Rate of Adriamycin Resistance Associated (ARA) long Noncoding RNA Expression in Sample of Hepatitis B Virus Iraqi Patients

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Abstract:

Numerous biological processes, including epigenetics regulation, control of cell cycle, beside transcriptional, translational and regulation of genes expression, have been linked to long non-coding RNAs lncRNAs . the disruption of expression for lncRNAs are important early stages in in hepatitis B virus disease.

Objectives: In order to investigate ARA lncRNA production in Hepatitis B patients, analyze its clinical importance, and examine the possible value as a predictive biomarker, Amplification of Adriamycin Resistance Associated Lnc-RNA and Glyceraldehyde 3-phosphate dehydrogenase (reference gene) was done by two steps reverase- transcriptase polymerase chain reaction ,No significant difference $P = 0.566$ was observed in the Adriamycin Resistance Associated lncRNA serum levels between chronic hepatitis B virus patient and control ,In the patients group with chronic HBV)infection, the expression of Adriamycin Resistance Associated lncRNAs was elevated to the 2 fold.

Key words: HBV, LncRNA, PCR, ARA LncRNA

Abbreviations: Lnc-RNA long non-coding RNA, HBV hepatitis B virus, ARA Adriamycin Resistance Associated, HCC hepatocyte carcinoma, HBV hepatitis B virus, HCV hepatitis C viruses HCV, GIT Hepatology and Gastroenterology Teaching, GAPDH Glyceraldehyde 3-phosphate dehydrogenase, CHB chronic hepatitis B, miR micro-RNA.

معدل مقاومة الأدرياميسين المرتبطة بتعبير الحمض النووي الريبوزي (ARA) الطويل غير المشفر في عينة من المرضى العراقيين المصابين بفيروس التهاب الكبد B.

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الخلاصة:

ترتبط العديد من العمليات البيولوجية، مثل تنظيم الوراثة اللا جينية، والتحكم في دورة الخلية، إلى جانب النسخ والانتقال وتنظيم التعبير الجيني، بالحوامض الريبية الطويلة غير المشفرة، ان الخلل في التعبير الجيني لتلك الحوامض قد يحدث في المراحل من الأولى من الإصابة بفيروس التهاب الكبد البائي. الأهداف: من أجل الكشف عن ARA lncRNA في مرضى التهاب الكبد البائي، وتحليل أهميته السريرية، والتنبؤ بقيمته المحتملة كمؤشر حيوي. تم إجراء تضخيم ARA Lnc-RNA و GAPDH الجين المرجعي بواسطة تفاعل البلمرة اللحظي ذو الخطوتين، ولم يلاحظ أي فرق كبير $P = 0.566$ في مستويات مصل ARA lncRNA بين مرضى التهاب الكبد البائي المزمن ومجموعة السيطرة، الا ان في مجموعة المرضى الذين يعانون من فيروس التهاب الكبد البائي المزمن قد ارتفع التعبير الجيني ARA lncRNAs إلى ضعفين.

الكلمات المفتاحية :التهاب الكبدالوبائي البائي، تفاعل البلمرة اللحظي، الاحماض الريبية الطويلة

Introduction

The double stranded DNA Hepatitis B virus HBV is a member of the hepadnavirus group. An international public health issue, HBV infection can cause both acute disease and chronic disorders. hepatitis B virus -related liver conditions are among the most frequent reasons for transplantation of liver (1). According to epidemiological data, a significant fraction of liver cancer cases, particularly in developing nations, are caused by hepatocyte carcinoma (HCC) connected to the HBV (2).

lncRNA was considered as, transcriptional fuss, are a class of transcript longer mor than 200 nucleotides without coding of protein functions (3). By interacting with RNA, DNA, or proteins, lncRNAs may have function as encouraging factors of epigenetic, gene regulation, and gene expression. They also play important roles in a number of physiological and pathological mechanisms and are tightly connected to diseases like tumor, and infections (4).

