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CTNNB1 polymorphism (*rs121913407*) in circulating tumor DNA (ctDNA) in Egyptian hepatocellular carcinoma patients

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Abstract

Background: Hepatocellular carcinoma (HCC) represents the sixth most common cancer worldwide and the fourth in Egypt. Persistent inflammation and specific somatic mutations in driving genes play a major role in the development of HCC. One of these somatic mutations is *CTNNB1* mutations with subsequent activation of β-catenin in HCC, associated with a risk of malignant transformation. In this study, we investigate the clinical utility of peripheral blood circulating tumor DNA (ctDNA) *CTNNB1* (*rs121913407*) in HCC patients compared to pathological chronic hepatitis C virus (HCV) patients and healthy controls.

Methods: Our study is a case-control study at the Ain Shams Centre for Organ Transplantation, Ain Shams University Hospitals, enrolling twenty-eight adult HCC patients (twelve early HCC patients and sixteen advanced HCC patients), ten patients with chronic hepatitis C as a disease control group, and ten healthy controls. We collected plasma and stored at −80 °C. We detected mutations in the gene locus *CTNNB1 rs121913407* by real-time PCR.

Results: All of our studied cases (early and advanced HCC) in addition to HCV and healthy control groups were *CTNNB1* wild (TT) genotype. There was statistical significant difference between early and late cases of HCC as regards AFP and AST.

Conclusions: None of our recruited subjects showed *CTNNB1 rs121913407* gene mutation. Further studies on larger number of patients are needed to clarify and confirm the clinical utility of *CTNNB1* single-nucleotide polymorphism in the pathogenesis of HCC related to HCV in Egyptian population.

Keywords: Chronic hepatitis C virus, ctDNA, *CTNNB1*, Hepatocellular carcinoma

Background

Hepatocellular carcinoma is a primary tumor of the liver, which arises from liver cells and constitutes about 90% of all primary liver cancer types that usually develops in the setting of chronic liver disease, particularly in patients with chronic hepatitis B and C. HCC has a rising incidence in Egypt mostly due to high prevalence of viral hepatitis and its complications [1, 2].

Over the past decade, advances in genomic research have increased our knowledge of HCC molecular pathogenesis. However, the exact molecular mechanisms underlying the development of HCC are still unclear [3].

According to the current European Association for Study of the Liver (EASL) guidelines, one of the unmet needs in HCC research is to develop new tools for early detection including the assessment of liquid biopsy [4]. In addition, without a liver biopsy, assessment of the genomic profile becomes a challenge. This can be

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addressed by a noninvasive liquid biopsy which provides actionable genomic information without the risk of complications. Recently, circulating tumor DNA (ctDNA) has attracted extensive attention as a promising component of liquid biopsy. Circulating tumor DNAs are mutant DNAs released into the circulation by tumor cells and can be assessed through analysis of plasma from a blood sample of HCC patient. A liquid biopsy, represented in a blood sample, can be used to assess ctDNA in a quest to comprehensively profile the tumor genome better than conventional sampling methods. This qualifies it as a better vehicle to provide information about abnormalities in genes for guiding targeted therapy, unveiling drug resistance, and monitoring treatment response [5, 6].

Recent studies have identified specific somatic mutations in driving genes that appear to contribute to tumor initiation and progression. One of these somatic mutations is *CTNNB1* mutations with subsequent activation of β-catenin in HCC, associated with a risk of malignant transformation. Notably, hepatitis B and C infections have different impacts on other driver genes frequently mutated in HCC and belong to key signaling pathways of oncogenesis as the WNT/β-catenin pathway and the P53 cell cycle pathway [3].

According to ClinGen Allele Registry, the variant CTNNB1 rs121913407:c.133T>C (p.Ser45Pro) lies within coding transcript of the gene CTNNB1, with substitution of cytosine for thymine at nucleotide position 133. This is a missense mutation causing the associated protein reference sequence amino acid number 45 to be changed from serine to proline. CTNNB1 maps to the short arm of chromosome 3 (3p22.1). The variant is present on chromosome 3 nucleotide number 41224645 (chr3: g.41224645T>C on assembly GRCh38). This SNP NM_001904.4 (CTNNB1): c.133T>C (p.Ser45Pro) is included in ClinVar and recorded on ensembl as rs121913407 SNP, with details about different synonyms for the same variant on OMIM, ClinGen, UniProtKB, and dbSNP. It is included in ClinVar as a somatic pathogenic variant in HCC and as a missense mutation that is pathogenic or likely pathogenic [7, 8].

