LONG NON-CODING RNA *H19* AS POTENTIAL BIOMARKER FOR HCV GENOTYPE 4 INDUCED HEPATOCELLULAR CARCINOMA PATIENTS

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ABSTRACT

The H19 gene is an oncofetal RNA expressed during embryo development and in several types of cancer. However, little is known about the role of the plasma *H19* in liver cancer diagnosis.

The current study aimed at measuring the plasma levels of long non-coding RNAs (H19) expression in chronic liver disease (CLD) due to HCV genotype 4 infections with/without cirrhosis and Hepatocellular carcinoma (HCC) patients in an attempt to evaluate the potential benefits of these new circulating, noninvasive, diagnostic, prognostic and epigenetic markers for liver cirrhosis and carcinogenesis of Egyptian patients.

A hundred subjects were included in this study, divided into two groups; Group I (50 patients) were classified into subgroup Ia (CLD without cirrhosis, n=25) and subgroup Ib (CLD with cirrhosis, n=25), Group II (CLD patients with HCC, n=25), and control (Healthy volunteer, n=25). The expression of lncRNAs (H19) genes was analyzed by Real-Time PCR.

LncRNAs (H19) showed upregulation in all diseased groups, which was in consistence with the progression of the disease toward the HCC stage. In addition, H19 showed a diagnostic ability to discriminate between cases of cirrhosis and HCC compared to healthy control ($p < 0.001$), while it did not show a discrimination significance differences between cirrhotic cases and non cirrhotic cases. By using receiver operating

characteristic curve (ROC) analysis, it was found that H19 could diagnose HCC with AUC (81.4%). The increased *H19* expression was associated with advanced tumor stages and higher grades.

Plasma level of *H19 marker* might serve as a potential non-invasive biomarker for diagnosis of HCC.

keywords: Biomarker, Cirrhosis, Epigenetic**,** Chronic liver disease, Hepatocellular carcinoma

INTRODUCTION

Hepatitis C virus (HCV) infection is a major global public health problem, costing billions per year in medical expenses ***(Cox, 2015)***. Although effective anti-HCV treatment was developed and put in to clinical use, the lack of preventive approaches and the recessive nature of the disease and its development result in a large number of patients who are diagnosed at late stage of HCV infection with progressed liver disorders or even hepatocellular carcinoma ***(Ippolito et al., 2015; Kohli et al., 2014)***. Hepatocellular carcinoma (HCC) and liver failure are the life-threatening conditions associated with untreated chronic HCV infection. Development of HCV-induced HCC is a multistep process that involves chronic liver inflammation and repetitive-cycles of hepatic fibrosis, which may occur over years leading to hepatic failure or cirrhosis and/or malignant transformation ***(Lok et al., 2009)***. Difficulty in early diagnosis and treatment is one of the important reasons for high morbidity and mortality of HCC ***(Tang at al., 2018)***.

Non-coding RNA has been confirmed to play a crucial role in the occurrence and progression of tumors. Among them, long non-coding RNA (lncRNA) is a kind of non-coding RNA with more than 200 nucleotides in length. LncRNA regulates gene expressions at transcriptional and post-transcriptional level. Functionally, lncRNA is widely involved in the physiological and pathological processes of the body ***(Daskalakis et al., 2018)***. LncRNAs are differentially expressed in various tumors with tissue specificity. Meanwhile, lncRNAs can promote the proliferation, invasion and metastasis of tumor cells through multiple mechanisms ***(Huarte, 2015)***. LncRNA has been found abnormally expressed in various malignancies, such as prostate cancer, colorectal cancer, bladder cancer, and kidney cancer ***(Martens-Uzunova et al., 2014; Misawa et al., 2017; Zhong et al., 2018)***.

The molecular regulation mechanism of lncRNAs in tumors has not been completely understood. In general, lncRNAs involve in chromosome recombination, gene imprinting, epigenetic regulation, nucleoplasm transport, mRNA splicing and translation ***(Li et al., 2019)***. Biological processes was found to be regulated by certain lncRNAs, such as proliferation, cell cycle, apoptosis, differentiation and metastasis of tumor cells ***(Winkle et al., 2017)***.

The characteristics of H19 are similar to other lncRNAs in both structures and their temporal and tissue-specific expression pattern. Structurally, the H19 gene contains five exons and four introns, producing a 2.3-kb lncRNA after splicing. The H19 gene contains shorter introns than most lncRNAs genes, each less than 100 base pairs ***(Kent et al., 2002)***. It is transcribed from chromosome 7 in the mouse and chromosome 11 in the human. It is adjacent to the protein-coding gene, ILGF-2, an important fetal growth factor. These two genes share regulatory sequences required for their expression, including two enhancers located 3' downstream of H19 ***(Leighton et al., 1995)***.

