Assessment expression of Serum Amyloid A and LncRNAAF085935 in patients with Hepatocellular Carcinoma, Hepatitis B and Hepatitis C Virus

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Original Article

ABSTRACT

Long non coding RNAs (Lnc RNAs) and serum Amyloid A (SAA) have been suggested to have a role in hepatocarcinogenesis. In this work, to investigated the role of lnc RNA AF085935 and SAA as diagnostic biomarkers in sera of HBV, HCV, HCC patients. Subjects and Methods: HBV genotyping was assessed by. Lnc RNAAF085935 quantitative gene expression in whole blood by real time PCR and the SAA expression was measured using ELISA. 149 adult Egyptian subjects were divided into 35 HCC patients on top of HCV and 24 HCC patients on top of HBV, 35patients with HBV, 35 patients with HCV and 20 controls. Results: HBV genotype D was found in all of the samples. In HCC patients, HCV patients, and HBV patients; the levels of LncRNA-AF085935 and SAA were significantly upregulated than in healthy controls. (p value = <0.001). In addition, higher expressions of both LncRNA-AF085935 and SAA (p value = <0.001) were observed in HCC/HBV and HCC/HCV patients than in HCV or HBV patients. Conclusion: Lnc RNA-AF085935 and SAA were observed with an aberrant level in sera of HCC, HCV and HBV patients. Lnc RNA-AF085935 and/or SAA can be considered to be used as potential biomarkers in HCC.

Key Words: Hepatitis B , Hepatitis C Virus , Hepatocellular Carcinoma , LncRNAAF085935, Serum Amyloid .

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the world's fifth most frequent malignancy and the third most common cause of cancer-related mortality [1]. with a male/female predominance of about 3:1 [2].

Cancer is widely recognized to develop in chronically inflamed tissue. The majority of HCC patients have preexisting chronic inflammatory liver disease, and liver cirrhosis is the major cause of HCC [3].

The release of cytokines and growth factors within the parenchyma and surrounding extracellular matrix (ECM), as well as chronic infection by hepatitis viruses, especially chronic infection by hepatitis B virus (HBV) and hepatitis C virus (HCV), leads to a complex micro - environment. The acute phase response is a nonspecific response to local or systemic abnormalities such as inflammation, tissue injury, immunological problems, or tumor development[4].

Serum amyloid A (SAA) is generated by the hepatocytes, as a major positive acute-phase reactant.

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SAA attracts immune cells such as monocytes, polymorph nuclear leukocytes, mast cells, and T-lymphocytes to the inflammatory sites by acting as a chemoattractant [5].

Inflammatory cytokines such as interleukin-1, interleukin-6, and tumor necrosis factor (TNF-) are the main regulators of SAA induction. As a consequence, SAA is involved in a variety of inflammatory processes and serves as a connection between immunity, inflammation, and cancer formation. Furthermore, SAA has been implicated in carcinogenesis in a number of studies, due to the ability to interact with extracellular matrix, which has a major effect on tumor onset and growth. Serum SAA levels were increased in patients with lung cancer [6], colorectal cancer[7] and renal cell carcinoma[8].

Recent study research suggest that elevated SAA levels and c- reactive protein (CRP) in the blood not only indicate the presence of cancer, but may also be linked to an increased risk of cancer development and progression[6].

Lnc RNAs are a class of newly found non-coding RNA that has recently been discovered. They are classified as transcribed RNA molecules with a length of more than 200 nucleotides [9].

Although the role of most lnc RNAs is unknown, growing evidence from community research findings has indicated their crucial roles in a variety of biological processes, including chromatin remodeling, transcriptional co-activation or co-repression, protein inhibition and posttranscriptional modification, or dealing as decoy aspects[10].

H19, HOTAIR, and MEG3 are types of Lnc RNAs that have been hypothesised to play a role in carcinogenesis and tumor growth. In HCC, it was discovered that H19 expression acts as an oncogene, which can promote cancer cell growth [11].