The aim of this work was to study the clinical utility of peripheral blood ctDNA *CTNNB1 rs121913407* c.133T>C in HCC patients compared to pathological chronic hepatitis C virus (HCV) patients and healthy controls.

Methods

Sample size

This is an exploratory study which was approved by the Faculty of Medicine Ain Shams University Research Ethics Committee (FMASU R92/2020).

Patient selection

In our study, we included Egyptian patients with HCV-related HCC attending the HCC clinic and Ain Shams Centre for Organ Transplantation (ASCOT), Ain Shams University Hospitals, between October 2020 and April 2021. The study included twenty-eight adult HCC patients, ten patients with chronic hepatitis C as a disease control group, and ten healthy controls.

Diagnosis of the studied cases

Based on EASL guidelines 2018, we diagnosed cirrhotic patients depending on noninvasive criteria and/or pathology. We based diagnosis on the identification of the typical hallmarks of HCC, which is the combination of hypervascularity in late arterial phase (defined as arterial phase hyperenhancement [APHE] according to LI-RADS [Liver Imaging Reporting and Data System] classification and washout on portal venous and/or delayed phases, which reflects the vascular derangement occurring during hepatocarcinogenesis [9]. We determined clinical staging of HCC according to Barcelona Clinic Liver Cancer (BCLC) staging classification and Child-Turcotte-Pugh staging (CTP) [10, 11].

We divided patients into two groups according to eligibility for liver transplantation into two groups: early HCC (EHCC) and advanced HCC patients (AHCC). *Group 1: early HCC (EHCC) patients (n = 12)* who fulfilled either Milan criteria, single lesion $\leq$ 5 cm or up to 3 lesions $\leq$ 3 cm each in the absence of tumor vascular invasion or evidence of extrahepatic metastases [12], or University of California, San Francisco (UCSF) criteria, which considered a single lesion $\leq$ 6.5 cm or 2–3 lesions $\leq$ 4.5 cm each, with total tumor diameter $\leq$ 8 cm are accepted [13]. Additional inclusion criteria were alpha-fetoprotein < 200 ng/mL and the absence of macrovascular invasion or distant metastatic spread as documented by triphasic pelviabdominal CT, CT chest, bone scan, and/or PET scan if needed [12, 13]. *Group 2: advanced HCC patients (AHCC) (n = 16)* and fulfilling HCC are beyond the abovementioned criteria (Milan or UCSF) and/or BCLC stage C, the presence of macrovascular invasion by duplex ultrasound, and/or triphasic CT. The presence of metastasis including lymph node invasion as documented by triphasic pelviabdominal CT, CT chest, bone scan, and/or PET scan if needed and alpha-fetoprotein > 1000 ng/mL are additional criteria for inclusion in this group.

Exclusion criteria were patients having other degenerative conditions affecting cfDNA concentrations like autoimmune diseases or other malignancies, non-HCV-related HCC, cystic liver focal lesions (hepatic abscesses, hydatid cysts), metastatic liver focal lesions

(cancer colon, cancer breast), and refusal to sign informed consent.

All subjects included in this study were subjected to the following: full history taking and thorough clinical examination and radiological workup including the following: abdominal ultrasound/duplex and spiral triphasic abdominal CT and/or MRI. In addition, we performed laboratory investigations, including complete blood count, international normalized ratio (INR), alpha-fetoprotein (AFP), renal function tests (BUN, creatinine), liver function tests including aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, and total bilirubin.

