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Diagnostic, Prognostic, and Therapeutic Significance of Long Noncoding RNAs MALAT1 and UCA1 in HCV-Complicated Hepatocellular Carcinoma

Shimaa Abdelsattar[1], Ashraf Abbass Basuni[1*], Eman S Sawan[2], Mona Gamal El-Abd[3], Esraa Elsayed Abdel Maksoud [4], Aliaa Sabry[5], Asmaa Mosbeh[6], Sally Mohammed El-Hefnawy[7], Ehab Darwish[8], Amira I Aldesoky[9], Alshimaa Mahmoud Elhanafy[9], Hytham M Ahmed[10], Suzan M. El-Morshedy[3]

[1]Clinical biochemistry and molecular diagnostics department, National Liver Institute, Menoufia University.
[2]Department of Clinical Pharmacy, Faculty of Pharmacy, Badr University in Cairo (BUC), Badr, Cairo 11828
[3]Clinical pathology department, National liver institute, Menoufia University
[4]Medical microbiology and immunology department, Faculty of medicine, Menoufia University
[5]Hepatology and gastroenterology department, National Liver institute, Menoufia University
[6]Fellow at pathology department, National liver institute, Menoufia University
[7]Department of Basic medical science, faculty of dentistry, Alryada University for Science and Technology (RST), Sadat city, Egypt
[8]Tropical Medicine department, Faculty of medicine, Zagazig University
[9]Clinical oncology and nuclear medicine department, Faculty of medicine, Menoufia University
[10]Pharmaceutical Analysis department, Faculty of Pharmacy, Menoufia University

Abstract

Long non-coding RNAs (lncRNAs) are involved in the development and progression of many cancers, including hepatocellular carcinoma (HCC). This study investigates two specific lncRNAs, MALAT1 (Metastasis-associated lung adenocarcinoma transcript 1) and UCA1 (Urothelial carcinoma-associated 1), as potential biomarkers for early HCC diagnosis, prognosis, and therapy evaluation.The study included three groups: HCC patients treated with sorafenib (n=120), HCV patients (n=120), and healthy controls (n=120). MALAT1 and UCA1 expression levels were measured in serum using Real Time PCR, alongside routine clinical evaluations and investigations. MALAT1 and UCA1 levels in HCC patients were significantly higher than those in the HCV group (223.9 vs. 13 and 24.8 vs. 2.34, respectively; $P < 0.001$). MALAT1 and UCA1 demonstrated high diagnostic accuracy for HCC with cutoff values of 87.76 and 12, respectively. MALAT1 showed a sensitivity of 96.67% and specificity of 95.0%, while UCA1 had a sensitivity of 93.33% and specificity of 92.5%. In addition, elevated levels of MALAT1 and UCA1 were associated with poor survival rates and resistance to sorafenib treatment, observed in 90% and 80% of cases, respectively. In conclusion, MALAT1 and UCA1 are promising non-invasive biomarkers for the diagnosis and prognosis of HCC. Their elevated expression levels correlate with poor survival and resistance to sorafenib treatment, underscoring their potential as targets for lncRNA-based therapies.

Keywords: HCC, lncRNAs, MALAT1, UCA1, Sorafenib

1. Introduction

Hepatocellular carcinoma (HCC) is one of the most aggressive liver malignancies that silently progress in more than 80 % of cases who are presented at advanced stages. Thus, Early detection and intervention are therefore essential for increasing HCC

*Corresponding author e-mail: ashrabasuni50@gmail.com.; (**Ashraf Abbass Basuni[1]**).
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survival rates [1].Despite advanced therapeutic methods to treat this disease, the 5-year overall survival (OS) for patients with HCC is still unsatisfactory. Moreover, patients with drug resistance continue to have lower survival rates.

Sorafenib (Nexavar)^{R}is among the most prosperous targeted therapies to improve patients' clinical results who have advanced HCC [2]. The most commonly reported side effects associated with sorafenib were fatigue, diarrhea, liver dysfunction, and palmar-plantar skin reaction. Sorafenib inhibits the proliferation of cancer cells by concentrating on several kinases involved in the Ras- Raf- MEK- ERK cascade. Additionally, sorafenib suppresses tumor vasculature by targeting tyrosine kinases that participate in the tumor's angiogenesis. This indirectly inhibits the proliferation of tumor cells. Sorafenib could potentially promote cell apoptosis through targeting Mcl-1 [3, 4]. Unfortunately, only thirty percent of HCC patients respond well to sorafenib and they frequently develop resistance within six months. This limited clinical response brought on by medication resistance still compromises patient survival. Furthermore, it remains unclear what causes sorafenib resistance[4, 5].

While the incidence of NASH and alcohol related HCC is rising, chronic viral hepatitis is still a prominent global cause of HCC development. Viral-related HCC is characterized by oxidative stress, inflammation of the liver, and dysregulation of cell signaling pathways [6]. This viral induced-chronic inflammation results in a gradual change in immune cells, which raises the release of pro-inflammatory cytokines, ROS and ultimately modifies the hepatic environment [7]. Typically, the primary processes behind HCV-associated hepatocarcinogenesis and malignant evolution are immune response dysregulation, persistent inflammation, and alteration to antigen-presenting cell function [8, 9].

Long non-coding RNAs (lncRNAs) are a group of newly discovered RNA molecules more than 200 nucleotides in length. lncRNAs can regulate gene expression at different levels mainly through epigenetic changes[10].lncRNAsperform crucial tasks in the emergence of numerous cancer types and influence the development of tumors through various mechanisms**.** Dysregulation of lncRNAs contributes to pathogenesis of HCC through complex mechanisms[11, 12]. Since their expressions are correlated with the disease's malignancy and response to treatment, lncRNAs have the potential to be employed as biomarkers for a variety of malignancies.

