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INVESTIGATION OF MICRO RNA RS11614913 GENE POLYMORPHISM IN PATIENTS INFECTED WITH HBV IN HILLA PROVINCE

Ruqaya Munther Ewadh

College of Pharmacy, University of Babylon, Iraq.
e-mail : rokia_84@yahoo.com

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ABSTRACT : MicroRNAs (miRNAs) are smaller RNAs that do not encrypt a particular protein and have a significant role to play in the regulation of several gene post-translation or post-transcription processes via mRNs denaturation or translation inhibition. Different studies have shown that miRNAs play an important role in gene expression and replication. Hepatitis B (HBV) and Hepatitis C virus (HCV) play a very important role in the relationship between the host and the virus. There is substantial evidence that viral infection of the hepatic tissue or circulatory system contributes significantly to changes in the profile of miRNA expression. A total of 30 patients and 30 control topics were completed between August to October 2018. In addition to the limiting fragment length polymorphism (FRLP), the genotyping of miR-196a2 was achieved by polymerase chain reaction (PCR). We appear to assess critical differences between HBV patients and controls using P = 0.043, OR = 0.46(0.21-1.02) within the miR-196a2 genotype range.

Key words : HBV, RFLP, miRNA, SNPs Polymorphisms.

INTRODUCTION

Viral hepatitis is a disease, which causes infections of your liver, and there are five types of hepatitis, namely types A, B, C, D and E. Hepatitis B (HBV) virus infects the liver, which carries DNA from the Hepadnaviridae family. A variety of illnesses, including chronic hepatitis B and cirrhosis (Ren *et al*, 2012).

The prevalence of hepatitis B in developing countries accounts for over 60% and less than a fifth of all hepatic cancer in developed countries (Jemal *et al*, 2012).

As part of important factors thought to play an important role in the development of liver cancer such as exposure to carcinogens, toxins and genetic factors. The new type of self-generated RNA that does not encode to particular proteins MicroRNAs (miR / miRNAS) are the processes that inhibit or diminish mRNA stabilisation, which regulate gene expression in post-transcript processes (Saito *et al*, 2013; He *et al*, 2012).

Numerous studies have shown that microRNAs play a vital role in a variety of regulatory processes in eukaryotic cells of different species, including growth, apostatis, proliferation and differentiation (Wilfred *et al*, 2007). Many studies have verified that miRNAs are linked to the sensitivity of and prediction of various kinds of human cancers (Saito *et al*, 2013). There are several studies that have indicated that the genetic diversity of microRNAs, including miR-196a2C> T and miR-499A> G, plays a large role in different types of human cancers, including lung, breast, colon, rectal and stomach (He *et al*, 2012). There is a possibility that these gene mutations are related to hepatitis (Sayan *et al*, 2011) and several mutations have been discovered in miRNA, miR-196a-2 and miR-106b-25, which are closely related to hepatitis B (Liu *et al*, 2012).

In addition, many recent studies indicated that miRNA inhibits many pathways of viral infection and viruses that possess miRNA molecules regulate the gene expression of these viruses and thus contribute to pathogenic events of the virus (Zheng *et al*, 2011). Therefore, miRNA may be a significant and important regulator in the interactions of the Host Virus and in controlling the replication of the virus. Microrneal genetic diversity is documented in a variety of studies, including miR — 146aG >C, miR — 134C > T, miR — 196a2 C > T and G — 499A > G; this may affect the risk of hepatitis B and liver cancer.

The abnormal expression of miRNA in the liver may play an important role in the pathological processes of

various liver disorders, due to the critical role that miRNA plays in many biological processes. It has also been found that the gene expression of miRNA has a role in liver diseases, including hepatitis B / HCV infection, cirrhosis and liver cancer, along with the function of gene expression for other molecules such as SIRNA (Wang *et al*, 2012).

The aim of this research is whether there is a relationship between the polymorphism in microrna single nucleotides and viral hepatitis in hospitalized patients in Babylon province.

MATERIALS AND METHODS

Sampling

In the Hepatic region, the Babylon Province and Iraq, thirty blood samples were collected and 30 blood samples were taken.

