NASH	S3A4F3	significant
188	NASH	NASH	S3A3F2	significant
189	NASH	NASH	S2A4F1	significant
190	NASH	NASH	S3A1F2	significant
191	NASH	NASH	S2A4F1	significant
192	steatosis	steatosis	S1A0F0	mild
193	NASH	NASH	S2A4F1	significant
194	steatosis	steatosis	S1A0F0	mild
195	NASH	NASH	S3A1F2	significant
196	NASH	NASH	S3A1F2	significant
197	steatosis	steatosis	S2A1F1	mild
198	steatosis	steatosis	S2A1F1	mild
199	NASH	NASH	S2A4F1	significant
200	NASH	NASH	S3A3F2	significant

Reference interpretation: is the initial evaluation done by pathologist using accepted criteria, algorithm and SAF score. Algorithmic classification: (steatosis vs. NASH) after using the algorithm. Based on SAF score: mild for A < 2 and F < 2 or significant for A > 2 and/or F > 2.

carcinogenesis CD24 is highly expressed in the liver progenitor cells [34].

Furthermore, *CD24* polymorphism may affect the immune/inflammatory response through T-cell activation. T-cell-mediated inflammation is one of the important mechanisms for the prevalence of HCC in HBV infected mice; it also affects the production of cytokines from liver necrotic cells [35] *CD24* P170 T/T is a higher cell-surface genotype than P170 C/T or P170 C/C genotypes, which increases the rapid progression and risk of multiple sclerosis (MS). Dinucleotide deletion in 3 untranslated regions (UTRs) is associated with protection from systemic lupus erythematosus and MS, as that deletion reduces the stability of *CD24* messenger RNA [36].

Talin-1 has an important role in the stimulation of integrin. Especially, the sensitivity of talin-1 for cancer diagnosis was stronger than that of AFP in Egyptian patients with HCC [37]. Obviously, these results indicate that talin-1 is a possible diagnostic indicator for HCC. This was also investigated by another recent study which was performed on 90 Egyptian subjects showing that talin-1 is involved in the carcinogenesis of HCC [14]. Even so, whether talin-1 stimulated HCC proliferation and metastases was still unknown, and the role of talin-1 in HCC proliferation remained under investigation [38].

Talin-1 has been shown to facilitate HCC progression by inhibiting the activity of apoptosis con-

Table VI. Evaluation of liver biopsies by the liver pathologists in hepatitis C virus patients without hepatocellular carcinoma

Case	Reference classification	Algorithmic classification	SAF	Disease severity
1	steatosis	steatosis	S1A1F1	mild
2	steatosis	steatosis	S1A1F1	mild
3	NASH	NASH	S1A4F3	significant
4	NASH	NASH	S3A1F2	significant
5	NASH	NASH	S2A4F1	significant
6	steatosis	steatosis	S1A1F1	mild
7	steatosis	steatosis	S1A1F1	mild
8	steatosis	steatosis	S1A1F1	mild
9	NASH	NASH	S3A1F2	significant
10	NASH	NASH	S2A4F1	significant
11	steatosis	steatosis	S1A0F0	mild
12	steatosis	steatosis	S1A1F1	mild
13	steatosis	steatosis	S1A1F1	mild
14	steatosis	steatosis	S1A1F1	mild
15	NASH	NASH	S3A1F2	significant
16	steatosis	steatosis	S1A1F1	mild
17	steatosis	steatosis	S1A1F1	mild
18	NASH	NASH	S3A4F3	significant
19	NASH	NASH	S3A3F2	significant
20	NASH	NASH	S2A4F1	significant
21	NASH	NASH	S2A4F1	significant
22	steatosis	steatosis	S1A1F1	mild
23	steatosis	steatosis	S1A0F0	mild
24	steatosis	steatosis	S1A1F1	mild
25	NASH	NASH	S3A3F2	significant
26	steatosis	steatosis	S2A1F1	mild
27	steatosis	steatosis	S1A1F1	mild
28	NASH	NASH	S2A4F1	significant
29	steatosis	steatosis	S1A0F0	mild
30	steatosis	steatosis	S1A1F1	mild
31	steatosis	steatosis	S1A0F0	mild
32	NASH	NASH	S3A3F2	significant
33	NASH	NASH	S3A1F2	significant
34	NASH	NASH	S3A1F2	significant
35	NASH	NASH	S2A4F1	significant
36	NASH	NASH	S3A1F2	significant
37	steatosis	steatosis	S1A1F1	mild
38	steatosis	steatosis	S1A0F0	mild
39	steatosis	steatosis	S1A0F0	mild
40	NASH	NASH	S3A3F2	significant
41	steatosis	steatosis	S3A1F0	mild
42	steatosis	steatosis	S3A1F0	mild
43	steatosis	steatosis	S1A0F0	mild
44	NASH	NASH	S3A1F2	significant
45	steatosis	steatosis	S3A1F0	mild