Recent studies have investigated the clinical implication of cell-free lncRNAs in cancer patients ***(Wang et al., 2016)***. LncRNA SNHG6 and MINCR were found to promote HCC ***(Cai et al., 2018; Cao et al., 2018)***. In contrast, lncRNA PVT1 and LNC473 were noticed to inhibit HCC ***(Ding et al., 2018; Chen et al., 2018)***. Similarly, other types of lncRNAs, also can be detected in body fluids, and may have diagnostic and prognostic roles in cancer ***(Wu et al., 2016)***.

The aim of the study is to develop a non-invasive diagnostic tool based on measuring the plasma levels of lncRNAs marker H19 in order to detect HCV (genotype 4) induced HCC at the early stages of the disease.

MATERIAL AND METHODS

Patient's criteria

Patients enrolled in this study were admitted to Gastroenterology and Hepatology Department TBRI, Giza, Egypt from November 2016 to August 2018. Diagnosis of patients was based on full medical history, thorough clinical examination, abdominal ultrasonography and laboratory assessment including CBC, and liver function tests, serological and HCV genotyping by HybProbe probes with the light cycler carousel-based system.

The study was approved by TBRI Ethics Review Board and an informed consent was obtained from all the subjects included in the study according to the roles of the Declaration of Helsinki 1975. Approval of local ethical committee (TBRI-REC number 01/19).

Inclusion criteria

All included patients were suffering from chronic hepatitis C genotype (4), persisting more than 6 months (HCV RNA Positive). They did not receive any specific treatment for HCV during the last 6 months.

Diagnosis of HCC was depending upon the presence of focal hepatic lesions diagnosed by abdominal ultrasound and confirmed by triphasic computed tomography and/or magnetic resonance imaging according to American Association for the Study of Liver Diseases 2011 guidelines ***(Bruix and Sherman, 2011)***.

Exclusion criteria

The exclusion criteria included, any concomitant cause CLD such as patients with history of schistosomiasis, chronic viral diseases other than HCV, dual HBV and HCV infection, Non-Alcoholic steato-Hepatitis (NASH), autoimmune hepatitis, biliary disorders, malignancies other than HCC, regular intake of hepatotoxic drugs, alcohol abuse, diabetes and HCV-infected patients receiving direct-acting antiviral or immunomodulatory interferon-α therapy.

Based on the inclusion and exclusion criteria 100 patients were included in this study. Seventy five patients with chronic hepatitis C, who were classified into two major groups: Group I, CLD without HCC (n = 50), and Group II, CLD with HCC (n = 25). Group I was further subdivided into two subgroups: (Ia) (CLD without cirrhosis) (n = 25) and, (Ib) (CLD with cirrhosis) (n = 25). In addition, twenty five age- and sex- matched healthy adults were chosen as a control group.

Specimen Collection and Handling

About 7 ml peripheral venous blood samples were collected under strict aseptic conditions by clean venipuncture using vacuum blood collection tubes and distributed as 2.5 ml in EDTA tubes for complete blood picture, 2.5 ml in another sterile EDTA tube (stored at -80oc) for viral RNA extraction for HCV genotyping, miRNA and mRNA extraction. In addition to, 2 ml in a plane tube were allowed to clot at 37°C, and then centrifuged at 3000 rpm for 10 minutes and the collected serum was stored at -80°C to be used for performing liver and kidney functions, and other specific serological tests.

Laboratory Investigations

All individuals were subjected to general investigations including; Haemogram, using automated cell counter (Celltac 5, Nihon Kohden, Tokyo, Japan). A battery of liver function tests was performed using standard methods. Alpha fetoprotein (AFP) ware determined using autoanalyzer (Hitachi 736, Hitachim

Japan). Coagulation tests were performed using Stago Compact Max, USA. Serological diagnosis of HCV infection were done using Murex anti-HCV, version IV, Murex Diagnostics limited, Dartford, England. HCV-RNA by PCR. Hepatitis B surface antigen and HBV core antibody were examined by enzyme-linked immunosorbent assay (ELISA) system assay. Autoimmune hepatitis was investigated by detecting anti-nuclear antibodies (ANA) using the immunospec ANA screen ELISA test system. A serological examination of schistosomiasis (schistosomal Ab) was done using antibody detection an in-house ELISA.

HCV Genotyping:

Viral RNA Extraction; using high pure viral RNA kit (version 18, 2011), cat. No: (11858882001) https://www.roche.com.

cDNA synthesis (Transcriptor First Strand); was done according to cDNA synthesis kit (Transcriptor first strand) (version 6.0, 2010), cat. No: (04379012001) https://www.roche.com.