Recently. MALAT1 (metastasis-correlated lung adenocarcinoma transcript 1) has gained a lot of interest due to its crucial role in several diseases, including cancer. Transcribing from human chromosome 11q13, MALAT1 is a highly conserved nuclear localized lncRNA. In addition to HCC, lncRNA MALAT1 was showed to be highly expressed in many human cancers [13]. It has been reported that MALAT1 could affect cell proliferation, migration, angiogenesis, and other vital biological processes [14].

Comparably, urothelial carcinoma-associated 1 (UCA1), a long noncoding RNA that was first found in cases of bladder cancer in humans, has been linked toa number of human malignancies such as gastric, colorectal and HCC. Notably, UCA1 overexpression in HCC tissues and cell lines reduces apoptosis and encourages HCC cell migration and proliferation, all of which are associated with an unfavorable prognosis and advanced disease characteristics [15].

In the current study, we investigated the possible roles of lncRNAs MALAT1 and UCA1 for assessing HCC patients as opposed to HCV patients and healthy individuals in order to evaluate MALAT1 and UCA1 as promising biomarkers for early diagnosis, severity assessment, and therapy evaluation. Early detection and proper intervention are essential for increasing HCC survival rates

2. Subjects and Methods:

2.1. Population of the Study:

This study was carried out from March 2022 to March 2024 and comprised 360 subjects, of which 240 were patients and 120 were healthy individuals. A written consent was taken from every study participant and the study protocol was approved by the ethics committee of the Menoufia University faculty of medicine (4/2024 ONCO 4). The two hundred forty patients were picked up from Health insurance Aboubaker clinics, the outpatient clinics of National Liver Institute, and department of clinical oncology and nuclear medicine, Faculty of Medicine, Menoufia University, Shebin El-Kom, Egypt. Of these patients, 120 had HCV and 120 had HCC treated with Sorafenib. All those patients had negative hepatitis B viral markers (HBsAg and HBcAb) but were positive for HCV Ab. In line with the American and European Association for the Study of Liver (AASLD & EASL), patients were identified by imaging methods and by the amount of AFP in the serum. The tumorcharacteristics (number, size, and invasion of the portal vein), were noted. We computed the MELD and Child Pugh scores. Liver cirrhosis was categorized in accordance with the Child score. The one hundred twenty healthy individuals, matched in terms of age and gender with the patient groups, were included as a control group for the study. All healthy subjects had perfectly normal biochemical and radiological function testing, and were negative for viral indicators. We excluded from the study those who had liver transplantation. Patients who came with renal insufficiency or with other forms of malignancy were also excluded. Verbal consent was given by each study participant, and a clinical examination and history were conducted. Response to sorafenib was assessed after 3-4 months of therapy by using Modified (RECIST) criteria of HCC [16]. Patient considered sorafenib resistant if had disease progression and sorafenib responsive if had stable disease, partial or complete response [17, 18].

2.2. Specimen collection:
Each participant had ten milliliters of blood collected, which were then divided into four tubes. Two milliliters were transferred into sodium citrate (3.8%) anticoagulant tubes to measure prothrombin time and INR, and the tubes were then immediately spun at 1500 x g for ten minutes before being sent to assay analysis. For the CBC assay, two ml were transferred to an EDTA tube. Three more ml were added to another EDTA tube. After centrifuging the mixture, the isolated plasma was retained for extraction of total RNA. Lastly,three ml were transferred to a plain tube, allowed to clot, and the serum was separated and used for biochemical examinations as well as tumor and virology markers.

2.3. Laboratory investigations:
Liver enzymes, serum total and direct bilirubin, albumin, kidney function tests (urea and creatinine) were analyzedon Cobas c501 Auto analyzer (Hitachi, High technologies cooperation, Tokyo, Japan). Tumor and viral markers (HCV Ab, HBsAg, HBcAb, HIV Abs) were measured by electrochemiluminescence (ECL) technology on Cobas e601 Auto analyzer (Hitachi, High technologies cooperation, Tokyo, Japan). The Sysmex Xn-1000 Automated Hematology Analyzer was used for CBC determination while Sysmex Cs-1600 automated analyzer was utilized to measure the prothrombin concentration and INR (Sysmex Corporation, Germany).

2.4. Detection of MALAT1 and UCA1 expression:

2.4.1. RNA extraction:
The RNeasy plus Universal Kit was used to extract total RNA (QIAGEN, USA). Nano Drop was utilized to measure the purity and concentration of RNA (Thermo-Scientific, Waltham, USA). Up to its utilization, the extract was kept at -80°C.

2.4.2.Reverse transcription reaction:
Sensi-FAST cDNA Kit was employed to perform reverse transcription (RT) reactions (Bio line, Germany). The following components were added to the reaction at a final volume of 20 µL: 5 µL nuclease-free water, 1 µL reverse transcriptase enzyme, 4 µL buffer, and 10 µL RNA template. To complete the reaction, the Applied Bio Systems' cycler "Singapore" was employed. Ten minutes at 42 ◦C, five minutes at 95 ◦C, and five minutes at 4 ◦C made up its single cycle. The cDNA was generated and then kept in a cold storage until the real-time PCR (qPCR) step.

2.4.3. Gene expression analysis:
Real Time Reverse Transcription PCR (RT-PCR) methodology was utilized to express the MALAT1 and UCA1 genes. The amplification conditions were adjusted as previously shown [19]. Sequences for primers were: MALAT1 (forward, 5′-CAG GCGTTGTGCGTAGAGGA-3′; reverse, 5′-TGCCGACCTCACGGATTTT-3′); (GAPDH forward, 5′-GTCAGCCGCATC TTCTTT-3′; and reverse, 5′-CGCCCAATACGACCAAAT-3′. UCA1 primers (forward: 5′ CTCTCCATTGGGTTCACCATTC-3′, reverse: 5′-GCGGCAGGTCTTAAGAGATGAG-3′). The 2−ΔΔCT technique was utilized to determine the degrees of MALAT1 and UCA1 expression, with GAPDH serving as the endogenous housekeeping gene (relative quantification).