Table VI. Cont.

Case	Reference classification	Algorithmic classification	SAF	Disease severity
46	steatosis	steatosis	S1A1F1	mild
47	NASH	NASH	S3A3F2	significant
48	NASH	NASH	S3A3F2	significant
49	NASH	NASH	S2A4F1	significant
50	steatosis	steatosis	S3A1F0	mild
51	steatosis	steatosis	S1A1F1	mild
52	steatosis	steatosis	S3A1F0	mild
53	NASH	NASH	S2A4F1	significant
54	steatosis	steatosis	S1A1F1	mild
55	NASH	NASH	S3A1F2	significant
56	NASH	NASH	S2A4F1	significant
57	steatosis	steatosis	S2A1F1	mild
58	steatosis	steatosis	S1A1F1	mild
59	NASH	NASH	S3A1F2	significant
60	steatosis	steatosis	S2A1F1	mild
61	steatosis	steatosis	S2A1F1	mild
62	NASH	NASH	S2A4F1	significant
63	NASH	NASH	S3A3F2	significant
64	NASH	NASH	S3A1F2	significant
65	NASH	NASH	S2A4F1	significant
66	steatosis	steatosis	S1A1F1	mild
67	steatosis	steatosis	S2A1F1	mild
68	steatosis	steatosis	S1A1F1	mild
69	NASH	NASH	S2A4F1	significant
70	steatosis	steatosis	S1A0F0	mild
71	NASH	NASH	S3A1F2	significant
72	NASH	NASH	S2A4F1	significant
73	steatosis	steatosis	S1A0F0	mild
74	steatosis	steatosis	S1A1F1	mild
75	steatosis	steatosis	S1A1F1	mild
76	steatosis	steatosis	S1A1F1	mild
77	NASH	NASH	S3A1F2	significant
78	steatosis	steatosis	S1A1F1	mild
79	steatosis	steatosis	S1A1F1	mild
80	NASH	NASH	S3A4F3	significant
81	NASH	NASH	S3A3F2	significant
82	NASH	NASH	S2A4F1	significant
83	NASH	NASH	S3A1F2	significant
84	NASH	NASH	S2A4F1	significant
85	steatosis	steatosis	S1A0F0	mild
86	NASH	NASH	S2A4F1	significant
87	steatosis	steatosis	S1A0F0	mild
88	NASH	NASH	S3A1F2	significant
89	NASH	NASH	S2A4F1	significant
90	steatosis	steatosis	S1A0F0	mild
91	steatosis	steatosis	S1A1F1	mild

Table VI. Cont.