For the purpose of genotyping, we collected 10 mL of blood on EDTA vacutainer tubes for ctDNA extraction. Circulating tumor DNA (ctDNA) was extracted from the plasma of at least 10 mL peripheral venous blood samples in EDTA. Blood samples were processed within 2 h after venipuncture by a two-step centrifugation method: the first spin at 1600 g for 10 min to remove the majority of blood cells and a second spin at 16,000 g for another 10 min to remove the cellular debris. The plasma was subpackaged in RNase DNase-free tubes and stored at −80 °C until use. The ctDNA was extracted using the GeneJET Whole Blood Genomic DNA Purification Mini Kit according to the manufacturer's instructions (Thermo Fisher Scientific, USA).

We assessed mutation in the gene locus *CTNNB1* c.133T>C (p.Ser45Pro) by real-time PCR (RT-PCR). The sequence information of the primers and probes primers and probes is illustrated in Table 1. Real-time PCR reactions were done in a final volume of 20 µL, using 20 ng of extracted DNA. We used TaqMan Genotyping PCR Master Mix and SNP Genotyping Assay (Thermo Fisher Scientific, USA). We used volumes for PCR reaction mix according to manufacturer's instructions (Table 2). Cycling conditions were as follows: initial activation at 95 °C for 10 min and then denaturation; 40 cycles of 95 °C for 15 s, annealing/extension; 57 °C for 15 s, and 72 °C for 1 min in a DT-Lite real-time PCR system (DNA technology, Russia).

Table 1 Sequence information of the primers and probes for the RT-PCR assay

Forward primer	5′-TCACTGGCAGCAACAGTCTT-3′
Reverse primer	5′-CAGGACTTGGGAGGTATCCA-3′
Mutant probe	5′-FAM-GTGCCACTGCCAC-MGB-3′
Wild-type probe	5′-VIC-GGTGCCACTACCAC-MGB-3′

Table 2 PCR reaction mix in each sample

Component	Volume
TaqMan genotyping Master mix (2×)	10 µL
TaqMan SNP genotyping assay (primers and probes) (40×)	0.5 µL
Extracted DNA	Equivalent to 20 ng
DNase-free water	To final volume 20 µL
Total volume per well	20 µL

Statistical analysis

For this purpose, we used a GraphPad Prism statistical software version (5.01). We expressed the values for the biochemical markers as mean and standard deviation in the case of parametric data and as median and interquartile ranges (IQR) in case of skewed data, while we summarized categorical variables using frequency measures. For comparative analysis, we used Wilcoxon's rank-sum test (Mann-Whitney U), chi-square test, and Kruskal-Wallis test. In all statistical analyses, $p < 0.05$ was considered significant.

Results

The demographic characteristics of all studied subjects and statistical comparison between the various studied parameters are included in Table 3. Regarding Child-Turcotte-Pugh (CTP), 39.3% of HCC patients were stage A, 39.3% stage B, and 21.4% of them at stage C. Also, regarding BCLC staging, 7.1% of HCC patients were at stage 0, 25% stage A, 21.4% stage B, 25% stage C, and 21.4% of them at stage D as demonstrated in Table 3.

The descriptive and comparative statistics of the demographic data and various studied parameters in healthy control, HCV, and HCC patients are shown in Table 4. Serum levels of AST, ALT, INR, and total bilirubin were highly significantly increased in the HCC group as compared to the healthy control group and HCV group ($p < 0.05$), while serum albumin was significantly decreased in the HCC group as compared to both healthy control group and HCV ($p < 0.05$).

The descriptive and comparative statistics of demographic data and various studied parameters in the EHCC patients and the AHCC patients are shown in Table 5. Serum levels of AFP were highly significantly increased in the AHCC group as compared to the EHCC group ($p < 0.01$). Also, serum AST, INR, BUN, and creatinine were statistically significantly increased in the AHCC group as compared to the EHCC group (p

Table 3 Tumor-related characteristics in HCC group ($n = 28$)

Parameters	*N* (%)
Number of focal lesions	1 (1–4)
Tumor size (cm)	4.8 (2.5–9.95)
Ascites	
No	12 (42.9%)
Yes	16 (57.1%)
Encephalopathy	
No	26 (92.9%)
Yes	2 (7.1%)
PVT	
No	16 (57.1%)
Yes	12 (42.9%)
Metastatic liver lesions	
No	27 (96.4%)
Yes	1 (3.6%)
Milan staging	
No	19 (67.9%)
Yes	9 (32.1%)
UCSF staging	
No	25 (89.3%)
Yes	3 (10.7%)
CTP stage A	11 (39.3%)
CTP stage B	11 (39.3%)
CTP stage C	6 (21.4%)
BCLC staging	0
0	2 (7.1%)
A	7 (25.0%)
B	6 (21.4%)
C	7 (25.0%)
D	6 (21.4%)