HCV Genotyping Detection; using hot start reaction mix detection for PCR using HybProbe probes with the lightcycler carousel-based system (version 15, 2011), cat. No: (03003248001) https://www.roche.com.

Target Gene Expression: Total RNA Extraction; was done according to high pure RNA isolation kit (version 12, 2011), Cat. No: (11828665001) https://www.roche.com. **Gene expression detections;** was performed using light cycler EvoScript RNA SYBR green I master (version 2, 2017), Easy to use reaction mix for one step RT-qPCR Cat. No: (07800134001) https://www.roche.com. The primers sequences are illustrated in (Table 1).

Analysis of result depending on the SYBR green I filter combination (465 – 510), on lightcycler EvoScript RNA SYBR green I master, comparative CT methods were applied to analyze data. Housekeeping gene B-actin was used as an endogenous control to normalize the amount of total mRNA in each sample of H19 between different samples. Gene expression were calculated relative to the control samples (used as the calibrator sample) using the formula $2^{-\Delta\Delta CT}$ and were expressed as fold change (https://bitesizebio.com).

Table (1): Primers of genes included in the study:

Gene		Sequence	Tm	Reference
H19				
	Forward	5'- TGC TGC ACT TTA CAA CCA CTG -3'	58°c	http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0000845.
	Reverse	5'-ATG GTG TCT TTG ATG TTG GGC -3'	58°c	
B-actin (used as an endogenous control to normalize the amount of total mRNA in each sample)				
	Forward	5-GCACCACACCTTCTACAATG-3	58°c	**http://hgsv.washington.edu**
	Reverse	5-TGCTTGCTGATCCACATCTG-3	58°c	

Statistical Analysis

The data were analysed using Microsoft Excel 2010 and statistical package for social science (SPSS version 24.0) for windows (SPSS IBM., Chicago, IL). Continuous normally distributed variables were represented as mean±SD. with 95% confidence interval, while non normal variables were summarized as median with 25 and 75 percentile, and using the frequencies and percentage for categorical variables; a p value < 0.05 was considered statistically significant. To compare the means of normally distributed variables between groups, the Student's t test was performed, and Mann-whitney test was used in non-normal variables. X^2 test or Fisher's exact test was used to determine the distribution of categorical variables between groups. The diagnostic performance of H19 was assessed by ROC curves. The area under the ROC (AUROC) was used as an index to compare the accuracy of tests. The cut-off for diagnosis of group of the study was taken from the point of maximum combined sensitivity and specificity. The sensitivity and specificity for relevant cut-offs were also displayed. Spearman's rank correlation coefficient (r) was done to show the correlation between different parameters in this study. An effect modification was evaluated by stratification, statistical interaction and was assessed by including main effect variables and their product terms in the logistic regression model.

RESULTS

Individual demographic and routine laboratory characteristics of the studied groups Table (2).

Table (2): Socio-demographic characteristics, laboratory investigations and ultra sound finding among patients of the studied groups

Items		Control N=25	Group I (CLD without HCC) N=50		Group II (CLD with HCC) N=25	*p* value
			Subgroup Ia (CHC without Cirrhosis) N=25	Subgroup Ib (CHC with Cirrhosis) N=25		
U.S Finding	Cirrhosis	0 (0.0%)	0 (0.0%)	25 (100.0%)	6 (24.0%)	0.01*
	Splenomegaly	0 (0.0%)	2 (11.11%)	17 (68.0%)	18 (72.0%)	
	Ascites	0 (0.0%)	0 (0.0%)	18 (72.0%)	25 (100.0%)	
ALT(N.10---40) (umol/dl)		27.5 (15.5 - 31.8)	40.0 (17.0- 51.0)	46.5 (24.3- 84.8)	55.5 (34.5- 82.5)	0.001**
AST(N.10----45) (umol/dl)		31.10±6.77	43.63±12.39	66.40±21.44	95.67±38.95	0.001**
AFP (ng/ml)		2.3 (1.5- 3.1)	2.2 (1.4- 4.5)	8.8 (6.5- 16.8)	224.0 (44.3- 597.5)	0.001**
Albumin (N.3.5- 5.5) (umol/dl)		4.1±0.5	4.1±0.5	3.1±1.1	2.6±0.7	0.001**
Total Bilirubin (N.UP TO 1.0) (umol/dl)		0.9 (0.8- 1.1)	0.6 (0.4- 0.9)	1.4 (0.7- 3.6)	1.9 (1.3- 5.0)	0.001**
Direct Bilirubin (N, UP TO 0.25) (umol/dl)		0.3 (0.2- 0.4)	0.2 (0.1- 0.3)	0.6 (0.2- 1.9)	1.1 (0.4- 2.5)	0.001**
ALP (N.96.0—276) (IU/L)		74.3±19.0	84.9±30.2	103.2±35.6	202.4±88.0	0.001**
PT (s)		12.4 (11.4- 12.8)	15.4 (13.2- 18.8)	17.8 (15.0- 20.4)	16.3 (14.5- 19.0)	0.001**
PC (%)		89.6 (78.2- 100.0)	64.0 (55.0- 80.0)	52.5 (44.0- 73.8)	71.5 (55.0- 75.3)	0.001**
INR (s)		1.1 (1.0- 1.1)	1.5 (1.0- 1.6)	1.5 (1.2- 1.8)	1.4 (1.2- 1.5)	0.001**
HB (N,12----16) (g/dl)		12.6±1.4	12.4±1.6	10.4±2.2	11.2±2.4	0.002**
WBCs (/mm3)		6.2±2.3	7.0±2.8	7.3±2.6	7.8±3.1	0.4
Platelets (/mm3)		241.50±56.48	183.58±35.32	97.35±19.42	140.1±69.5	0.001**
APRI Score		0.34±0.13	0.61±0.17	1.69±0.31	2.1±1.3	0.001**