Case	Reference classification	Algorithmic classification	SAF	Disease severity
92	steatosis	steatosis	S1A1F1	mild
93	steatosis	steatosis	S1A1F1	mild
94	NASH	NASH	S3A1F2	significant
95	NASH	NASH	S2A4F1	significant
96	steatosis	steatosis	S1A0F0	mild
97	steatosis	steatosis	S1A0F0	mild
98	NASH	NASH	S3A1F2	significant
99	NASH	NASH	S2A4F1	significant
100	steatosis	steatosis	S1A0F0	mild
101	steatosis	steatosis	S1A1F1	mild
102	steatosis	steatosis	S1A1F1	mild
103	steatosis	steatosis	S1A1F1	mild
104	NASH	NASH	S3A1F2	significant
105	steatosis	steatosis	S1A1F1	mild
106	steatosis	steatosis	S1A1F1	mild
107	NASH	NASH	S3A4F3	significant
108	NASH	NASH	S3A3F2	significant
109	NASH	NASH	S2A4F1	significant
110	NASH	NASH	S3A1F2	significant
111	NASH	NASH	S2A4F1	significant
112	steatosis	steatosis	S1A0F0	mild
113	NASH	NASH	S2A4F1	significant
114	steatosis	steatosis	S1A0F0	mild
115	NASH	NASH	S3A1F2	significant
116	NASH	NASH	S2A4F1	significant
117	steatosis	steatosis	S1A0F0	mild
118	steatosis	steatosis	S1A1F1	mild
119	steatosis	steatosis	S1A1F1	mild
120	steatosis	steatosis	S1A1F1	mild
121	NASH	NASH	S3A1F2	significant
122	NASH	NASH	S2A4F1	significant
123	steatosis	steatosis	S1A0F0	mild
124	NASH	NASH	S3A3F2	significant
125	NASH	NASH	S2A4F1	significant
126	NASH	NASH	S3A1F2	significant
127	NASH	NASH	S2A4F1	significant
128	steatosis	steatosis	S1A0F0	mild
129	NASH	NASH	S2A4F1	significant
130	steatosis	steatosis	S1A0F0	mild
131	NASH	NASH	S3A1F2	significant
132	NASH	NASH	S2A4F1	significant
133	steatosis	steatosis	S1A0F0	mild
134	steatosis	steatosis	S1A1F1	mild
135	steatosis	steatosis	S1A1F1	mild
136	steatosis	steatosis	S1A1F1	mild
137	NASH	NASH	S3A1F2	significant

Table VI. Cont.

Case	Reference classification	Algorithmic classification	SAF	Disease severity
138	NASH	NASH	S2A4F1	significant
139	steatosis	steatosis	S1A0F0	mild
140	steatosis	steatosis	S1A0F0	mild
141	NASH	NASH	S3A1F2	significant
142	NASH	NASH	S2A4F1	significant
143	steatosis	steatosis	S1A0F0	mild
144	steatosis	steatosis	S1A1F1	mild
145	NASH	NASH	S3A1F2	significant
146	NASH	NASH	S2A4F1	significant
147	steatosis	steatosis	S1A0F0	mild
148	NASH	NASH	S3A3F2	significant
149	NASH	NASH	S2A4F1	significant
150	NASH	NASH	S3A1F2	significant
151	NASH	NASH	S2A4F1	significant
152	steatosis	steatosis	S1A0F0	mild
153	NASH	NASH	S2A4F1	significant
154	steatosis	steatosis	S1A0F0	mild
155	NASH	NASH	S3A1F2	significant
156	NASH	NASH	S2A4F1	significant
157	steatosis	steatosis	S1A0F0	mild
158	steatosis	steatosis	S1A1F1	mild
159	steatosis	steatosis	S1A1F1	mild
160	steatosis	steatosis	S1A1F1	mild
161	NASH	NASH	S3A1F2	significant
162	NASH	NASH	S2A4F1	significant
163	steatosis	steatosis	S1A0F0	mild
164	steatosis	steatosis	S1A0F0	mild
165	NASH	NASH	S3A1F2	significant
166	NASH	NASH	S2A4F1	significant
167	steatosis	steatosis	S1A0F0	mild
168	steatosis	steatosis	S1A1F1	mild
169	steatosis	steatosis	S1A0F0	mild
170	NASH	NASH	S3A3F2	significant
171	NASH	NASH	S2A4F1	significant
172	NASH	NASH	S3A1F2	significant
173	NASH	NASH	S2A4F1	significant
174	steatosis	steatosis	S1A0F0	mild
175	NASH	NASH	S2A4F1	significant
176	steatosis	steatosis	S1A0F0	mild
177	NASH	NASH	S3A1F2	significant
178	NASH	NASH	S2A4F1	significant
179	steatosis	steatosis	S1A0F0	mild
180	steatosis	steatosis	S1A1F1	mild
181	steatosis	steatosis	S1A1F1	mild
182	steatosis	steatosis	S1A1F1	mild
183	NASH	NASH	S3A1F2	significant

Table VI. Cont.