BCLC Barcelona clinic liver cancer, *CTP* Child-Turcotte-Pugh, *HCC* hepatocellular carcinoma, *PVT* portal vein thrombosis, *UCSF* Liver Center of the University of California, San Francisco. Data are presented as number (%)

< 0.05, respectively). On the contrary, serum albumin was significantly decreased in AHCC group ($p < 0.05$). Regarding *CTNNB1* c.133T>C genotyping, all of our studied cases (early and advanced HCC) in addition to HCV and healthy control groups were *CTNNB1* wild (TT) genotype (Table 6). We demonstrate a representative of results of the cycle threshold (Ct) for a run on 5 EHCC, and 6 AHCC samples in the study in Table 7, and the fluorescence curves showing the Ct in Fig. 1.

Discussion

Hepatocellular carcinoma frequently arises in the context of chronic cellular injury with consequent DNA damage and genetic alterations [14].

The fundamental pathogenic event in the development of HCC is genetic mutation resulting in aberrant activation of signal transduction Wnt/β-catenin pathway, which plays a critical role in initiating and sustaining hepatic carcinogenesis [15, 16].

β-catenin, encoded by *CTNNB1* gene, is the main effector signaling molecule in the canonical Wnt pathway [17]. In malignant hepatocytes, β-catenin loses its physiological function as a cell-adhesion molecule accumulates resulting in cellular proliferation and metastasis [18]. *CTNNB1* mutations were identified in about 20–40% of liver cancers [19].

A meta-analysis including 2334 liver cancer cases from twenty-two studies showed that accumulation of β-catenin in the cytoplasm and/or nucleus significantly correlated with poor prognosis [18]. Hence, we examined the polymorphism of *CTNNB1* c.133T>C in early and advanced HCC patients compared to pathological chronic HCV patients and healthy controls.

All of our studied cases (early and advanced HCC) in addition to HCV and healthy control groups were *CTNNB1* wild (TT) genotype. None of our recruited HCC cases showed *CTNNB1* gene mutation. The reason for these negative results may be due to the fact that all cases analyzed in our study were cirrhotic HCC patients, and *CTNNB1* mutations were described to be particularly prevalent in non-cirrhotic HCC patients [20].

Our results are in agreement with Lombardo et al. [3] who revealed the absence of *CTNNB1* mutation in exon 3 of all frozen tumor liver specimens from 67 HCC Italian patients using Sanger sequencing. Forty (59.7%) of the 67 patients with HCC had HCV. They mentioned that although somatic mutations are expected to be found in *CTNNB1* gene which is considered a driver gene for HCC development, these mutations show variable frequencies in different geographic areas, possibly depending on liver disease etiology and environmental factors. They added that in the absence of *CTNNB1* somatic mutations, possibly in these patients, activation of Wnt/β-catenin could be induced independently as in previous studies on adrenal aldosterone-producing adenomas [3].

Our results are in disagreement with Tornesello et al. [21] who found that mutations in exon 3 of *CTNNB1* gene occurred in HCV-related HCCs (17.5%) from Italians in Naples. Tornesello et al. [21] performed the analysis of *CTNNB1* exon 3 using the direct sequencing analysis. A similar lower mutation frequency was previously observed among French HCC cases using whole-exome DNA sequencing [22]. This discrepancy between our results and earlier studies may be due to other researchers using a more sensitive next-generation sequencing technique by the researchers on a different sample represented in HCC tissue from a dissimilar population of European patients. Moreover, it is well known that genetic origin and heterogeneity affect the mutation rates. Previous study has indicated that mutation pattern

Table 4 Demographic characteristics of all studied subjects and statistical comparison between the various studied parameters in HCC patients versus healthy ccontrol and HCV patients[ab]