2.5. Statistical Analysis:
Version 20.0 of IBM SPSS software was used for analysis. To show the categorical variables, numbers and percentages were employed. To compare two groups, the Chi-square test was utilized. For quantitative variables that were regularly distributed, the mean, median and standard deviation were used. We used the median expression values of MALAT1 and UCA1 to equally subdivide the HCC and HCV patients into low and high expression groups. When comparing more than two groups, the ANOVA test was utilized, and for pairwise comparisons, the Post Hoc (Tukey) test was employed. In contrast, for numerical variables that did not follow a regular distribution, the Kruskal Wallis test was employed and pairwise comparisons were performed using Dunn's multiple comparisons test. Finally, the ROC and Kaplan-Meier methods were introduced for analysis. In order to identify the independent variables for low survival, univariate and multivariate regression analysis were conducted after the Kaplan–Meier curve. The degree and direction of the relationship between the markers under study were evaluated using the spearman correlation.

3. Results

3.1. Demographic features of the research groups:
Table 1 displays the study participants' basic data. The total number of subjects enrolled in our studywere 360 who were split up as follows: there were 120 HCC Sorafenib treated patients (86 male and 34 females) with an average age of 56 years, 120 HCV infected patientwho didn't receive treatment (84 male and 36 females), with an age of 54.96 ± 11.04 years and 120 healthy individuals (80 men and 40 women), with an age of 57.98 ± 9.13 years. Age and gender differences between the groups under study were not statistically significant.

Supplementary table (1) provides an overview of participants' laboratory results. Regarding the laboratory parameters, there was a notable distinction between the groups under study. While hemoglobin level, platelet count, and albumin showed lower values in the HCC group, the values for liver enzymes, INR, bilirubin, and tumor markers were raised in HCC patients compared to HCV patients. All P for these parameters were <0.05.

Table 1. Demographic data of the study participants

	HCC treated (n = 120)	HCV (n = 120)	Control (n = 120)	Test of Sig.	P value
Age (years)					
Mean ± SD.	56.58 ± 9.82	54.96 ± 11.04	57.98 ± 9.13	F= 2.734	0.066
Median (Min.- Max.)	59 (40 – 75)	56 (19 – 70)	60 (21 – 75)		
Gender					
	86 (71.7%)	84 (70.0%)	80 (66.7%)	χ^2= 0.952	0.621
	34 (28.3%)	36 (30.0%)	40 (33.3%)		
Smoking	28 (23.3%)	32 (26.7%)	20 (16.7%)	χ^2=3.600	0.165
Diabetes	40 (33.3%)	25 (20.8%)	0 (0.0%)	χ^2=45.997*	<0.001*
Hypertension	37 (30.8%)	27 (22.5%)	0 (0.0%)	χ^2=1.769*	<0.001*