Previous studies have reported that Lnc RNAs are elevated in liver cancer [1], suggesting that LncRNA AF085935 expression in HCC could be employed as a predictive biomarker[12].

AIM OF THE STUDY

The aim of the present work was to assess the level of Serum Amyloid A and lncRNA-AF085935 in Hepatocellular Carcinoma, hepatitis B virus and hepatitis C virus patients compare to healthy subjects, in addition to find correlation among different groups.

PATIENTS AND METHODS

Patients

Our study included 149 adult Egyptian individuals. All of the participants were recruited between June 2022 and February 2023 from endemic medicine and gastroenterology department. Faculty of Medicine Cairo University.

Subjects were divided into five groups: group (I): 20 healthy volunteered for the study and served as controls, group (II): 35 HCV infected patients, group (III): 35 HBV infected patients, group (IV): 35 patients with HCC on top of HCV infection, Group (V): 24 patients with HCC on top of HBV infection. HCV patients' inclusion criteria were positive HCV antibodies, detectable HCV-RNA and they were never treated with interferon before. HBV patients' inclusion criteria were positive HBV surface markers, detectable HBV-DNA and they were never treated before their enrollment in the study. All HCC patients were selected from HCC unit of the Endemic Medicine and Gastroenterology Department, Kasr El-Ainy Hospital, Cairo University. Patients were diagnosed as HCC according to physical examination, radiological findings (including unequivocal clinical and imaging information, ultrasonic CT scanning); histological diagnosis (defined by means of percutaneous biopsy) and laboratory findings (total and direct bilirubin, transaminases, alkaline phosphatase, albumin, prothrombin time and concentration, AFP levels). 20 healthy subjects, age and sex matched were included as a control group; they had normal values of serum alanine aminotransferase (ALT) and were seronegative for hepatitis B surface markers and HCV antibodies.

The study was authorized by the ethical committee at Kasr Al-Ainy Hospitals in accordance with the ethical guidelines of Declaration of Helsinki. consent form was taken from all eligible individuals.

All participants were performed to thorough clinical examination, history taking and complete routine laboratory findings such as liver function tests, kidney function tests, markers of chronic hepatitis virus: (Anti-HCV and HBsAg), HCV-RNA, HBV–DNA, thyroid function tests, autoantibodies, prothrombin time (PT), prothrombin concentration (PC), international normalized ratio (INR), random blood glucose and estimation of Alpha Fetoprotein in serum.

Sample collection

5 ml venous blood was withdrawn and divided as following; a) 2 ml were collected in sterile citrate vacutainer to assess PT, PC and INR, b) 1 ml were collected on EDTA vacutainer tubes for assessment of RNA extraction and qRT-PCR of lncRNA-AF085935gene expression and c) the reminder 2 ml were collected into sterile vacutainer tube with gel left to clot, centrifuged at 3000xg for 10 minutes and then the sera were separated for the blood biochemical analysis and serum Amyloid A level assessment by ELISA.

HBV Genotyping

The Alto Star® HBV PCR Kit 1.5 (Altona Diagnostics, GmbH, Hamburg, MAN-AS0201510-EN-S03) is

diagnostic test, based on real-time PCR technique, for the quantification of human HBV specific DNA (genotypes A to H) in human blood. Briefly, the steps were summarized as following: DNA Purification using the Gene JET Whole Blood Genomic DNA Purification Mini Kit, Cat No. (K0781) followed by Real-time PCR (applied biosystem using the Alto Star® HBV PCR Kit 1.5).

HBV DNA Sequencing

The HBV genotyping Sequencing assay targets the preS1/preS2 regions of the HBV viral genome. Sequencing (Applied Biosystems 4 and 16 capillary systems. ABI SeqScape Software version 2.5 or later). HBV PCR products were purified using Nucleospin Extract II columns (Centri-sep) and sequenced using the bigdye terminator v3.1 kit (life technologies). After purification sequences of amplified nucleic acids were determined using an ABI 310 sequencer.