Albumin, Alkaline phosphatase (ALP.), Hemoglobin (HB), White blood corpuscles (WBCs) and Platelets are represented as mean ± SD; the data were analyzed by ANOVA Test. But U/S finding is represented as frequency and percent; the data were analyzed by x2 Test. While alanine aminotransferase (ALT), aspartate aminotransferase (AST)}, alpha fetoprotein (AFP), T. Bilirubin., D. Bilirubin, PT, PC and International normalized ratio (INR) are represented as Median and interquartile range (25%-75%); the data were analyzed by Kruskal Wallis Test.

APRI score calculated regarding AST to Platelet Ratio Index (APRI) = [AST Level (IU/L)/ AST (Upper Limit of Normal) (IU/L)]/ Platelet Count (109/L) X100. (Normal < 0.05, CLD without cirrhosis 0.5 – 1.5 and Cirrhosis ≤ 1.5).

* *p* value <0.01 is significant, ** *p* value <0.001 is highly significant

H19 gene expression examined in plasma regarding subgroup (Ib) and group II comparing to healthy controls, as well as when compared to subgroup (Ia). besides that, actual fold change of up and downregulated genes among subgroups (Ia), (Ib) and Group II are illustrated in (Table 3).

H19 expression when compared to control group showed a high significant upregulation in all diseased groups (p value <0.001). On comparing to subgroup Ia, the expression of H19 in subgroup Ib and group II showed significant upregulation (p <0.01, 0.001) respectively, as well as in group II when compared to subgroup Ib (p <0.001) and group I as general (including Ia and Ib) (p <0.001) (Table 3), figure (1).

Table (3): H19 gene expression in the studied groups.

Biomarkers	Control N=25	Group I CLD without HCC		Group II CLD with HCC N=25
		(Subgroup Ia) CLD without cirrhosis N=25	(Subgroup Ib) CLD with cirrhosis N=25	
H19	0	1.6(0.5- 8.65)[aa]	8.9(0.3- 16.95) [aa,b]	21(4- 40.65) [aa, bb, c, ##]

The fold change results depend on the fold change low: Fold-Change (2^(- Delta Delta Ct)) is the normalized gene expression (2^(- Delta Ct)) in the Test Sample divided the normalized gene expression (2^(- Delta Ct)) in the Control Sample. (Fold-change values less than one indicate a negative or downregulation).

H19 is represented as Median with Interquartile range (25–75%) of the fold-change of the studied groups, the data were analyzed by Mann Whitney U test

**P* value is significantly different comparing with CLD without HCC group (I)

[a]*P* value is significantly different comparing with control group

[b]*P* value is significantly different comparing with CLD without cirrhosis group

[c]*P* value is significantly different comparing with CLD with cirrhosis group

[#]*P*. value significantly different comparing with CLD without HCC group.

1 initial *P* value < 0.01 is significant, 2 initial *P* value < 0.001 is highly significant.

Diagnostic performance of H19 gene expression as markers of subgroup (Ib) and group II at different cut off points using (ROC) curve. The calculated sensitivity, specificity, and diagnostic accuracies for studied parameters to discriminate group II and subgroup (Ib) from subgroup (Ia) (Table 4).