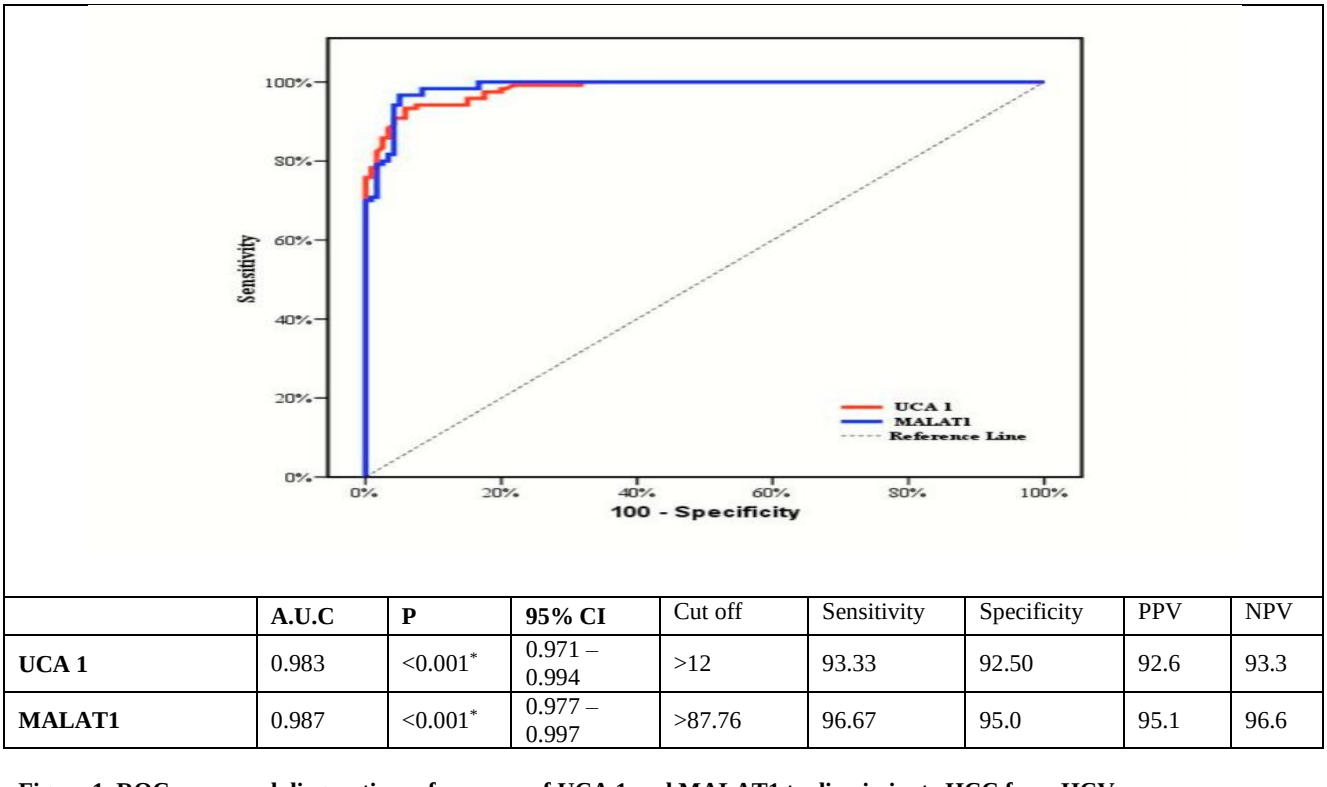
SD: Standard deviation

3.2. The relative expression levels of UCA1 and MALAT1 in studied groups

The values of UCA1 and MALAT1 relative expression levels are shown in table (2). Patients with HCC had considerably higher UCA1 values than the HCV group (24.8 versus 2.34, P < 0.001). Additionally, compared to the HCV group, the HCC patients had noticeably higher serum levels of MALAT1 (223.9 versus 13, P<0.001). Finally, The HCV group's UCA1 and MALAT1 values were notably greater than those of the healthy control group (P < 0.001). Table 3 defines the clinical findings for the patient group with HCC. 51.7% of patients are classified as Child B under the Child PUGH categorization, compared to 32.5% Child A and 15.8% as Child C. Multiple focal lesions are present in one-third of HCC patients. The MELD score's mean value is 11.5. At the end of the study (two-year follow-up period), 35.8% of the patients had died, and the average survival time was 20.4 ± 4.98 months.

3.3. The diagnostic value of MALAT1 and UCA1:

The ROC curve demonstrated that UCA 1 and MALAT1 performed well in terms of diagnostic performance when separating patients with HCC from those with HCV infection, with cutoff values of >12 and >87.79, respectively. UCA1 displayed an AUC of 0.983 with sensitivity of 93.33% and specificity of 92.5%), whereas MALAT1 showed an AUC of 0.987 with sensitivity of 96.67% and specificity of 95.0%. (Figure 1).



	A.U.C	P	95% CI	Cut off	Sensitivity	Specificity	PPV	NPV
UCA 1	0.983	<0.001*	0.971 – 0.994	>12	93.33	92.50	92.6	93.3
MALAT1	0.987	<0.001*	0.977 – 0.997	>87.76	96.67	95.0	95.1	96.6

Figure 1. ROC curve and diagnostic performance of UCA 1 and MALAT1 to discriminate HCC from HCV

Table 2. The expression level of UCA1 and MALAT1 in the studied groups

	HCC treated (n = 120)	HCV (n = 120)	Control (n = 120)	P value	Post Hoc Test
UCA 1 Median (Min. –Max.)	24.8 (4 – 44.9)	2.34 (0.12 – 19.3)	1.0 (0.02 – 2.40)	<0.001*	p1, p2, p3 <0.001*
MALAT1 Median (Min. –Max.)	223.9 (46 – 596.6)	13 (0.02 – 142.2)	1.10 (0.20 – 5.66)	<0.001*	p1, p2, p3 <0.001*

Pairwise comparison between each 2 groups was done using **Post Hoc Test**
p: p value for comparing between the three studied groups
p1: between HCC treated and HCV patients.
p2: between HCC treated patients and healthy control.
p3: between HCV patients and healthy control.
*: Statistically significant at $p \leq 0.05$

Table 3. Distribution of the studied cases according to different parameters in HCC Group

	No. (%)
Number of foci	
Unifocal	78 (65.0%)
Multifocal	42 (35.0%)
Child PUGH	
A	39 (32.5%)
B	62 (51.7%)
C	19 (15.8%)
MELD score	
Mean ± SD.	11.5 ± 3.98
Median (Min. – Max.)	11 (6 – 30)
Ascites	
None	54 (45.0%)
Mild	50 (41.7%)
Moderate	16 (13.3%)
Encephalopathy	
None	24 (20.0%)
Grade I	54 (45.0%)
Grade II	34 (28.3%)
Grade III	6 (5.0%)
Grade IV	2 (1.7%)
Fate	
Live	77 (64.2%)
Dead	43 (35.8%)
Overall survival time (months)	
Mean ± SD.	20.4 ± 4.98
Median (Min. – Max.)	24 (6 – 24)

SD: **Standard deviation**

3.4. Association analysis between clinical, biochemical and molecular markers:

Table 4 shows that, for the HCC group, the expression values of UCA1 demonstrated negligible differences with respect to most criteria, with the exception of child classification, fate, and sorafenib response. By the end of the research, 75% of patients who had low expression levels were responding to sorafenib, and 78.3% of patients were still alive. Notably, high levels of UCA1 expression were linked to 80% sorafenib resistance.

In contrast, patients with higher blood MALAT1 values displayed a substantial increase in serum AFP and total bilirubin as well as lower albumin levels than those with lower MALAT1 values. In terms of clinical findings, patients with lower serum

MALAT1 values have lower MELD scores, no or mild degree of ascites; the majority of these patients are Child A, as Table 5 illustrates. Patients' outcomes and the impacts of sorafenib response were more prominent; 85% of patients with low MALAT1 expression levels responded well to sorafenib and 81.7% of patients were still alive. Of note, 90% sorafenib resistance was correlated with high expression levels of MALAT1.

Table 4. Expression levels of UCA 1 and different parameters in HCC treated patients