(Life technologies) and the nucleotide sequences analyzed with SeqScape v2.6 software (life technologies). Nucleotide sequences of the polymerase region were compared with reference strains representing each of the genotypes A-H obtained from GenBank. Genotyping of HBV was then determined by blast analysis and full genotypes were used as references.

ELISA

Serum Amyloid (A) ELISA kit was provided by bioneovan co. ltd, Daxing industry zone, Beijing, China. SAA was expressed as (ng/ml) regarding to manufactures protocol.

RNA extraction and Real time PCR

RNA was extracted and purified from the whole blood of all enrolled subjects. The kit was provided by Thermo Fisher Scientific Inc. Germany. (GeneJET,Kit,#K0732) according to manufactures instructions.

qRT-PCR was applied by kit named Bioline, a median life science company, UK (SensiFAST™ SYBRR Hi-ROX One-Step Kit, Cat No. PI-50217 V). Primer sequence for the studied target genes Lnc RNA-AF085935 and housekeeping gene (18sRNA) were shown in Table (1). RT-PCR thermal profile was programmed in Step One instrument (Applied Biosystem, USA) as follows: 15 minutes at 45ºC for one cycle, 10 minutes at 95 ºC for Taq polymerase activation followed by 40 cycles of 15 seconds at 95ºC, 20 seconds at 55 ºC and 30 second at 72 ºC for the amplification step. After the RT-PCR run the data were expressed in Cycle threshold (Ct). The PCR data sheet includes Ct values of assessed target gene (lncRNA-AF085935) and the house keeping (reference) gene (18rsRNA). The relative quantitation (RQ) of each target gene is quantified by the calculation of delta-delta Ct (ΔΔCt).

Table (1): Primer sequences used in the study

Gene	Primer sequence from 5′- 3′	Gene bank accession number
LncRNA-AF085935	F: CAGGGCAGCAAGGTGTTTTC R: TTGGTGGGTTGCCTGATACC	NG009286.1
18sRNA	F: CAGCCACCCGAGATTGAGCA R: TAGTAGCGACGGGCGGGTGT	JX132355.1

Statistical analysis

The statistical tool SPSS version 24 was used to analyze the data. In quantitative data, mean, standard deviation, median, minimum, and maximum were used to describe the data. The non-parametric Kruskal-Wallis and Mann-Whitney tests were used to compare quantitative variables. The Spearman correlation coefficient was used to calculate correlations between quantitative variables. The best cutoff value of long noncoding RNA and serum amyloid for predicting cancer cases was achieved using a ROC curve and area under curve analysis. Statistical significance was considered as a P-value of less than 0.05.

RESULTS

Comparison between mean values ± SD of some demographic and biochemical data among control, HCV and HCC+HCV groups. The levels of AST (45.37 ±15.45) and ALT (52.66 ±10.42) in HCV patients were significantly higher than control group and HCC+HCV group. The level of AFP in HCC+HCV patients (4661.98±21406.84) was significantly higher than control group (5.14± 2.99) and HCV group (3.35±2.18). The level of T-bilirubin (0.97± 0.26) in HCC+HCV patients was significantly higher than control group as level of T-bilirubin (0.84±0.21) and HCV group as level of T-bilirubin (0.74±0.17). The level of D-bilirubin, albumin, alkaline phosphatase, prothrombin concentration and creatinine in HCC+HCV patients, control group and HCV group showed no statistically significant difference. The level of hemoglobin (11.13± 1.33) in HCC+HCV patients was significantly lower than control group as level of hemoglobin (14.06±0.93) and HCV group as level of hemoglobin (13.52±0.96). The level of TLC (3.89± 0.83) in HCC+HCV patients was significantly lower than control group as level of TLC (6.38±1.90) and HCV group as level of TLC (6.43±1.95). The level of Platelets count (199.29± 75.42) in HCC+HCV patients was significantly lower than control group as level of platelets count (279.65±88.70) and HCV group as level of platelets count (276.54±103.47). The level of HCV RNA (484297.97± 414098.89) in HCC+HCV patients was higher than the level of HCV RNA in HCV group which was (443986.34± 373743.00) with no statistically significant difference (p value =0.618). Table (2).