Numerous epigenetic modifications and chromosomal mutations in the genes regulating the circadian rhythm have indeed been demonstrated to be strongly related with the onset of tumor. There are variations in liver cancer incidence across the globe. The hepatitis B (HBV)and C viruses HCV, exposure to specific chemicals, consumption of alcohol, and metabolic illnesses including diabetes and obesity are all significant risk causes for hepatic cancer (5). Differential lncRNA expression and the breakdown of these regulatory mechanisms have been identified as crucial stages in the growth of cancer. Additionally, several studies suggested the utilization of lncRNAs for medical and diagnostic applications, in other words, as targets for the regulation of lncRNA expression, options for therapeutic treatments, and biomarkers for particular malignancies (6).

Cells that have been infected may produce viral, cellular and hybrid lncRNAs as a result of transcription of virus or proliferation during the course of infection. The interaction between the virus and host is therefore affected in different ways by various lncRNAs, which ultimately affects the clinical result (7).

Many lncRNA top player of different phases of CHB infection have been registered up to this point (8), Furthermore unique functional and mechanistic investigations show that inappropriately expressed lncRNAs may be crucial in the progression of Hepatitis B virus infection (9). An intron in p21 activated -kinase three PAK-3 gene, which is located on chromosome (Xq23), produces the (ARA – lncRNA), which stimulates cell division, apoptosis suppression and cell -cycle inhibition (10).

In order to investigate ARA lncRNA expression by (qRT-PCR) in Hepatitis B patients, analyze its clinical importance, and examine the possible value of ARA lncRNA as a predictive biomarker.

Patients & Methods

Patients

The current study was carried out between January and September 2022. Fifty participants in all, including 20 healthy volunteers and 30 chronic HBV patients, were sent to the Hepatology and Gastroenterology Teaching (GIT) center in the Baghdad-Iraq. According to clinical records, every patient tested positive for (HBs Ag) and had no other liver conditions (liver fibrosis or HCC hepatocyte carcinoma).

Sample collection

From each individual 5mL of peripheral blood were withdrawn, which then separated into serum and utilized to identify all serological indicators for HBV and to quantify RNA using real-time PCR. Serum was preserved in 0.75 ml of TRIzol reagent and kept in a refrigerator before being utilized for ARA Lnc-RNA isolation and

fold change measurement. Ribonucleic acid was extracted from the sample in accordance with the TRIzol Reagent's instructions.

Molecular Detection Expression of Adriamycin Resistance Associated Lnc-RNA

Complementary DNAs for ARA Lnc-RNA and GAPDH as reference gene were created. SYBR Green, RT-qPCR system (addedbio - Korea), primers (Table-1) and according to the producer's protocol. Quantus Florometer was utilized to assist the concentration of extracted cDNA in fact to checking the quality of samples for molecular downstream application. For 1 µl of ARA Lnc-RNA or cDNA, 199 µl of diluted QuantyFlour Dye was added. After 5 mins of incubation at room conditions in dark space, RNA concentration was asset.

Polymerase chain reaction Amplification of ARA Lnc-RNA and GAPDH was conducted by 2 Steps RT PCR as follow 1st step: 16C for 30 min, (42C) for (30min), (85C) for 5 min and (4C) for 10 min for one cycle. 2nd step (95C) for 5 min for one cycle., 95C for (20 sec), (55C) for (30 sec) and (72C) for (20 sec) for 45 cycles.

The mean value for each triplicate was used to evaluate the relative lncRNA concentrations ΔCt = Ct mean lncRNA-Ct mean GAPDH. the expression of fold change was counted using $2^{-\Delta\Delta Ct}$ methods (11). The Statistical Analysis System 2018 programs was utilized to estimate the effects of differences factors in the study parameter.