	UCA1		Test of Sig	p
	Low expression (n = 60)	High expression (n = 60)		
Total bilirubin (mg/dl)				
Mean ± SD.	1.5 ± 1	1.6 ± 1.2	U= 1731.500	0.719
Median (Min. – Max.)	1.3 (0.6 – 7.1)	1.3 (0.5 – 8)		
ALT (IU/L)				
Mean ± SD.	58.4 ± 27.9	54.6 ± 24.5	U= 1644.500	0.414
Median (Min. – Max.)	55.5 (9 – 156)	51 (15 – 150)		
Albumin (gm/dl)				
Mean ± SD.	3.4 ± 0.5	3.5 ± 0.6	U= 1773.000	0.887
Median (Min. – Max.)	3.4 (2.4 – 5)	3.3 (2 – 5)		
ALP (IU/L)				
Mean ± SD.	120.3 ± 43.3	130.9 ± 68	U= 1666.000	0.482
Median (Min. – Max.)	118 (22 – 202)	127 (22 – 443)		
GGT (IU/L)				
Mean ± SD.	122.8 ± 102.8	176.6 ± 393.1	U= 1732.500	0.723
Median (Min. – Max.)	83.5 (35 – 522)	94.5 (18 – 2250)		
CEA (mg/dl)				
Mean ± SD.	10.4 ± 8.2	12.7 ± 9.5	U= 1567.500	0.222
Median (Min. – Max.)	7.8 (2 – 34)	10.6 (1.2 – 33)		
AFP (ng/ml)				
Mean ± SD.	468.7 ± 764.4	428.4 ± 603.1	U= 1691.500	0.569
Median (Min. – Max.)	36.5 (4.5 – 3130)	116 (3.8 – 2230)		
HCV Ab	60 (100%)	60 (100%)	–	–
Number of foci				
Unifocal	40 (66.7%)	38 (63.3%)	χ^2= 0.147	0.702
Multifocal	20 (33.3%)	22 (36.7%)		
Child PUGH				
A	24 (40%)	15 (25%)	χ^2= 6.340*	0.042*
B	31 (51.7%)	31 (51.7%)		
C	5 (8.3%)	14 (23.3%)		
MELD score				
Mean ± SD.	10.67 ± 2.76	12.25 ± 4.81	U= 1500.500	0.114
Median (Min. – Max.)	10.0 (7.0 – 18.0)	11.0 (6.0 – 30.0)		
Ascites				
None	27 (45%)	27 (45%)	χ^2= 1.320	0.517
Mild	27 (45%)	23 (38.3%)		
Moderate	6 (10%)	10 (16.7%)		
Encephalopathy				
None	13 (21.7%)	11 (18.3%)	χ^2= 2.099	^{MC}p= 0.791
Grade I	26 (43.3%)	28 (46.7%)		
Grade II	16 (26.7%)	18 (30%)		
Grade III	3 (5%)	3 (5%)		
Grade IV	2 (3.3%)	0 (0%)		
Fate				
Live	47 (78.3%)	30 (50%)	χ^2= 10.474*	0.001*
Dead	13 (21.7%)	30 (50%)		
Sorafenib response				
resistant	15 (25 %)	48 (80 %)	χ^2= 10.79*	0.001*
responsive	45 (75%)	12 (20%)		

SD: Standard deviation Statistically significant at $p \leq 0.05$

p: p value for relation between UCA1 expression and different parameters

Table 5. Expression levels of MALAT1 and different parameters in HCC treated patients

	MALAT1		Test of Sig	p
	Low expression (n = 60)	High expression (n = 60)		
Total bilirubin (mg/dl)				
Mean ± SD.	1.2 ± 0.6	1.9 ± 1.3	U= 1015.000*	<0.001*
Median (Min. – Max.)	1.1 (0.5 – 4)	1.5 (0.6 – 8)		
ALT (IU/L)				
Mean ± SD.	53.4 ± 25.9	59.6 ± 26.3	U= 1462.500	0.076
Median (Min. – Max.)	48.5 (9 – 156)	56 (10 – 150)		
Albumin (gm/dl)				
Mean ± SD.	3.6 ± 0.6	3.3 ± 0.5	U= 1260.500*	0.005*
Median (Min. – Max.)	3.5 (2 – 5)	3.2 (2.2 – 4.5)		
ALP (IU/L)				
Mean ± SD.	131.8 ± 58.6	119.4 ± 55.2	U= 1516.000	0.136
Median (Min. – Max.)	120 (22 – 443)	126.5 (22 – 443)		
GGT (IU/L)				
Mean ± SD.	93 ± 84.8	206.3 ± 391	U= 963.000*	<0.001*
Median (Min. – Max.)	73 (18 – 522)	114.5 (19 – 2250)		
CEA (mg/dl)				
Mean ± SD.	10.5 ± 8.3	12.7 ± 9.4	U= 1519.500	0.141
Median (Min. – Max.)	8.3 (1.5 – 32)	9.5 (1.2 – 34)		
AFP (ng/ml)				
Mean ± SD.	297.2 ± 546.6	599.9 ± 776.9	U= 1285.500*	0.007*
Median (Min. – Max.)	32 (3.8 – 2176)	240.6 (4 – 3130)		
HCV Ab	60 (100%)	60 (100%)	–	–
Number of foci				
Unifocal	38 (63.3%)	40 (66.7%)	χ^2= 0.147	0.702
Multifocal	22 (36.7%)	20 (33.3%)		
Child PUGH				
A	29 (48.3%)	10 (16.7%)	χ^2= 15.132*	0.001*
B	26 (43.3%)	36 (60%)		
C	5 (8.3%)	14 (23.3%)		
MELD score				
Mean ± SD.	10.53 ± 3.69	12.38 ± 4.08	U= 1226.000*	0.002*
Median (Min. – Max.)	10.0 (7.0 –30.0)	12.0 (6.0 – 29.0)		
Ascites				
None	35 (58.3%)	19 (31.7%)	χ^2= 10.021*	0.007*
Mild	21 (35%)	29 (48.3%)		
Moderate	4 (6.7%)	12 (20%)		
Encephalopathy				
None	16 (26.7%)	8 (13.3%)	χ^2= 8.626*	^{MC}p= 0.049*
Grade I	28 (46.7%)	26 (43.3%)		
Grade II	11 (18.3%)	23 (38.3%)		
Grade III	3 (5%)	3 (5%)		
Grade IV	2 (3.3%)	0 (0%)		
Fate				
Live	49 (81.7%)	28 (46.7%)	χ^2= 15.983*	<0.001*
Dead	11 (18.3%)	32 (53.3%)		
Sorafenib response				
Resistant	9 (15 %)	54 (90%)	χ^2= 13.19*	0.001*
responsive	51 (85%)	6 (10%)		

SD: Standard deviation Statistically significant at $p \leq 0.05$

p: p value for relation between MALAT1 expression and different parameters

3.5. Expression Levels of UCA1 and MALAT1 in HCV Patients

Supplementary tables 2 & 3 display the association of UCA1 and MALAT1 expression levels with the laboratory parameters in the HCV group. The median values for UCA1 and MALAT1 were lower than the cut-off values obtained from the roc

curve, indicating that most HCV patients had low expression levels of these genes. Notably, the UCA1 expression values showed minimal significant correlation with studied parameters (all p> 0.05). In contrast, higher MALAT1 level significantly correlatedwith most biochemical parameters (P<0.05) except for AFP and albumin levels (p> 0.05). These findings suggest that while UCA1 may have limited prognostic value in this cohort, MALAT1 could be a valuable marker for monitoring biochemical changes in HCV patients, potentially aiding in the early identification of those at risk for progression to HCC.