Table (2): Age distribution and laboratory data among control, HCV and HCC/ HCV groups

Variable	Group						
	control group		HCV group		HCC/HCV group		P value
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	
AST(U/L)	30.25	6.12	45.37	15.45	40.66	14.43	< 0.001*
ALT(U/L)	24.50	7.47	52.66	10.42	51.51	10.47	< 0.001*
AFP(ng/ml)	5.14	2.99	3.35	2.18	4661.98	21406.84	< 0.001*
T. bilirubin(mg/dl)	.84	.21	.74	.17	.97	.26	< 0.001*
D. bilirubin(mg/dl)	.23	.09	.23	.11	.29	.17	0.149
Albumin(g/dl)	4.27	.61	4.04	.49	4.15	.51	0.328
ALK Phos(U/L)	73.60	31.38	70.51	25.17	76.37	30.53	0.667
Creatinine(mg/dl)	.89	.35	.90	.29	.94	.33	0.801
RBS	88.50	16.24	87.86	20.91	95.29	47.08	0.944
PC(%)	.92	.07	.88	.07	.85	.12	0.104
INR	1.05	.19	1.07	.11	1.07	.11	0.874
HB(g/dl)	14.06	.93	13.52	.96	11.13	1.31	< 0.001*
TLC	6.38	1.90	6.43	1.95	3.89	.83	< 0.001*
Platelets count	279.65	88.70	276.54	103.47	199.29	75.42	< 0.001*
BMI	28.45	2.52	27.63	2.10	.	.	0.216
HCV RNA (IU/ml)	.	.	443986.34	373743.00	484297.97	414098.89	0.618
HBV PCR(IU/ml)	.	.	.	.	.	.	.

Data were reported as Mean ± SD, and p value < 0.05 was considered significant.
(*) Denotes a statistical significant difference
T-bilirubin: total bilirubin, D-bilirubin: direct bilirubin, AST: aspartate transaminase, ALT: alanine transaminase, ALKPhos: alkaline phosphatase, RBS: Random blood sugar, PC: prothrombin concentration, AFP: alpha fetoprotein, HB: Hemoglobin, TLC: Total leucocytes count, BMI: Body mass index

The gender (M/F) of HCC+HCV patients (31/4), HCV patients (26/9) & controls (13/7), showed no statistically significant difference from each other (p value > 0.05). Score A of child score for patients with HCC+HCV represents the highest percentage among the group (65.7%) followed by score B (31.4%) then score C represents lowest percentage (2.9%) as shown in table (3)

Table (3): Sex distribution and child score level among control, HCV and HCC/HCV groups

Variable		Group						
		control group		HCV group		HCC/HCV group		P value
		Count	%	Count	%	Count	%	
Sex	Female	7	35.0%	9	25.7%	4	11.4%	0.111
	Male	13	65.0%	26	74.3%	31	88.6%	
Child score	A	0	.0%	0	.0%	23	65.7%	---
	B	0	.0%	0	.0%	11	31.4%	
	C	0	.0%	0	.0%	1	2.9%	