Table (1): Sequence of primer utilized for amplification Adriamycin Resistance Associated Lnc-RNA and GAPDH genes

Primer	Sequence
GAPDH -F	5′-GAA GGT GAA GGT CGG AGT C-3′
GAPDH- R	5′-GAA GAT GGT GAT GGG ATT C-3′
ARA Lnc-RNA -F	5′-TGC TGC ACT TGA GCA TTA GG-3′
ARA Lnc-RNA -R	5′-GCC TCC ATG AAA AAG GAT CA-3′

ARA: Adriamycin Resistance Associated. GAPDH: Glyceraldehyde 3-phosphate dehydrogenase

viral load of 30 Chronic Hepatitis B patient were divided in to high viral load which is more than (100,000 copies/ml) 16.66% and intermediate viral load which the viral load ranged from (2000 to 100,000) copies/ml 83.33% (12).

Table 2: Distribution of Viral load Of Chronic Hepatitis B patient.

	Quantity of virus	number	percent
Viral load group	Intermediate viral load	25	83.33
	High viral load	5	16.66

Results

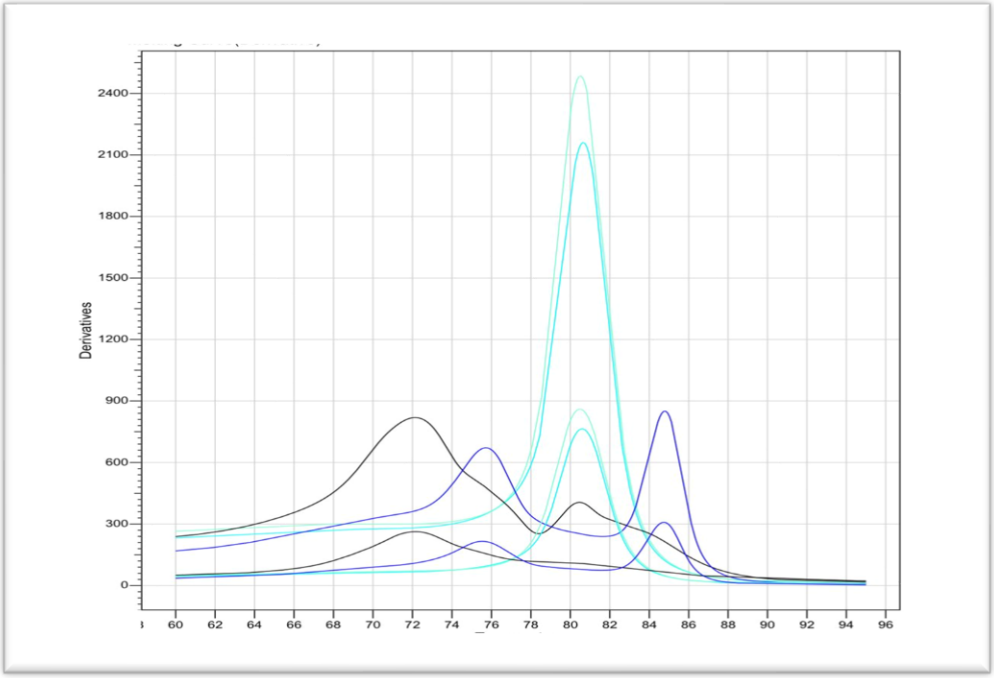
The viral load results showed (83.33%) of HBV patients inwards intermediate viral load group and (16.66%) within high viral load groups, as show in table (2).

It has been known that viral load of HBV can stay stable for long time tel HBeAg serum sero conversion or a hepatitis develop occur (13). On other studies, 2 landmark investigation of the HBV study group have showed alik between the viral

load of HBV and progressing disease of liver, like liver cancer and fibrosis (14,15).

Expression profile of Adriamycin Resistance Associated lncRNA.