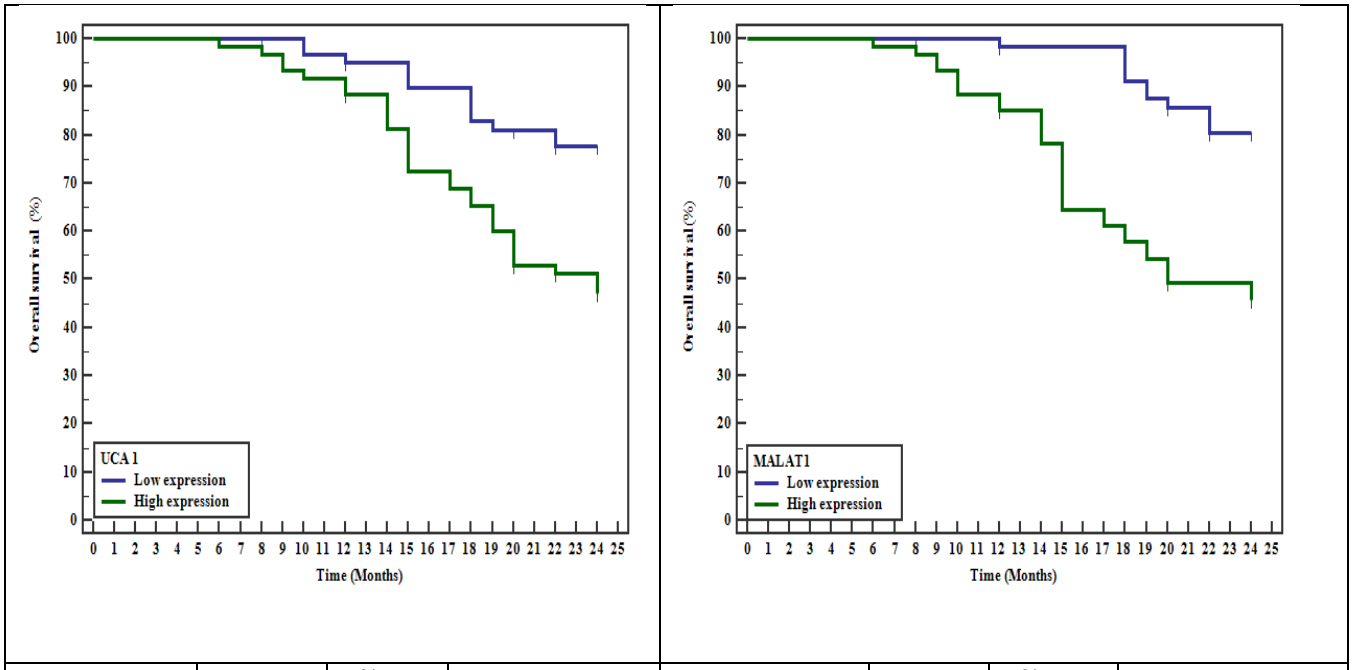
3.6. Role of MALAT1 and UCA1 Expression on the Survival of HCC patients and associated risk factors:

In addition, we monitored the survival of HCC patients for a period of 24 months, and we used Kaplan-Meier survival tests to determine the overall survival. Figure 2 illustrates that HCC patients with greater blood levels of MALAT1 and UCA1 had a shorter overall survival than those with lower serum levels (P=0.001). In an effort to confirm the risk factors affecting HCC patients' survival, we performed a univariate and multivariate analysis. Table 6 presents univariate and multivariate analyses of factors associated with outcomes. In the univariate analysis, significant associations were found for age, female gender, total bilirubin, UCA1, and MALAT1 (all p < 0.05). In the multivariate analysis, only MALAT1 (p < 0.05) remained significant. Non-significant factors included smoking, diabetes, hypertension, Hb level, TLC, platelets, INR, ALT, AST, ALP, AFP, CEA, albumin, direct bilirubin, BUN, and creatinine (all p > 0.05). The hazard ratio (HR) for MALAT1 was significant in both univariate (HR: 1.004, p<0.001) and multivariate (HR: 1.003, p=0.005) analyses. However, UCA1 only showed a significant HR in univariate analysis (HR: 1.055, p=0.001). The hazard ratio (HR) provides a measure of how much the risk of an event increases with each unit increase in the predictor variable.

Table 6. Univariate and multivariate COX regression analysis (demographic, laboratory and clinical parameters)