Case	Reference classification	Algorithmic classification	SAF	Disease severity
184	NASH	NASH	S2A4F1	significant
185	steatosis	steatosis	S1A0F0	mild
186	NASH	NASH	S3A3F2	significant
187	NASH	NASH	S2A4F1	significant
188	NASH	NASH	S3A1F2	significant
189	NASH	NASH	S2A4F1	significant
190	steatosis	steatosis	S1A0F0	mild
191	NASH	NASH	S2A4F1	significant
192	steatosis	steatosis	S1A0F0	mild
193	NASH	NASH	S3A1F2	significant
194	NASH	NASH	S2A4F1	significant
195	steatosis	steatosis	S1A0F0	mild
196	steatosis	steatosis	S1A1F1	mild
197	steatosis	steatosis	S1A1F1	mild
198	steatosis	steatosis	S1A1F1	mild
199	NASH	NASH	S3A1F2	significant
200	NASH	NASH	S2A4F1	significant

Reference interpretation: is the initial evaluation done by the pathologist using accepted criteria, algorithm and SAF score. Algorithmic classification: (steatosis vs. NASH) after using the algorithm. Based on SAF score: mild for A < 2 and F < 2 or significant for A > 2 and/or F > 2.

Table VII. Odds ratio (OR) and 95% confidence interval (CI) of clinical status and of *CD24* C/T SNP 170 genotypic frequencies in 400 hepatitis C virus patients

Variable	Genotypic frequencies			
	CC	CT + TT	OR (95% CI)	*P*-value
Clinical stage:				
I/II	160 (45.2%)	38 (53.4%)	1.00	0.537
III/IV	30 (32.7%)	88 (78.8%)	1.121 (0.371–1.640)	
Lymph node metastasis:				
No	230 (83.2%)	64 (74.4%)	1.00	0.627*
Yes	2 (3.1%)	10 (4.6%)	0.722 (0.158–2.568)	
Distant metastasis				
No	220 (85.1%)	65 (83.4%)	1.00	0.526
Yes	9 (4.9%)	16 (4.6%)	1.453 (0.542–2.786)	
Vascular invasion:				
No	220 (82.5%)	60 (55.9%)	1.00	0.452*
Yes	41 (16.5%)	200 (83.1%)	1.521 (0.652–1.987)	
Liver cirrhosis:				
Negative	52 (19.8%)	12 (19.5%)	1.00	0.821*
Positive	80 (20.1%)	320 (80.2%)	1.562 (0.634–1.823)	

The ORs with analyzed by their 95% CIs were estimated by logistic regression models.
**P-value < 0.05 indicates statistical significance.*

siderations and the anti-apoptotic BCL_2 members [39]. It also encourages HCC metastasis by inducing the release of mesenchymal epithelial-to-mesenchymal transition (EMT) markers and by reducing the activity of epithelial molecules. Talin-1 can stimulate HCC expansion and metastases through the regulation of electrical cell signaling and function as a promising bioelectricity target therapeutically [40]. In our research, the histological characteristics of HCC are prominent acinar patterns, mitotic activity, pseudoglandular or acinar and compact or solid patterns. Septae are observed

and giant cells are also seen. The occurrence of liver biopsy steatosis in HCV patients is more expressed compared to other liver diseases such as autoimmune hepatitis and chronic hepatitis B. Steatosis is suggested to be 2.5 times more common in HCV patients relative to the normal community. Macrovesicular steatosis occurring in HCV patients is often distributed in the periportal and non-centrilobular regions, which are most frequently seen in NAFLD. All this implies that HCV can directly induce steatosis in these patients [41].

In summary, our study suggested that the *CD24* polymorphism P170 CT/TT may affect both the prevalence of chronic HCV infection and HCC. Moreover, talin-1 gene expression was shown to facilitate HCC progression by reducing the activity of epithelial cells and through regulation of electrical cell signaling and inhibition of apoptosis.

In conclusions, *CD24* polymorphism and the talin-1 gene expression outside the hematopoietic cells recently raised interest as a promising prognostic marker for progression of chronic HCV infection and HCC with better accuracy than serum AFP due to their high correlation with invasion and malignant growth of hepatocytes and the immune/inflammatory response in the liver tissue.