Parameters	Healthy control ($n = 10$)	HCV patients ($n = 10$)	HCC patients ($n = 28$)	*p*-value HCC vs healthy control	*p*-value HCC vs HCV
Gender					
Female *n* (%)	1 (10)	1 (10)	3 (10.7)	0.950[b]	0.095[b]
Male *n* (%)	9 (90)	9 (90)	25 (89.3)		
Age (years)	60.5 (56.5–62)	64 (60–69.3)	60 (58–63.7)	0.950[a]	0.075[a]
AST (U/L)	12.5 (11.75–18.25)	24.0 (18.7–35.2)	50.5 (35–107)	< 0.0001[a]	0.001[a]
ALT (U/L)	17.0 (12.50–21.25)	24.0 (12.0–51.7)	47.5 (27.75–63.60)	0.0002[a]	0.0414[a]
INR	0.85 (0.61–1.10)	1.04 (1.0–1.29)	1.20 (1.10–1.39)	< 0.0001[a]	0.0158[a]
Albumin (g/dl)	3.90 (3.15–4.60)	3.90 (3.30–4.65)	3.35 (2.62–3.60)	0.0018[a]	0.104[a]
Total bilirubin (mg/dl)	0.40 (0.20–0.85)	0.50 (0.27–0.92)	1.30 (0.90–2.30)	< 0.0001[a]	0.0002[a]
BUN (mg/dl)	17.5 (9.2–20.2)	20.5 (17.0–24.0)	20.0 (12.0–32.0)	0.2801[a]	0.8941[a]
Creatinine (mg/dl)	0.90 (0.70–1.03)	0.95 (0.77–1.12)	0.95 (0.80–1.20)	0.4487[a]	0.752[a]

AFP alpha-fetoprotein, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *BUN* blood urea nitrogen, *HCC* hepatocellular carcinoma, *INR* international normalized ratio. Data are presented as median (interquartile range) or number (%). $p < 0.05$ is significant

[a] Statistical comparison using Wilcoxon's rank-sum test

[b] Statistical comparison using chi-square test

Table 5 Demographic characteristics of all studied subjects and statistical comparison between EHCC and AHCC[a] using Wilcoxon's rank-sum test and between all the studied groups using Kruskal-Wallis test[b]

Parameters	EHCC patients ($n = 12$)	AHCC patients ($n = 16$)	*p*-value	HCV patients ($n = 10$)	Healthy control ($n = 10$)	*p*-value
Age (years)	60 (55–63)	57 (52–60)	0.193[a]	64 (60–69)	60 (56–62)	0.0509[b]
Albumin (g/dl)	3.6 (3.2–3.6)	2.9 (2.5–3.4)	0.0013[a]	3.9 (3.3–4.7)	3.9 (3.2–4.6)	0.0037[b]
Total bilirubin (mg/dl)	1.15 (0.9–1.9)	1.4 (0.8–4.3)	0.0105[a]	0.5 (0.3–0.9)	0.4 (0.2–0.9)	< 0.0001[b]
INR	1.15 (1.1–1.2)	1.39 (1.2–1.65)	0.1635[a]	1.05 (1–1.29)	0.85 (0.62–1.1)	0.0002[b]
BUN (mg/dl)	12 (10–18)	13 (10–26)	0.0298[a]	20 (17–24)	17 (9–20)	0.0012[b]
Creatinine (mg/dl)	0.75 (0.63–1)	1.05 (0.9–1.63)	0.0173[a]	0.95 (0.8–1.1)	0.9 (0.7–1)	0.0344[b]
AST (U/L)	40 (33–47)	93 (59–190)	0.2746[a]	24 (19–35)	13 (12–18)	< 0.0001[b]
ALT (U/L)	40 (31–50)	66 (32–95)	0.0029[a]	24 (12–52)	17 (12–21)	0.0006[b]
AFP (ng/ml)	5.5 (3.3–136)	250 (65–9784)	0.0117[a]	4.6 (3.1–6.1)	3.6 (2.5–4.6)	< 0.0001[b]