The role of lncRNAs in HBV infections is not clear. Here we estimate the expression profile of ARA lncRNAs in sample of 30 HBV patients and 20 healthy controls, The levels of serum ARA Lnc RNA are varied in CHB patients, and control. The expression of ARA Lnc RNA was detected in the serum from patients and control by quantitative real time (PCR). Real time PCR results were quantified base on cycle threshold Ct values that are inversely related with amount of the starting templates so elevated Ct values equivalent with depressed level expression of gene, and so on and so forth as in figure (1) and (2)



(Figure 1) The Adriamycin Resistance Associated lncRNA gene expression Melt on the Green, Melt from 72°C to 95°C.

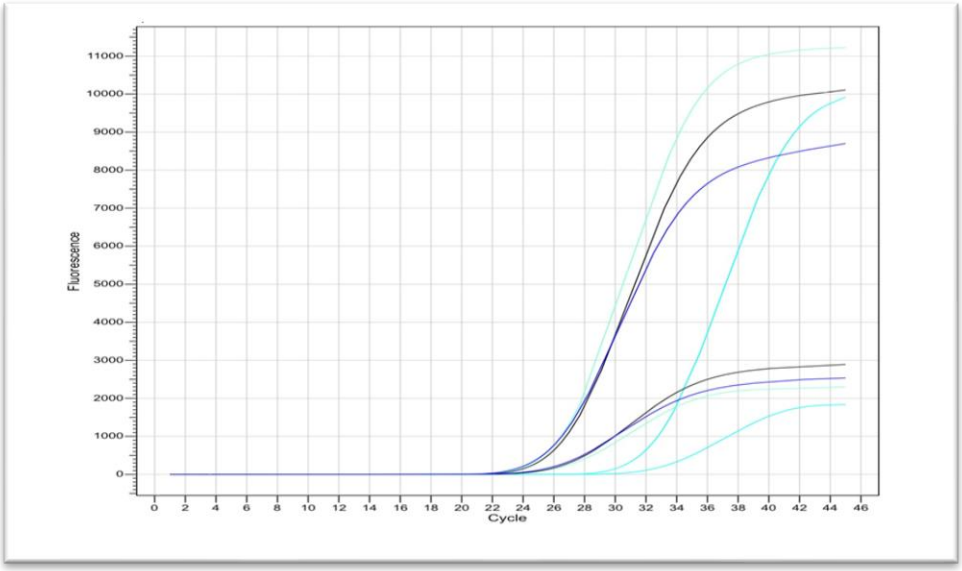


Figure (2) Amplification plot for Adriamycin Resistance Associated lncRNA expression.

No significant difference (P = 0.566) was observed in the ARA lncRNA serum levels between the CHB patients and healthy control (table 3) Nevertheless, the relationships between ARA lncRNA in CHB patients and healthy subjects could not be fully considered because to the small number of HBV samples available.

Table (3) Adriamycin Resistance Associated lncRNA serum levels in chronic hepatitis B and control.

	Patients	**Control**	**P-value ©**
Mean	8.29	5.49	0.566 NS
Standard Deviation	19.35	11.04	--
Median	1.41	0.667	--
Standard Error	3.72	2.47	--
Range	0.08-82.31	0.0064-41.73	--
No	27	20	--
NS: Non-Significant.			

On other hand the results obtained from the current study were showed that the serum ARA lncRNA was upregulated in the CHB compared to the healthy subjects (Figure 3).