Comparison between mean values ± SD of some demographic and biochemical data among control, HBV and HCC+HBV groups. The levels of AST (48.71 ±15.52) and ALT (56.66 ±12.87) in HBV patients were significantly higher than control group as levels of AST (30.25±6.12) and ALT (24.50 ±7.47) and HCC+HBV group as levels of AST (35.83±11.43) and ALT (44.83±18.31). The level of AFP in HCC+HBV patients (1248.24±3272.48) was significantly higher than control group (5.14± 2.99) and HBV group (4.73±2.14). The level of alkaline phosphatase (81.48± 29.09) in HCC+HBV patients was significantly higher than control group as level of alkaline phosphatase (73.60±31.38) and HBV group as level of alkaline phosphatase (62.60±23.54). The level of T-bilirubin, D-bilirubin, Albumin and creatinine in HCC+HBV patients, control group and HBV group showed no statistically significant difference. The level of prothrombin concentration (82± 13) in HCC+HBV patients was significantly lower than control group as level of prothrombin concentration (92±7) and HBV group as level of prothrombin concentration (89±8). The level of hemoglobin (10.85± 1.19) in HCC+HBV patients was significantly lower than control group as level of hemoglobin (14.06±0.93) and HBV group as level of hemoglobin (12.05±1.06). The level of TLC (4.25± 1.19)

in HCC+HBV patients was significantly lower than control group as level of TLC (6.38±1.90) and HBV group as level of TLC (5.08±1.71). The level of platelets count (177.48± 72.81) in HCC+HBV patients was significantly lower than control group as level of Platelets count (279.65±88.70) and HBV group as level of platelets count (210.97±63.33). The level of HBV DNA (3728935.74± 5412325.82) in HBV patients was higher than the level of HBV DNA in HCC+HBV group which was (1198916.78± 989786.74) with a statistical significant difference (p value =0.037) as shown in table (4).

Table (4): Age distribution and laboratory data among control, HBV and HCC/HBV groups

	Groups						
P value	HCC/HBV group		HBV group		Control group		Variable
	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	Mean	
< 0.001*	11.43	35.83	15.52	48.71	6.12	30.25	AST (U/L)
< 0.001*	18.31	44.83	12.87	56.66	7.47	24.50	ALT(U/L)
< 0.001*	3272.48	1248.24	2.14	4.73	2.99	5.14	AFP(ng/ml)
0.217	.22	.95	.17	.93	.21	.84	T. bilirubin(mg/dl)
0.41	.13	.26	.07	.22	.09	.23	D. bilirubin(mg/dl)
0.921	.56	4.21	.58	4.20	.61	4.27	Albumin(g/dl)
0.048*	29.09	81.48	23.54	62.60	31.38	73.60	ALK Phos(U/L)
0.668	.30	.97	.28	.91	.35	.89	Creatinine(mg/dl)
0.837	15.17	90.87	44.09	99.00	16.24	88.50	RBS
0.025*	.13	.82	.08	.89	.07	.92	PC(%)
0.318	.13	1.12	.11	1.08	.19	1.05	INR
< 0.001*	1.19	10.85	1.06	12.05	.93	14.06	HB(g/dl)
0.001*	1.19	4.25	1.71	5.08	1.90	6.38	TLC
< 0.001*	72.81	177.48	63.33	210.97	88.70	279.65	Platelets count
.	.	.	.	.	2.52	28.45	BMI
.	.	.	.	.	.	.	HCV RNA(IU/ml)
0.037*	989786.74	1198916.78	5412325.82	3728935.74	.	.	HBV PCR(IU/ml)

Data were reported as Mean ± SD, p value <0.05 was significant
(*) Denotes a statistically significant difference

The gender (M/F) of HCC+HBV patients (18/6), HBV patients (31/4) & controls (13/7), showed no statistically significant difference from each other (p value > 0.05). Score C of child score for patients with HCC+HBV represents the highest percentage among the group (43.5%) followed by score A (30.4%) then score B represents lowest percentage (26.1%) as shown in table (5)

Table (5): Sex distribution and child score level among control, HBV and HCC/HBV groups

Variable		Groups						
		control group		HBV group		HCC/HBV group		P value
		Count	%	Count	%	Count	%	
Sex	Female	7	35.0%	4	11.4%	6	25%	0.117
	Male	13	65.0%	31	88.6%	18	75%	
Child score	A	0	.0%	0	.0%	7	30.4%	---
	B	0	.0%	0	.0%	6	26.1%	
	C	0	.0%	0	.0%	10	43.5%	

p value <0.05 means statistically significant difference

Lnc RNA AF085935 expression level in HCC+HCV patients (11.74±2.77) was significantly higher than control group (2.52± 1) and HCV group (3.66±1.26) with p value < 0.001 as shown in figure (1).