AHCC advanced hepatocellular carcinoma, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *BUN* blood urea nitrogen, *EHCC* early hepatocellular carcinoma, *HCC* hepatocellular carcinoma, *HCV* hepatitis C, *INR* international normalized ratio. Data are presented as median (interquartile range).. $p < 0.05$ is significant

[a] Statistical comparison between EHCC and AHCC using Wilcoxon's rank-sum test

[b] Statistical comparison between all groups using Kruskal-Wallis test

Table 6 *CTNNB1* c.133T>C genotyping in HCC, HCV, and healthy control groups

CTNNB1 c.133T>C	HCC	HCV	Healthy control
Wild	28 (100%)	10 (100%)	10 (100%)
Mutant	0	0	0

and frequencies could be variable according to heterogeneity in host genetic, etiology, and geographical regions (23). This is the first study on Egyptian patients for SNPs in gene locus *CTNNB1* c.133T>C in a liquid biopsy, a different population from those done in previous studies [23].

Another relevant observation by Tornesello et al. [21] is the presence of *CTNNB1* gene mutations in HCC patients in a comparable frequency in Asia and Europe. They observed that these HCC patients with mutated gene were significantly younger relative to those with wild type. In North Africa, only 4 out of 42 HCC patients revealed *CTNNB1* mutations. The mean age in our studied patients was 60 years old which could contribute in addition to the different geographic regions of our population to the negative results.

Table 7 Cycle thresholds for EHCC and AHCC patients for wild genotype (VIC probe) on DT-Lite PCR system

Well number	ID of the tube	Ct, FAM	Ct, VIC
C2	EHCC6		26.7
C3	EHCC7		27.3
C4	EHCC8		26.8
C5	EHCC9		26.5
C6	EHCC10		27.8
C7	AHCC7		27.1
C8	AHCC8		26.0
D1	AHCC9		26.5
D2	AHCC10		23.4
D3	AHCC11		26.0
D4	AHCC12		24.2

In discordance with our results, Lee et al. [24] reported the presence of *CTNNB1* mutations in HCC patients which were not associated with any clinicopathologic factors. Lee et al. [24] performed the analysis of *CTNNB1* mutations using PCR amplification and Sanger sequencing analysis. Moreover, *CTNNB1* mutations were present in 13.2% (10/76) of HCC related to HBV and in 14.3% (1/7) HCC related to HCV. The small number of cases in our study, although greater than HCC-HCV patients in Lee et al. (2016), in addition to their use of a more sensitive NGS technology on a tissue sample, may explain the absence of concordance between our results [24].

As a justification to our results which failed to identify any mutation, Park et al. [25] described that *CTNNB1* mutations were not found in precursor lesions of HCC and were not uniformly present in all tumors, indicating that these mutations are late events in hepatocarcinogenesis. Furthermore, intratumoral genetic heterogeneity is a practical challenge, and it is known that HCC shows morphological and immunophenotypical heterogeneity, which most probably presents different genetic alterations among HCC patients [26].

The limitation of this study is its nature as a small pilot study without previous genetic information on the frequency of CTNNB1 mutation in the Egyptian population. Such a situation makes it mandatory to enroll a larger number of patients to explore the frequency and the role of such mutation in HCC.

Conclusion

Our study fails to prove evidence for the clinical utility of *CTNNB1 rs121913407* c.133T>C (p.Ser45Pro) in Egyptian HCC patients. We attribute our observed negative results to the small number of cases in our study and the need for the study of more patients to reach a definitive conclusion. Furthermore, we need to support our speculations by sequencing either ctDNA from blood samples or DNA from tissue samples from the same patients to confirm the absence of mutations or their presence with low frequencies and using spatial sequencing which studies single cells to reveal cancer genome obtained from separate multiple cells in HCC tumor tissue.