	Univariate p	HR (LL – UL 95%C.I)	#Multivariate p	HR (LL – UL 95%C.I)
Age (years)	0.**032***	0.967(0.938 – 0.997)	0.075	0.972(0.942 – 1.003)
Gender(female)	**0.046***	0.438(0.195 – 0.985)		
Smoking	**0.604**	1.199(0.604 – 2.379)		
Diabetes	**0.810**	0.925(0.488 – 1.750)		
Hypertension	**0.799**	1.086(0.574 – 2.056)		
Hb level (gm/dl)	**0.934**	0.994(0.859 – 1.150)		
TLC (x103/ul)	**0.404**	0.934(0.797 – 1.096)		
Platelets (x103/ul)	**0.480**	1.002(0.996 – 1.008)		
INR	**0.482**	0.502(0.074 – 3.426)		
ALT (IU/L)	**0.564**	0.997(0.985 – 1.008)		
AST (IU/L)	**0.215**	0.994(0.985 – 1.003)		
ALP (IU/L)	**0.334**	0.997(0.991 – 1.003)		
GGT (IU/L)	**0.049***	0.995(0.990 – 1.0)	0.155	0.997(0.992 – 1.001)
AFP (ng/ml)	**0.268**	1.0(0.999 – 1.0)		
CEA (mg/dl)	**0.242**	1.020(0.987 – 1.053)		
Albumin (gm/dl)	**0.134**	1.486(0.885 – 2.495)		
Total bilirubin (mg/dl)	**0.038***	1.261(1.012 – 1.570)	0.374	1.125(0.868 – 1.459)
Direct bilirubin (mg/dl)	**0.075**	1.412(0.965 – 2.066)		
BUN (mg/dl)	**0.731**	1.005(0.976 – 1.035)		
Creatinine (mg/dl)	**0.466**	1.106(0.843 – 1.453)		
UCA 1	**0.001***	1.055(1.022 – 1.089)	0.111	1.028(0.994 – 1.063)
MALAT1	**<0.001***	1.004(1.002 – 1.006)	0.005*	1.003(1.001 – 1.006)

C.I: Confidence interval LL: Lower limit UL: Upper Limit
HR: Hazard ratio *: Statistically significant at p ≤ 0.05
#: All variables with p<0.05 was included in the multivariate



UCA 1	Mean	% End of study	Log rank		MALAT1	Mean	% End of study	Log rank	
Low expression	22.285	77.6	χ^2	p	Low expression	23.008	80.3	χ^2	p
High expression	19.755	47.2	11.264*	0.001*	High expression	19.113	45.8	17.364*	0.001*

Figure 2. Kaplan-Meier survival curve for overall survival with UCA 1 and MALAAT1 expression levels

4. Discussion

Hepatocellular carcinoma is a leading contributor of death from cancer. Lately, non-invasive lncRNA biomarkers for hepatocellular carcinoma have proven to have prospective roles for aiding in the diagnosis, selection of treatment, and management of cancer recurrence. Several studies have emphasized the significance of TLR4/NF κBsignaling in the initiation of fibrosis. Furthermore, HSCs apoptosis is inhibited and the NF-κBsignaling pathway is kept perpetually active. Inflammatory mediators and adhesion molecules such as monocyte chemoattractant protein-1 were also increased by TLR4/NF κBsignaling [20]. Since liver fibrosis is greatly impacted by the TLR4/NF κB pathway, upstream compounds that alter this pathway should be thoroughly investigated [21]. The current study investigated the noninvasive diagnostic, prognostic and therapeutic utility of circulating MALAT1 and UCA1 in the plasma of HCC patients receiving sorafenib, as well as HCV infected and uninfected controls.

The present study revealed extremely elevated MALAT1 expression values in patients with HCC. This was consistent with a prior cell investigation that reported lncRNA MALAT1 may regulate glucose metabolism in cancer, aerobic glycolysis is known to be a sign of a tumor cell's capacity to evade apoptosis, by elevating the translation of transcription factor TCF7L2 [22, 23]. In hyperglycemia, inflammatory cytokines may also be released due to MALAT1 [24]. Furthermore, our results were strengthened by earlier clinical trials. Toraih et al., 2018 [25] showed that Patients with HCC had higher serum levels of MALAT1 than did cirrhosis patients or controls. Huang et al., 2020 [26] demonstrated that MALAT1 and UCA1 were greatly expressed in HCC patients compared to other hepatic patients or healthy controls. In addition, our results align with a recent 90-person case-control study, which found that patients with HCC had considerably higher serum levels of UCA1 than those with liver cirrhosis and healthy controls [27]. Qin et al., 2018 [28] underscored that UCA1 levels were significantly increased in HCC tissues. Where, it was lower in the former stages compared to later stages and reduction of UCA1 expression levels decreased HCC cell division and apoptosis.

Remarkably, analysis of receiver operating characteristic (ROC) curve in the current study revealed that MALAT1 and UCA1 performed well as diagnostic tools, separating HCC patients from other HCV patients with high sensitivity (96.67% & 93.33%), specificity (95.0% & 92.5%) and AUC (0.987& 0.983) respectively. This finding regarding UCA1 is consistent with

that of Abdelmoety et al., 2023 [27] who reported that using ROC analysis, UCA1 shown strong diagnostic capacity for HCC. (73.3% sensitivity, 100% specificity and 0.9 AUC).

Our results for MALAT1 are in line with those of Li et al., 2023 [29] who discovered that MALAT1 showed good specificity (0.85), sensitivity (0.76), and AUC (0.89) for HCC, indicating good diagnostic accuracy. In addition to its role in HCC, reports have shown the diagnostic and prognostic significance of MALAT1 for several malignancies [30]. lncRNA MALAT1, first identified in 2003, was showed to upregulate TLR4 and NF κB expression in hematopoietic stem cell line. This aggravated the release of inflammatory cytokines and improved TGF β1-induced migration, adhesion, and proliferation when exposed to LPS [21]. Consequently, its diagnostic value lies mainly for differentiating HCC cases from non-cases either HCV patients or control.

In the current study, HCV patients showed greatly higher expression levels of MALAT1 and UCA1 than those in healthy control group. However, most HCV patients had low expression values of these genes and did not show significant correlation with biochemical parameters for UCA1. In contrast, low MALAT1 expression levels in HCV patients were as relevant as they are in HCC regarding the clinical features. It seems that MALAT1 expression levels are more indicative of disease state than UCA1 in HCV infection. It has been reported that both liver cirrhosis (LC) and chronic hepatitis B (CHB) patients had elevated plasma MALAT1 [31]. Furthermore, patients with CHB showed to have higher values of MALAT1 and UCA1 than healthy controls suggesting that they may have a role in the initial stages of liver damage. Toraih et al., 2018 [25] verified the carcinogenic significance of MALAT1 in HCC caused by HCV in their study that was implemented on 70 subjects, in addition to meta-analysis research results.