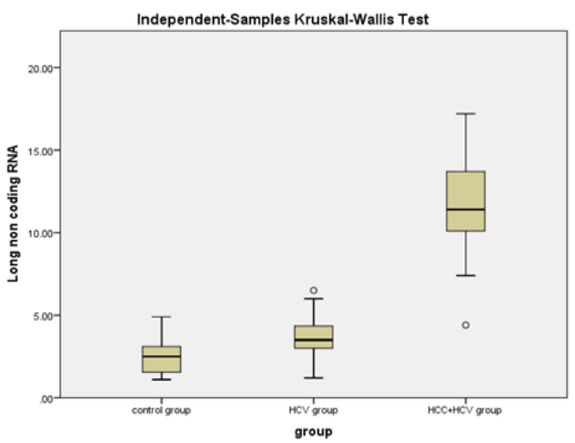

Fig. (1): Lnc RNA AF085935 gene expression level among control, HCV and HCC/HCV groups.

The SAA level in HCC+HCV patients (3.15±1.06) was significantly higher than control group (1.38± 0.68) and HCV group (2.73±1.08) with p value < 0.001

Lnc RNA AF085935 expression level in HCC+HBV patients (22.76±4.81) was significantly higher than control group (2.52± 1) and HBV group (14.49±3.24) with p value < 0.001 as shown in figure (2).

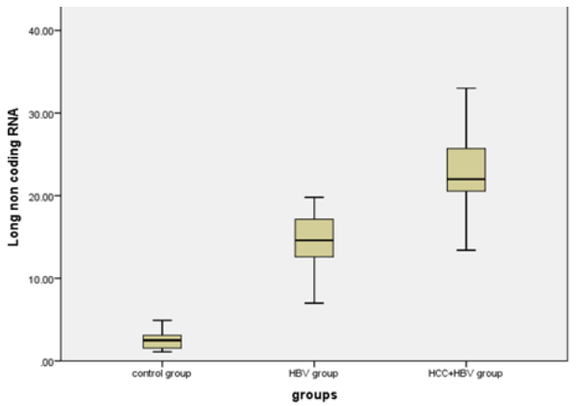

Fig. (2): Lnc RNA AF085935 expression level among control, HBV and HCC/HBV groups.

The SAA level in HCC+HBV patients (4.22±0.76) was significantly higher than control group (1.38± 0.68) and HBV group (3.55±1.05) with p value < 0.001 as shown in figure (3). HBV genotype D was found in all of the samples with DNA gene sequence of mutant genotype D1 HBsAG PreS1 region aligned at Blast gene bank website as shown in figure (4).

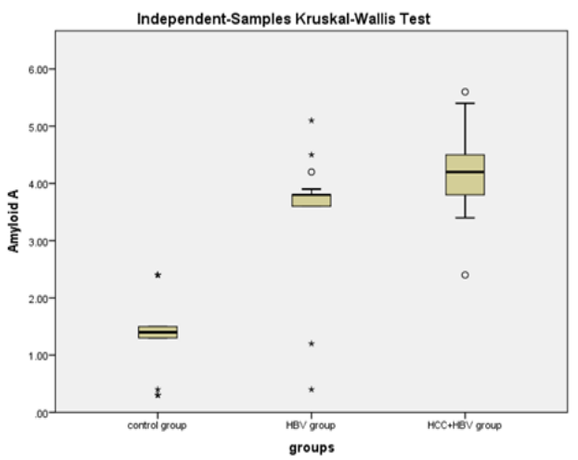

Fig. (3): Level of SAA among control, HBV and HCC/HBV groups.