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The study protocol was approved by the Ethics Committee of the Faculty of Pharmacy, Ahram Canadian University (ACU) Human Ethics Committee (PBC-2020-04).

Conflict of interest

The authors declare no conflict of interest.

References

1. Shawky EM, El-Beih N, El-Hussieny E, El-Ahwany E, Hassan M, Zoheiry M. Effects of free and nanoparticulate curcumin on chemically induced liver carcinoma in an animal model. Arch Med Sci 2021; 17: 218-27.
2. Abd-Elsalam S, Elwan N, Soliman H, et al. Epidemiology of liver cancer in Nile delta over a decade: a single-center study. South Asian J Cancer 2018; 7: 24-6.
3. Sherman M. Hepatocellular carcinoma: epidemiology, surveillance, and diagnosis. Semin Liver Dis 2010; 30: 3-16.
4. Forner A, Lovet JM, Bruix J. Hepatocellular carcinoma. Lancet 2012; 379: 1245-55.
5. Gao J, Xie L, Yang WS, et al. Risk factors of hepatocellular carcinoma – current status and perspectives. Asian Pac J Cancer Prev 2012; 13: 743-52.
6. Sharma SD. Hepatitis C virus: molecular biology & current therapeutic options. Indian J Med Res 2010; 131: 17-34.
7. Gottwein JM, Scheel TK, Jensen TB, et al. Development and characterization of hepatitis C virus genotype 1-7 cell culture systems: role of CD81 and scavenger receptor class B type I and effect of antiviral drugs. Hepatology 2009; 49: 364-77.
8. Miki D, Ochi H, Hayes CN, Aikata H, Chayama K. Hepatocellular carcinoma: towards personalized medicine. Cancer Sci 2012; 103: 846-50.
9. Nahon P, Zucman-Rossi J. Single nucleotide polymorphisms and risk of hepatocellular carcinoma in cirrhosis. J Hepatol 2012; 57: 663-74.
10. Jin F, Xiong W, Jing JC, Feng Z, Qu LS, Shen X. Evaluation of the association studies of single nucleotide polymorphisms and hepatocellular carcinoma: a systematic review. J Cancer Res 2011; 137: 1095-104.
11. Simmonds P, Bukh J, Combet C, et al. Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. Hepatology 2005; 42: 962-73.
12. Siegel R, Desantis C, Jemal A. Colorectal cancer statistics. Cancer J Clin 2014; 64: 104-17.
13. Braliou GG, Pantavou KG, Kontou PI, Bagos PG. Polymorphisms of the CD24 gene are associated with wisk of multiple sclerosis: a meta-analysis. Int J Mol Sci 2015; 16: 12368-81.
14. Austen K, Ringer P, Mehlich A, et al. Extracellular rigidity sensing by talin isoform-specific mechanical linkages. Nat Cell Biol 2015; 17: 1597-606.
15. Mashaly AH, Anwar R, Ebrahim MA, Eissa LA, Shishtawy MME. Diagnostic and prognostic value of talin-1 and midkine as tumor markers in hepatocellular carcinoma in Egyptian patients. Asian Pac J Cancer Prev 2018; 19: 1503-8.
16. Vauthey JN, Lauwers G, Esnaola N, et al. Simplified staging for hepatocellular carcinoma. J Clin Oncol 2002; 20: 1527-36.
17. Kim HJ, Kim SY, Shin SP, et al. Immunological measurement of aspartate/alanine aminotransferase in predicting liver fibrosis and inflammation. Korean J Intern Med 2020; 35: 320-30.
18. Karmen A. A note on the spectrometric assay of glutamic-oxalacetic transaminase in human blood serum. J Clin Invest 1955; 34: 131-3.
19. Yoon S, Lim N, Ha S, et al. The human cervical cancer oncogene protein is a biomarker for human hepatocellular carcinoma. Cancer Res 2004; 64: 5434- 41.
20. Hall P, Cash J. What is the Real Function of the Liver Function Tests? Ulster Med J 2012; 81: 30-6.
21. Huang G, Yang L, Lu W. Expression of hypoxia-inducible factor and vascular endothelial growth factor in hepatocellular carcinoma: Impact on neovascularization and survival. World J Gastroenterol 2005; 11: 1705-8.
22. Kawada N. Evolution of hepatic fibrosis research. Hepatol Res 2011; 41: 199-208.
23. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 2001; 25: 402-8.
24. Sun J, Fang K, Shen H, Qian Y. MicroRNA-9 is a ponderable index for the prognosis of human hepatocellular carcinoma. Int J Clin Exp Med 2015; 8: 17748-56.
25. Alwahaibi NY, Alkhatri AS, Kumar JS. Hematoxylin and eosin stain shows a high sensitivity but sub-optimal specificity in demonstrating iron pigment in liver biopsies. Int J Appl Basic Med Res 2015; 5: 169-71.
26. Forlano R, Mullish BH, Giannakeas N, et al. High-throughput, machine learning-based quantification of steatosis, inflammation, ballooning, and fibrosis in biopsies from patients with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol 2020; 18: 2081-90.