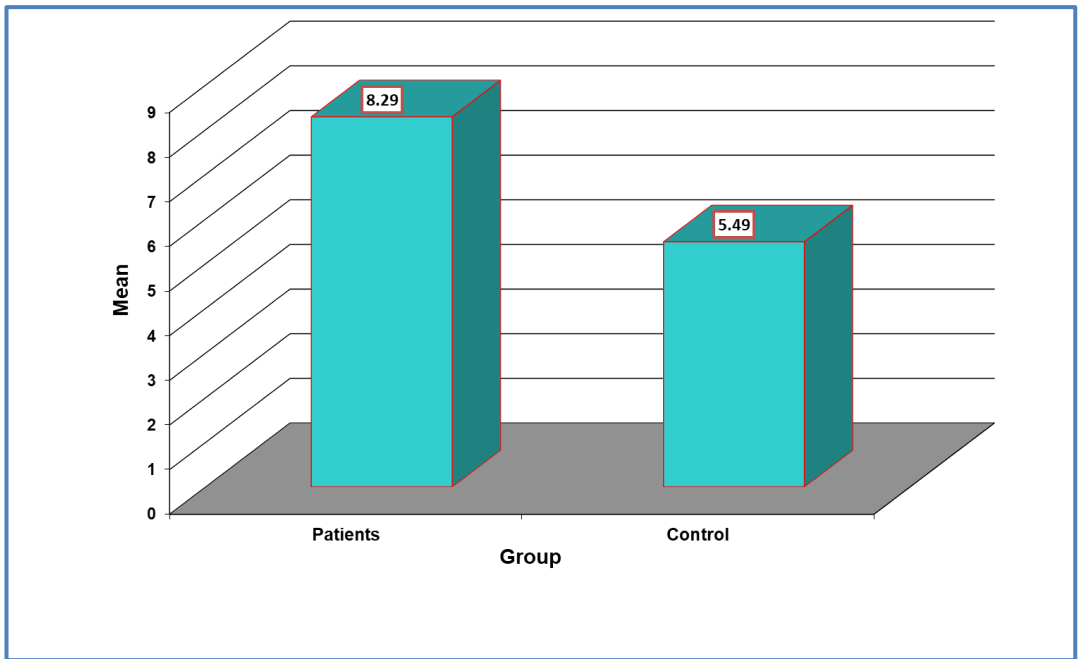


Figure (3) Adriamycin Resistance Associated lncRNA expression in the CHB compared with the healthy subjects.

Discussion

LncRNAs can be behave as oncogene and (TSG) tumor suppressor gene , so has showed in research, which these molecule can play an crucial role in tumor formation ,differentiation, proliferation of cell and program cell death or lncRNA , greatly action on the malignant conduct of tumors (16) Adriamycin Resistance Associated lncRNA was included to be in hepatocyte cell line (17).Our findings demonstrated an alterations in its expression in the CHB group as compared to the control participants,In the group of patients with CHB infection, the expression of ARA lncRNAs increased by 2 fold comparatively , which suggested that serum ARA lncRNAs might act as a standalone marker of infection independently of the state of the HBV development. on a different study When compared to the control, the expression of twenty-one lncRNAs was substantially greater at the time of the starting of HBV diagnosis and at twelve months after treatment and follow-up P 0.05, suggesting the potential use of lncRNAs as assessment is used to determine in HBV patients (18). *In vitro* silencing of this lncRNA was used to examine the roles of ARA, and it was discovered that ARA suppression resulted in decreased proliferation, caused apoptosis, G2/M arrest, and migratory abnormalities (19). The crucial functions of miRNAs in adriamycin resistance, including miR-34a, have also been shown in recent investigations. (20), miR-128 (21). Therefore, it is critical to find new markers or methods to enhance HBV detection. New studies have shown that lncRNAs have enormous promise as disease markers for a variety of disorders (22,23). It is now understood that lncRNA play a critcal role in the etiology of liver cancer. Some lncRNAs are abundantly expressed and take involvement in the development of liver cancer, including the MALAT1, HULC, and also H19 lncRNAs (24, 25).

Similar to miRNAs, lncRNA release may be a competitive process, and the amount of lncRNAs in blood serum may not accurately represent those present inside cells (25). This is the first paper that specifically discusses ARA lncRNA in

HBV individuals in Iraq, to the to our knowledge.

Conclusion

Current study was demonstrated the ARA lncRNA expression patterns for HBV patients and healthy subjects were different, and the downregulation ARA lncRNA may constitute prospective HBV treatment targets and diagnostic markers., To determine if the variations in the tested lncRNAs are in fact connected to HBV, additional research is needed.

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