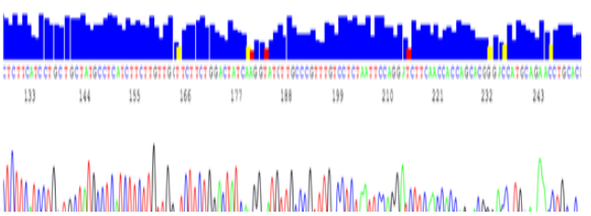

Fig. (4): DNA gene sequence of mutant genotype D1 HBsAG PreS1 region aligned at Blast gene bank website.

Table (6a) represents diagnostic accuracy of Lnc RNA AF085935 as a tumor biomarker, the chosen cut-off level for long non coding RNA AF085935 expression level was (4.15). ROC curve analysis for plasma level of long non coding RNA AF085935 had significantly high predictive accuracy (AUC= 0.999, CI = 0.996–1.002, sensitivity =100% and specificity =95%). Table (6b) represents diagnostic accuracy of SAA as a tumor biomarker, the chosen cut-off level for SAA expression level was (2.9). ROC curve analysis for plasma level of SAA had significantly high predictive accuracy (AUC= 0.927, CI = 0.858–0.997, sensitivity =87.8% and specificity =87.8%). Table (6c) represents diagnostic accuracy of SAA in HCC patients with HBV, the chosen cut-off level for SAA expression level was (3.85). ROC curve analysis for plasma level of SAA had significantly high predictive accuracy (AUC= 0.740, CI = 0.587–0.894, sensitivity =69.6% and specificity =78.9%). Table (6d) represents diagnostic accuracy of Lnc RNA AF085935 in HCC patients with HBV, the chosen cut-off level for Lnc RNA AF085935 expression level was (20.15). ROC curve analysis for plasma level of Lnc RNA AF085935 had significantly high predictive accuracy (AUC= 0.931, CI = 0.862–1, sensitivity =78.3% and specificity =100%).

Table (6) Diagnostic accuracy of Lnc RNA AF085935 and SAA in different groups

a	Area under curve	P value	95% Confidence Interval		Cutoff value	Sensitivity (%)	Specificity (%)
		Lower Bound	Upper Bound				
	.999	<0.001*	996.	1.002	4.15	100	95
b	Area under curve	P value	95% Confidence Interval		Cutoff value	Sensitivity (%)	Specificity (%)
			Lower Bound	Upper Bound			
	.927	<0.001*	.858	.997	2.9	87.8	100
c	Area under curve	P value	95% Confidence Interval		Cutoff value	Sensitivity (%)	Specificity (%)
		Lower Bound	Upper Bound				
	.740	<0.001*	.587	.894	3.85	69.6	78.9
d	Area under curve	P value	95% Confidence Interval		Cutoff value	Sensitivity (%)	(%) Specificity
		Lower Bound	Upper Bound				
	.931	<0.001*	.862	1.000	20.15	78.3	100

DISCUSSION

Although the clinical diagnostic performance of AFP is insufficient due to its limited sensitivity and specificity and there is a critical need to find more HCC-specific biomarkers. AFP can still be used in the surveillance of HCC for its high sensitivity value [13].

Some studies had identified other potential biomarkers for screening of HCC and HBV including the circulating lncRNA-AF085935 [12] and new prognostic biomarkers of HCC including the circulating SAA [7].

Other recent study has succeeded using lncRNAs as biomarkers in the descriptive of serious disease especially in carcinoma. Unlike mRNAs, lncRNAs are effector molecules themselves and can also be detected in body fluids which makes them an attractive diagnostic target. LncRNAs, such as H19, HOTAIR, and MEG3, have been suggested to have a functional role in tumor genesis and tumor progression [14].