27. Munteanu M, Tiniakos D, Anstee Q, et al. Diagnostic performance of Fibro-Test, Steato-Test and Acti-Test in patients with NAFLD using the SAF score as histological reference. Aliment Pharmacol Ther 2016; 44: 877-89.
28. Liu J, Chen L, Pan J, et al. Comprehensive analysis of key lncRNAs in HCV-positive hepatocellular carcinoma. Arch Med Sci 2021; 17: 142-51
29. Ibrahim SM, Bastawy AA. The relevance of single-nucleotide polymorphism +62 G>A to the expression of resistin gene affecting serum resistin levels in metabolic syndrome in the Egyptian population. Curr Pharm Biotechnol 2020; 21: 626-34.
30. Wang L, Lin S, Rammohan KW, et al. A dinucleotide deletion in CD24 confers protection against autoimmune diseases. PLoS Genet 2007; 3: e49.
31. Li D, Zheng L, Jin L, et al. CD24 polymorphisms affect risk and progression of chronic hepatitis B virus infection. Hepatology 2009; 19: 735-42.
32. Robert F, Pelletier J. Exploring the impact of single-nucleotide polymorphisms on translation. Front Genet 2018; 9: 507.
33. Kristiansen G, Machado E, Bretz N, et al. Molecular and clinical dissection of CD24 antibody specificity by a comprehensive comparative analysis. Lab Invest 2010; 90: 1102-16.
34. Ochsner SA, Strick-Marchand H, Qiu Q, et al. Transcriptional profiling of bipotential embryonic liver cells to identify liver progenitor cell surface markers. Stem Cells 2007; 25: 2476-87.
35. Mihm S. Hepatitis C virus, diabetes and steatosis: clinical evidence in favor of a linkage and role of genotypes. Dig Dis 2010; 28: 280-4.
36. Mirza S, Siddiqui AR, Hamid S, Umar M, Bashir S. Extent of liver inflammation in predicting response to interferon α & Ribavirin in chronic hepatitis C patients: a cohort study. BMC Gastroenterol 2012; 12: 71.
37. Youns MM, Abdel Wahab AH, Hassan ZA, Attia MS. Serum talin-1 is a potential novel biomarker for diagnosis of hepatocellular carcinoma in Egyptian patients. Asian Pac J Cancer Prev 2013; 14: 3819-23.
38. Bostanci O, Kemik O, Kemik A, et al. A novel screening test for colon cancer: Talin-1. Eur Rev Med Pharmacol Sci 2014; 18: 2533-7.
39. Sakamoto S, McCann RO, Dhir R, Kyprianou N. Talin1 promotes tumor invasion and metastasis via focal adhesion signaling and anoikis resistance. Cancer Res 2010; 70: 1885-95.
40. Chen P, Zheng X, Zhou Y, Xu Y, Zhu L, Qian Y. Talin-1 interaction network promotes hepatocellular carcinoma progression. Oncotarget 2017; 8: 13003-14.
41. Zampino R, Marrone A, Rinaldi L, et al. Endotoxinemia contributes to steatosis, insulin resistance and atherosclerosis in chronic hepatitis C: the role of pro-inflammatory cytokines and oxidative stress. Infection 2018; 46: 793-9.