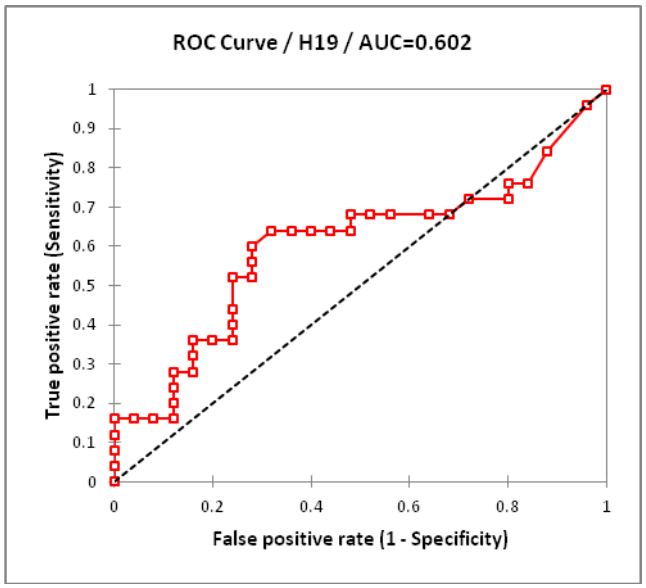
Table (4). Diagnostic performances of H19 to discriminate (Cirrhotic and HCC) patients from CLD patients.

Biomarkers in the studied groups	**Cutoff**	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Accuracy**	**AUC**	**95% CI**	**p-value**
Subgroup Ib Vs Subgroup Ia	**4.6**	**60.0%**	**72.0%**	**68.2%**	**64.3%**	**66.0%**	60.2%	44.5% - 76.0%	0.203
Group II Vs Subgroup Ib	**> 18.5**	**56.0%**	**84.0%**	**77.8%**	**65.6%**	**70.0%**	68.7%	54.3% - 83.1%	0.011*
Group II Vs Group I	**> 19.6**	**56.0%**	**90.0%**	**73.7%**	**80.4%**	**78.7%**	75.0%	63.4% - 86.7%	< 0.0001**

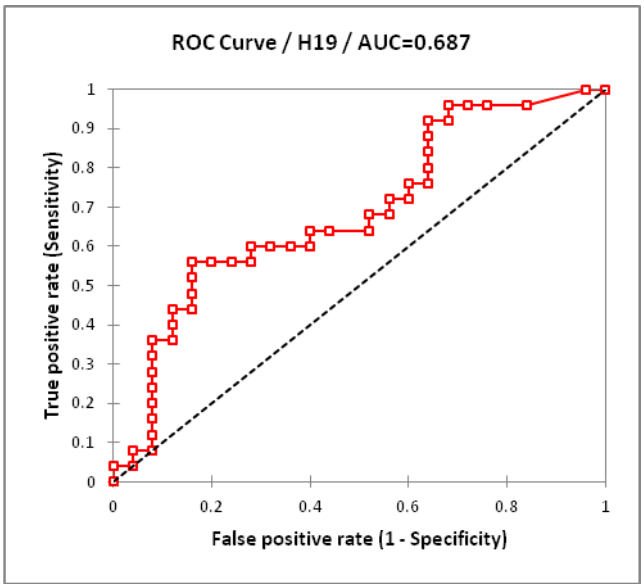
PPV: Positive predictive value, NPV: Negative predictive value and AUC Area under curve.

For discrimination of subgroup (Ib) versus subgroup (Ia), the AUC =60.2% with (95% CI 44.5% – 76.0%, P = 0.203) figure (2a). While in case group II versus subgroup (Ib), the results indicated that H19 could be considered as a diagnostic parameter with weak significant results or in borderline, AUC of 68.7% (95% CI 54.3% – 83.1%, P = 0.011) figure (2b).

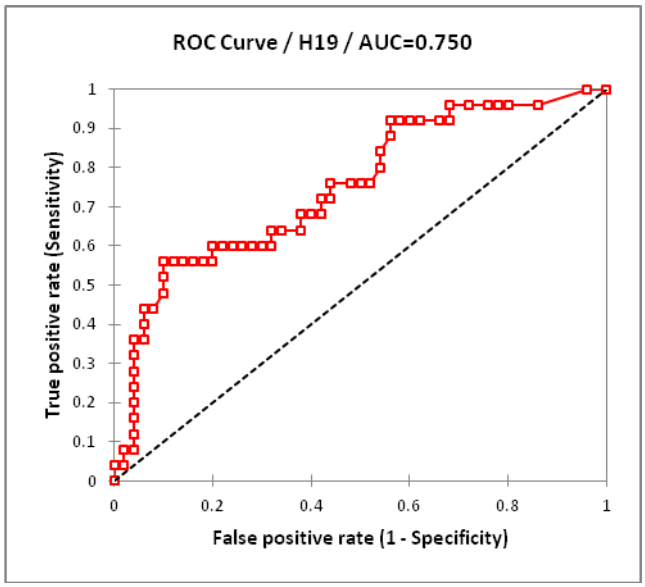
On discrimination between group II versus group I, AUC = 75.0% (95% CI 63.4% – 86.7%, P = 0.05), figure (2c). These results indicated that H19 could be considered as a diagnostic parameter with a significant result and could be used for discrimination between group II versus group I.



a) CLD with cirrhosis Vs CLD without cirrhosis



b) HCC Vs CLD with cirrhosis



HCC Vs CLD without HCC

Figure (2): ROC curve of H19 in the studied groups.