Comparing HCV with HCC/HCV as regards the genes expression of lncRNA-AF085935, the present finding demonstrated that there is a significant elevation of lncRNA-AF085935gene expression level in HCC/HCV patients (11.74±2.77) than control group (2.52± 1) and HCV group (3.66±1.26) with p value < 0.001.

In comparing HBV with HCC/HBV as regards the genes expression of lncRNA-AF085935by real time qPCR amplification, the present results revealed that there is a significant elevation oflncRNA-AF085935gene expression level in HCC+HBV patients (22.76±4.81) than control group (2.52± 1) and HBV group (14.49±3.24) with p value < 0.001.

Through the genetic expression profile and qRT-PCR. Lu et al. (2015) [12] have shown similar results to our work regarding to serum level of lncRNA-AF085935in HCC and HBV patients. The study showed that the levels of serum lncRNA-uc003wbd and lncRNA-AF085935 were significantly elevated in HCC patients with HBV infection compared with those in normal controls.

Other studies have displayed several lncRNAs that are associated with HCC. Such as, HULC (Highly upregulated

in Liver Cancer) is expressed at elevated levels in HCC and colorectal carcinomas that have metastasized to the liver[15, 16].

Other, less well-known lncRNAs have also been studied in the detection of HCC. Tang et al. (2015) [17] found upregulation of RP11-160H22.5, XLOC 014172, and LOC149086 transcripts in the plasma of HCC patients compared to cancer-free controls, and found that merging the three lncRNAs yielded better HCC diagnosis scores than each individual lncRNA, with a merged AUC of 0.896, a sensitivity of 82 %, and a specificity of 73 %

Furthermore, the lncRNAs XLOC 014172 and LOC149086 had predictive value for metastasis prediction; these lncRNAs were able to discriminate HCC patients with metastasis from those who did not, with a sensitivity and specificity of 91% and 90%, respectively (AUC = 0.934) [17].

Comparing HCV with HCC/HCV as regards the SAA level, the present results revealed that there is a significant elevation of SAA level in HCC+HCV patients (3.15±1.06) than control group (1.38± 0.68) and HCV group (2.73±1.08) with p value < 0.001.

And Comparing HBV with HCC+HBV as regards the SAA level, the present results revealed that there is a significant elevation of SAA level in HCC+HBV patients (4.22±0.76) than control group (1.38± 0.68) and HBV group (3.55±1.05) with p value < 0.001.

Ni et al. (2014) [7] reported similar findings to the current study in terms of serum SAA levels in HCC patients, finding that levels of SAA were significantly higher in HCC patients than in patients with benign liver lesions, and that there were strong correlations between presurgical serum SAA level and tumor size.

Another study performed immune-histochemical staining to detect the role of SAA in hepatitis. Biopsy samples with little fibrosis and/or mild systemic inflammation stain more than those with cirrhosis (P= 0.001) or at least moderate inflammatory activity (P 0.001). This suggests that liver biopsy specimens with mild chronic hepatitis, initial fibrosis, and normal serum transaminases had higher serum amyloid levels. When compared to subjects with greater inflammatory activity and fibrosis, immune- positivity was observed [18].

Recent studies showed association between SAA and different types of cancer. As in [19] it was found that CRP and SAA values were considerably greater in both serum and pleural effusion of cancer vs. non-malignant group (P < 0.003). A statistically significant positive correlation between pleural fluid CRP and pleural fluid SAA in malignant and non-malignant effusions was found (r = 0.315 and P = 0.002 respectively).But in contrast to our study [20] reported that among patients with hepatitis C and hepatitis B virus patients SAA stay normal.

CONCLUSION

The level of lncRNA-AF085935 and SAA can be possibly used as a clinical utility for detecting HCC, HCCV and HBV. Lnc RNA AF085935 and SAA were high and significantly upregulated compared to healthy subjects. In addition, higher expressions of lncRNA-AF085935 and SAA were found in HCC patients than in HCV and HBV patients.

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