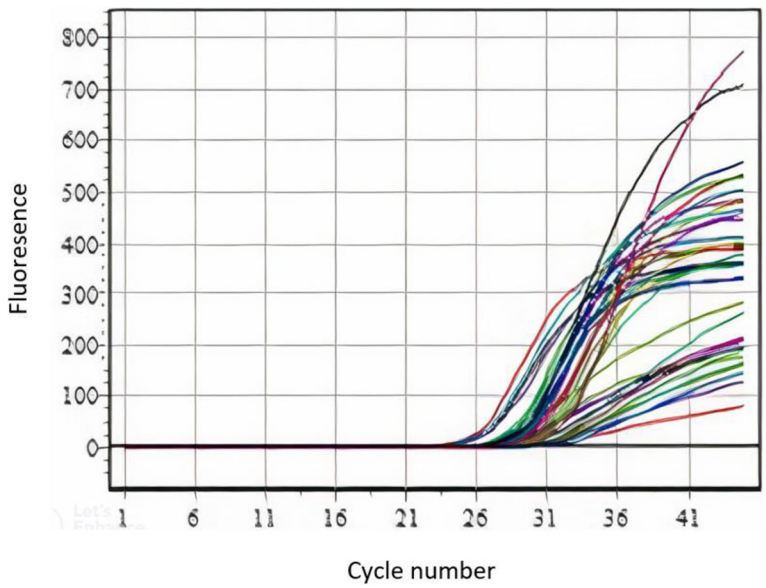

Fig. 1 Showing the fluorescence curves for the studied samples on DT-Lite PCR system graphical display

Abbreviations
AHCC: Advanced HCC patients; AFP: Alpha-fetoprotein; ALT: Alanine aminotransferase; APHE: Arterial phase hyperenhancement; AST: Aspartate aminotransferase; ASCOT: Ain Shams Centre for Organ Transplantation (ASCOT); BCLC: Barcelona Clinic Liver Cancer; ctDNA: Circulating tumor DNA; cfDNA: Cell-free DNA; *CTNNB1*: Catenin beta-1; CTP: Child-Turcotte-Pugh staging; EASL: European Association for Study of the Liver; EHCC: Early HCC; HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus; INR: International normalized ratio; LI-RADS: Liver Imaging Reporting and Data System; LT: Liver transplantation; RT-PCR: Real-time PCR; UCSF: University of California, San Francisco.

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Authors' contributions
MA wrote the manuscript and contributed to data collection and interpretation. IM, YM, and EM contributed to patients' recruitment, sample, and data collection. RM contributed to samples collection, laboratory processing of samples, data interpretation, and drafted the paper. PH and IM planned and designed the study. PH contributed to sample collection, laboratory processing of samples, data interpretation, and reviewed the manuscript. The authors read and approved the final manuscript.

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Availability of data and materials
All data generated or analyzed during this study are included in this published article, and if any data is needed, it will be available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The study was conducted according to the World Medical Association Declaration of Helsinki, after the approval of the local research ethics. This study was approved by the Ethics Committee of the Faculty of Medicine, Ain Shams University, Egypt (FMASU R 92/2020).

Consent for publication
Written informed consent for publication regarding the data of the studied patients was obtained from the ASCOT unit.

Competing interests
The authors declare that they have no competing interests.