Regarding univariate logistic regression analysis: the H19 were considered as predictor and/or prognostic parameter for cirrhotic progression, an increase in 1 degree of H19 increased the odds of being cirrhosis by a factor of 1.065 with (*p* value = 0.04). As for HCC progression, an increase in 1 degree of its expression level increased the odds of being HCC by a factor of 1.034 with (*p* value = 0.04).

Concerning CLD as general groups I (subgroups Ia and Ib) had, an increase in 1 degree of H19 which increased the odds of being HCC by a factor of 1.054 with (*p* value = 0.002), (Table 5).

Table (5): Univariate analysis showing the predictive power of different biomarkers for Cirrhosis and HCC diagnosis

Biomarkers in the studied groups		OR	95% C.I	P-value
H19	**Subgroup Ib *Vs* Subgroup Ia**	1.065	1.00-1.133	0.04*
	Group II *Vs* Subgroup Ib	1.034	1.001-1.069	0.04*
	Group II *Vs* Group I	1.054	1.02-1.089	0.002**

AOR; Adjusted Odd Ratio, C.I; Confidence Interval, p-value calculated depend on logistic regression analysis.

Correlation analysis revealed, H19 expression showed a significant direct correlation with AFP(r = 0.424 and p value = 0.0001) (Figure 3), ALT (r = 0.242 and p value = 0.034), and ALK (r = 0.481 and p value = 0.0001), while there were inverse correlations with albumin (r = -0.403 and p value = 0.0001) and platelets count (r = -0.373 and p value = 0.001) (Table 6) .

Table (6). Correlation study of H19 gene regarding laboratory investigations.

	H19	
	Correlation Coefficient	**Sig. (2-tailed)**
Age	0.228*	0.046
AFP	0.424**	0.0001
Albumin	-0.403**	0.0001
T. Bil	0.139	0.229
D. Bil	0.122	0.289
ALT	0.242*	0.034
AST	0.159	0.166
ALK	0.481**	0.0001
UREA	0.285*	0.012
CREAT	0.280*	0.014
PT	0.425**	0.0001
PC	-0.252*	0.027
INR	0.302**	0.008
HB	-0.276*	0.015
WBCs	0.112	0.334
Platelets	-0.373**	0.001

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

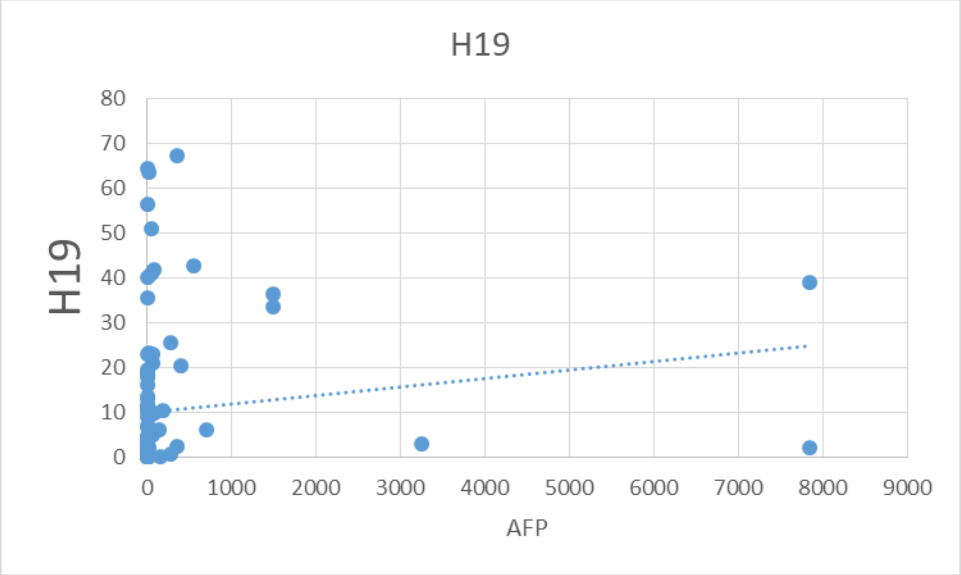


Fig. (3): Correlation between H19 and AFP.