In the current study, Kaplan-Meier survival curve with relative quantification of both genes and follow-up data showed that Patients with HCC who had high expression levels of MALAT1 and UCA1 had a worse overall survival compared to those with low expressions. This was in line with some earlier experiments [32-34]. Furthermore, elevated serum expression of both MALAT1 and UCA1 was associated with a poor prognosis in clinical or biochemical aspects, according to association analysis conducted in our work. By the end of this study, 78.3% of patients with low expression levels of UCA1 were still alive, compared to 50% of patients with high expression levels. This is because high expression levels of UCA1 were significantly correlated with a higher Child score and fate.

This result is consistent with the findings of Wang et al., 2015 [35] who reported that UCA1 overexpression was linked to poor TNM stage and postoperative survival. Wang et al. further clarified this result by pointing to UCA1 TO's capacity to counteract miR-216b's inhibitory effect on HCC metastasis and cell growth. In parallel, patients whose serum MALAT1 expression was high showed significant increase in AFP levels compared to those with low MALAT1 expression. In terms of clinical findings, patients with low expression levels of MALAT1 had lower MELD scores and are primarily Child A patients compared to those with greater expression levels. This finding is agreed with a recent meta-analysis, which showed that over-expression of MALAT1 may be a predictor of both lymph node and distant metastasis.A larger tumor size and a worse TNM stage had been linked to MALAT1 over-expression. In addition, Li et al., 2018 [34] have shown that MALAT1 positively controls the production of transcription factor II, B related factor 2 (BRF2), which has been linked to a poor prognosis for HCC recurrence.

Sorafenib resistance in hepatocellular carcinoma was inevitable and might be attributed to a variety of signaling mechanisms[3,17]. Nonetheless, a large body of research has demonstrated the crucial role that non-coding RNAs, particularly miRNAs and lncRNAs, play in controlling HCC resistance to sorafenib via several signaling pathways. This suggests that ncRNAs may be novel prognostic markers and offer intriguing therapeutic options in the context of sorafenib resistance [5]. According to the current investigation, a strong correlation existed between high serum expression of MALAT1 and UCA1 and sorafenib resistance in many HCC patients (90% and 80%, respectively).

This result is consistent with that of Huang et al., 2021 [32]who reported that the downregulation of miR-138. 5p and the stimulation of the AKT-mTOR signaling route by UCA1 in HCC cells accounted for the molecular basis of such resistance. However, earlier studies revealed that several lncRNAs, such as UCA1 and sorafenib resistance associated lncRNA in renal cell carcinoma (SRLR) may be involved in sorafenib resistance [36, 37].

Similarly, former cohort studies demonstrated that the suppression of MALAT-1 and HOTAIR (HOX antisense intergenic RNA) exerted important role in improving HCC chemotherapeutic sensitivity suggesting them as a promising treatment target option for HCC [38,39]. Parallel to this, Lai et al., 2012 [39] established a connection between chemoresistance and MALAT1 overexpression. Invitro studies in HepG2 cells treated with doxorubicin or cisplatin demonstrated a higher proportion of apoptotic cells when treated with small interfering RNA (siRNA) against MALAT1. The matter that reduced cell growth and hindered cell migration.

In the current study, the hazard ratio (HR) for MALAT1 was significant in both univariate and multivariate analyses, indicating that higher levels of MALAT1 are associated with a higher risk of adverse outcomes in HCC patients. In contrast, while UCA1 showed a significant HR in univariate analysis, it did not maintain significance in multivariate analysis, suggesting that its prognostic value is influenced by other factors. This is in line with the findings of Zhang et al., 2019 [40]

who used univariate analysis to show a robust association between low overall survival and high expression of UCA1 and GGT levels in primary hepatic carcinoma. This outcome is also consistent with a Zhao et al., 2022 [41] who used multivariate analysis to indicate that increased expression of MALAT1 has a substantial potential prognostic value for HCC. This highlights the relative risk associated with higher levels of MALAT1 and UCA1, suggesting that patients with elevated levels of these markers are at a significantly higher risk of adverse outcomes.

Finally, it is important to recognize that this study had a few limitations: the study's sample size, comprising 240 patients and 120 healthy individuals, may not be sufficient to generalize the findings across broader populations. Additionally, the grouping of patients into those with HCV and those with HCC treated with Sorafenib might not account for other variables or comorbidities that could influence the outcomes. This limitation suggests the need for larger, more diverse cohorts to validate the results and ensure they are representative of the general population.

5. Conclusions

This study demonstrates the promising role of MALAT1 and UCA1 as non-invasive biomarkers in the diagnosis and prognosis of HCC. Their potential as targets for lncRNA-based therapeutics is highlighted by the correlation between their higher expression levels and poor survival as well as resistance to sorafenib treatment. Future clinical investigations are warranted to augment our finding and to give more evidences for actual application of lncRNAs competing this fatal illness.

6. List of Abbreviations

AFP: Alpha-fetoprotein, ALP: Alkaline phosphatase, ALT: Alanine aminotransferase, AST: Aspartate amino transferase, CBC: Complete blood count, cDNA: Complementary DNA, CEA: Carcinoembryonic antigen, D Bil: direct bilirubin, ECL: Electrochemiluminescence, GGT: gamma glutamyl transferase, Hb: Hemoglobin, HCC: Hepatocellular carcinoma, HOTAIR: HOX antisense intergenic RNA, HSCs: Hematopoietic stem cells, INR: international normalized ratios, Mcl 1, Myeloid cell leukemia-1, MELD: Model for End stage liver disease, NASH: Nonalcoholic steatohepatitis, NFκB: nuclear factor κB, RECIST: Response evaluation criteria in solid tumors, ROS: Reactive oxygen species, RT: Reverse transcription, T Bil: Total bilirubin, TLC: Total leukocytic count, TLR4: Toll-like receptor 4.

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8. Conflict of interest: There aren't any competing interests.

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