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References

1. Tawfik M, Gomaa A, Hassan E, Fouad E (2019) Presentation of hepatocelluler carcinoma at time of discovery in Egyptian patients with liver cirrhosis secondary to chronic hepatitis C. FUMJ 4(1):1–10
2. Yehia SA, Morad WS, Hendy OM, Dorgham LS (2020) Effect of health education intervention on hepatocellular carcinoma risk factor prevention in Menoufia Governorate. Egypt Egyptian Liver J 10:1-8.
3. Lombardo D, Saitta C, Giosa D et al (2020) Frequency of TP53, CTNNB1, and TERT promoter mutations in patients with hepatocellular carcinoma. Dig Liver Dis 52:e52–e53
4. Pawlotsky J-M, Negro F, Aghemo A et al (2018) EASL recommendations on treatment of hepatitis C 2018. J Hepatol 69(2):461–511
5. Mocan T, Simão A, Castro R et al (2020) Liquid biopsies in hepatocellular carcinoma: are we winning? J Clin Med 9(5):1541
6. Morishita A, Iwama H, Fujihara S et al (2018) Targeted sequencing of cancer-associated genes in hepatocellular carcinoma using next-generation sequencing. Oncol Lett 15:528–532
7. Legoix P, Bluteau O, Bayer J et al (1999) Beta-catenin mutations in hepatocellular carcinoma correlate with a low rate of loss of heterozygosity. Oncogene 18(27):4044–4046
8. Chang MT, Asthana S, Gao SP et al (2016) Identifying recurrent mutations in cancer reveals widespread lineage diversity and mutational specificity. Nat Biotechnol 34(2):155–163
9. Mazzaferro V, Regalia E, Doci R et al (1996) Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. N Engl J Med 334(11):693–700
10. Yao FY, Ferrell L, Bass NM et al (2001) Liver transplantation for hepatocellular carcinoma: expansion of the tumor size limits does not adversely impact survival. Hepatology 33(6):1394–1403
11. Matsui O, Kobayashi S, Sanada J et al (2011) Hepatocelluar nodules in liver cirrhosis: hemodynamic evaluation (angiography-assisted CT) with special reference to multi-step hepatocarcinogenesis. Abdom Imaging 36(3):264–272
12. Llovet J, Burroughs A, Bruix J (2003) Hepatocellular carcinoma. Lancet 362(9399):1907–1917
13. Child CG, Turcotte JG (1964) Surgery and portal hypertension. In: Child CG (ed) The Liver and Portal hypertension. Saunders, Philadelphia
14. Ogunwobi OO, Harricharran T, Huaman J et al (2019) Mechanisms of hepatocellular carcinoma progression. World J Gastroenterol 25(19):2279–2293
15. Khalaf A, Fuentes D, Morshid A et al (2018) Role of Wnt/β-catenin signaling in hepatocellular carcinoma, pathogenesis, and clinical significance. J Hepatocell Carcinoma 5:61–73
16. Li Q, Zhang F, Li Y, Xian Q, Zhang Y, Li P (2017) Influence of polymorphisms in the Wnt/β-catenin pathway genes on hepatocellular carcinoma risk in a Chinese Han population. Medicine 96(12):e6127
17. MacDonald B, Tamai K, He X (2009) Wnt/β-catenin signaling: components, mechanisms, and diseases. Dev Cell 17(1):9–26
18. Chen J, Liu J, Jin R et al (2014) Cytoplasmic and/or nuclear expression of β-catenin correlate with poor prognosis and unfavorable clinicopathological factors in hepatocellular carcinoma: a meta-analysis. PLoS One 9(11):e111885
19. Wang W, Pan Q, Fuhler GM, Smits R, Peppelenbosch MP (2017) Action and function of Wnt/β-catenin signaling in the progression from chronic hepatitis C to hepatocellular carcinoma. J Gastroenterol 52(4):419–431
20. Cieply B, Zeng G, Proverbs-Singh T, Geller DA, Monga SP (2008) Unique phenotype of hepatocellular cancers with exon-3 mutations in beta-catenin gene. Hepatology 49(3):821–831
21. Tornesello ML, Buonaguro L, Tatangelo F, Botti G, Izzo F, Buonaguro FM (2013) Mutations in TP53, CTNNB1 and PIK3CA genes in hepatocellular carcinoma associated with hepatitis B and hepatitis C virus infections. Genomics 102(2):74–83
22. Guichard C, Amaddeo G, Imbeaud S et al (2012) Integrated analysis of somatic mutations and focal copy-number changes identifies key genes and pathways in hepatocellular carcinoma. Nat Genet 44(6):694–698
23. Pezzuto F, Izzo F, Buonaguro L et al (2016) Tumor specific mutations in TERT promoter and CTNNB1 gene in hepatitis B and hepatitis C related hepatocellular carcinoma. Oncotarget 7(34):54253–54262
24. Lee S, Chang S, Kim W et al (2016) Frequent somatic TERT promoter mutations and CTNNB1 mutations in hepatocellular carcinoma. Oncotarget 7(43):69267–69275
25. Park JY, Park WS, Nam SW et al (2005) Mutations of β-cateninandaxin igenes are a late event in human hepatocellular carcinogenesis. Liver Int 25(1):70–76
26. Friemel J, Rechsteiner M, Frick L et al (2015) Intratumor heterogeneity in hepatocellular carcinoma. Clin Cancer Res 21(8):1951–1961

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