DISCUSSION

HCC is among the most prevalent and lethal cancers worldwide ***(Jemal et al., 2011)***. HCC initiation and progression are greatly affected by the liver milieus in which it arises. More than 80% of HCCs develop on the basis of a CLD ***(Farazi and DePinho, 2006; Hernandez-Gea et al., 2013; Marquardt et al., 2015)***. As results of the constant injuries, hepatocyte necrosis and high cellular turnover perpetuate error prone chronic repair processes that exert selective pressure on proliferating hepatocytes in the background liver. This pathological process not only causes hepatocyte genomic instability, but also creates a pro-oncogenic hepatic microenvironment. Such combined effects importantly contribute to the clinically heterogeneous phenotypes and genotypes of HCC ***(Marquardt et al., 2015)***.

The aim of this study is to develop a non-invasive diagnostic tool based on measuring the plasma level of long non-coding RNAs H19 in order to detect HCV-induced HCC at the early stages of the disease.

H19 was mapped on the short arm of chromosome 11, band 15.5, homologous to a region of murine chromosome 7 (***Matouk et al., 2005)***. Results of the present study, revealed significant upregulation of H19 gene in cirrhotic and HCC patients when compared to controls ($P< 0.001$). Similarly significant increase ($P<0.001$, $P<0.01$) was noticed on comparing HCC with chronic HCV without and with cirrhosis patients respectively. No significant difference was found between chronic HCV with and without cirrhosis. These results are not in agreements with ***Iizuka et al. (2004)*** which revealed a decreased expression of H19 in human HCC tissue compared to non-tumorous tissue. ***Schultheiss et al. (2017)*** used four independent patient cohorts which revealed a decreased expression of

H19 in human HCC tissue compared to non-tumorous tissue. However, interestingly, although H19 was downregulated in HCC, in each of the investigated patient cohorts a high H19 expression was observed in a small patient subgroup. ***Lv et al. (2014***) also found H19 was to be downregulated in hepatocellular cancer. On the other hand increased expression of H19 has been observed in some cancers, such as gastric cancer ***(Yang et al., 2012; Song et al., 2013; Arita et al., 2013)*** and bladder cancer ***(Luo et al., 2013),*** where it was reported that H19 levels were significantly higher in the patients than in the controls.

H19 was shown to be regulated under inflammatory conditions ***(Wang et al., 2016)*** and has been linked with HCC that represents a type of tumor that is associated with inflammatory conditions such as found in viral hepatitis as well as in alcoholic and non-alcoholic steatohepatitis ***(El-Serag and Rudolph, 2007; Yoshimizu et al., 2008; Matouk et al., 2007)***. H19 expression can be regulated by loss of imprinting (LOI) and by differential promoter methylation ***(Gabory et al., 2006)***. ***Schultheiss et al.(2017)*** showed that the decreased H19 expression was not due to LOI. Interestingly, H19 promoter methylation was decreased in HCC compared to normal liver tissue, suggesting that decreased expression of H19 correlates with decreased promotor methylation. However, elevated gene expression is normally based on decreased promoter methylation. Nevertheless, an association of hypermethylation and increased gene expression has been described as well ***(Wu et al., 2008)***.

A recent meta-analysis showed that H19 expression might be a novel molecular marker for predicting prognosis and could also be a predictive factor of clinicopathological features in various cancers ***(Liu et al., 2016)***.

The diagnostic performance of H19 gene expression as markers in cirrhotic patients at different cut off points using ROC curve showed no significant difference between CLD with and without cirrhosis at the cut-off value of 4.6, with sensitivity of 60.0% and specificity of 72.0% with areas under curve (AUC) were 60.2% ($p = 0.203$, 95% Confidence interval C.I 44.5% - 76.0%) and accuracy 66.0% regarding H19. In case of HCC at the cut-off value of >18.5, with sensitivity of 65.0% and specificity of 84.0% with areas under curve (AUC) were 68.7% ($p = 0.011$, 95% confidence interval C.I 54.3% - 83.1%) and accuracy 70.0% regarding HCC group when compared to cirrhotic group, the results indicated that H19 could be considered as a diagnostic parameter with weak significant results or in borderline. This could be explained due to the relatively small sample size. Evidently, for discrimination of HCC group from CLD patients without HCC as one group it was found plasma H19 at the cut-off value of >19.6, with sensitivity of 56.0% and specificity of 90.0% with areas under curve (AUC) were 75.0% ($p <0.001$, 95% Confidence interval C.I 63.4% - 86.7%) and accuracy 78.7%. These results indicated that H19 could be considered as a diagnostic parameter with a significant result and could be used for discrimination between HCC versus CLD without HCC but weak versus CLD with cirrhosis.

Interestingly, according to regression analysis, the expression levels of H19 could be considered as significant predictors associated with the changes of the cirrhotic group

versus non-cirrhotic group. Evidently, the expression levels H19 increased the odds of being cirrhosis when selected as significant predictors associated with the chances of diagnosis HCC versus cirrhosis patients and CLD without HCC patients as one group.

Correlation analysis revealed, a significant direct correlation of H19 with AFP, ALT and ALK ($r = 0.424$ and $p= 0.0001$, $r = 0.242$ and $p= 0.034$, $r = 0.481$ and $p= 0.0001$) respectively, and, a significant inverse correlation with albumin ($r = -0.403$ and $p= 0.0001$), but no significant correlation with the AST and Total Bilirubin ($r = 0.159$ and $p = 0.166$, $r = 0.139$ and $p = 0.229$) respectively.

CONCLUSIONS

The results obtained in this study highlighted the presence of an association between the over expression level of H19 marker and HCC. H19 can be used to detect the presence of HCC. These results prompt the use of this parameter as noninvasive diagnostic biomarker and targets for new therapeutic modalities for HCV Genotype 4 induced HCC.

Abbreviations

AFP	Alpha fetoprotein
ALT	Alanine aminotrasferase
ANA	Anti-nuclear antibodies
APRI	AST, platelet ratio index
AST	Aspartate aminotransferase
AUC	Area under the ROC
CLD	Chronic liver disease
CT	Computed tomography
CT	Threshold
ELISA	Enzyme-linked immunosorbent assay
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HSCs	Hepatic stellate cells
INR	International normalized ratio
LNCRNAs	Long non-coding RNAs
LOI	loss of imprinting

Author contributions

All authors conceived and designed the study, contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work

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Availability of data and materials

The datasets used and analyzed in the current study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

Procedures were reviewed and approved by the Committee of the Affiliated

Hospital of Theodor Bilharz Research Institute.

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الحامض النووي الريبوزي الطويل (H19) غير المشفر كأداة تشخيص جديدة غير نافذه لأورام الكبد التي قد تنتج عن فيروس الكبد (C)

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يعد سرطان الكبد أو تليفه من الأمراض الخطيرة التى قد تنتج عن الاصابه بفيروس الكبد سى لذا انشغل الباحثون بكيفية ايجاد طريقه للكشف المبكر عن امكانية اصابة المريض بفيروس الكبد سى بالتليف او بالسرطان ومن ثم كان اتجاه الباحثين الى دلالات غير جينيه يمكن من خلال قياس مستوياتها تحديد ذلك دون الحاجه الى التدخل الجراحى وأخذ عينة الكبد والتى تسبب معاناه للمرضى. ولقد وجد أن الحامض النووى الريبوزى الطويل غير المشفر H19 لديه العديد من الوظائف البيولوجية المتنوعة.

الهدف من البحث:

تطوير اداة تشخيص غير نافذة من خلال قياس H19 فى الدم من أجل الكشف عن اورام الكبد التى قد تنتج عن فيروس سى فى المراحل المبكرة من المرض.

وقد شملت الدراسة الحالية :-

- أجريت هذه الدراسة على ٧٥ من الأشخاص المرضى بفيروس التهاب الكبد (سى) بالإضافة إلى ٢٥ من الأشخاص الأصحاء البالغين الذين تتفاوت أعمارهم من الجنسين.
- - تم تقسيم المرضى إلى مجموعتين (العدد = ٧٥) ,المجموعة الأولى: الأشخاص الذين يعانون من التهاب الكبد الفيروسى المزمن بدون وجود سرطان الكبدHCC (العدد = ٥٠) وتنقسم هذه المجموعة إلى المجموعة الفرعية Ia : الأشخاص الذين يعانون من التهاب الكبد الفيروسى المزمن بدون تليف الكبد (العدد = ٢٥) والمجموعة الفرعية Ib : الأشخاص الذين يعانون من التهاب الكبد الفيروسى المزمن مع تليف الكبد (العدد = ٢٥)
- المجموعة الثانية: الأشخاص الذين يعانون من التهاب الكبد الفيروسى المزمن مع وجود سرطان فى الكبد HCC (العدد=٢٥)
- تم عمل HCV-RNA genotyping and target gene expression بواسطة جهاز Real Time PCR (lightCycler 480 II-Roch).

و قد أسفرت نتائج البحث عن :-

١ - H19 يتم التعبير عنه بشكل مفرط في مرضى التهاب الكبد المزمن مع تطور سرطان الكبد.

٢- H19 من منحنى ROC والانحدار اللوجستي يمكن استخدامه كأداة تشخيص جديدة ويمكن أن يكون تنبئي أو علامة تنذر بتطور تليف الكبد وسرطان الكبد.