DNA extraction

Genomic DNA from whole blood cells was extracted and purified using the Favergen Company (Taiwan) Extraction and Purification Kit.

Genotypic Identification using RFLP-PCR amplification

A primary was applied to classify pre-microRna196a2 (rsss11614913) from Bioneer, IDTDNA(USA) and to enhance targeted DNA sites by unique primers. Prime: 5-CCCCTTCCCCTCCCA-3 and reverse 5-CGAAACCGACTGATGAAC-3 series are forwards. Primary:

PCRs were done in 20 × 1 ul reverse and forward primer, 12,5 liters of green mix, 3 liters of genomic DNA and 20 liters of volume of reaction, including 2.5 liters of free nuclear water. A two minute intensification has been carried out with a thermocycler at 94°C; 35 cycles, 5 minutes each with 94°C; 57.8°C at one point; 72°C at one minute; PCR elements were electrophoresized with an electrophoresis of 1 per cent agarose gel at 75 V for 1 hour and imaged with ethidiumbromide. Using the gel documentation system, photographs have been taken. The PCR product was canceled using MSPI endonuclease control. With the Promega Company Protocol, the PCR-RFLP approach was compatible. Following digestion with MSPI the electrophorosis gel electrophoresis (Cleaver Science, UK) was linked to: 75 V and 20 Am for 160 mins after the ethidium bromide gels in 3% agarosis gels at 75 V for 1 hour electroPhoresis control. A Usage of the gel recording system (EBOXCX-UK) to take the photos.

Statistical analysis

The P- values < 0. 05 were statistically meaningful by the thought-about statistical meaning of all realistic math analysis of the SPSS program (17; SPSS Inc., Chicago, IL).

RESULTS

Genotyping study

A pre-microRna196a2 (rs11614913) electrophoresis agarose gel showed up to 149bp (Fig. 1). MSPI restriction enzyme was found to have three forms of genotic polymorphisms, including two-group homozygous genotypes (CC) of 125bp and 24bp, the second was one DNA-band homozygous (TT) genotype (149bp) and the third example was heterozygus (TT) genotype.

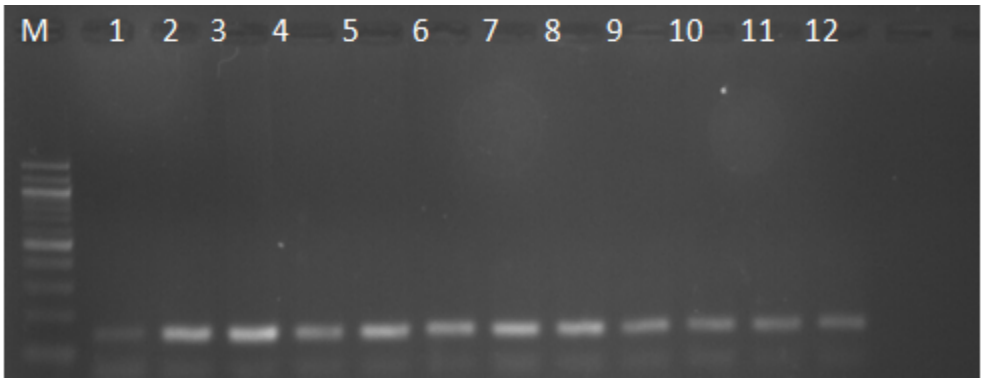

Fig. 1 : Agarose gel electrophoresis of pre-microRna196a2 (rs 11614913) amplification products of patients and control groups.

Table 1 : Genotyping distribution of pre-microRna196a2 (rs s11614913) polymorphism and there association in control and patients groups.

Genotype	Patients %	Control %	P Value	OR=(95%CI)
TT[a]	11(36.6)	17(56.6)		
TC	15(50)	9(30)	0.08	0.38(0.12- 1.19)
CC	4(13.3)	4(13.3)	0.44	0.64(0.13-3.14)
Allele				
T	27	43	0.043*	0.46(0.21-1.02)
C	23	17		

P ≤ 0.05, OR = (95%CI), [a]reference.

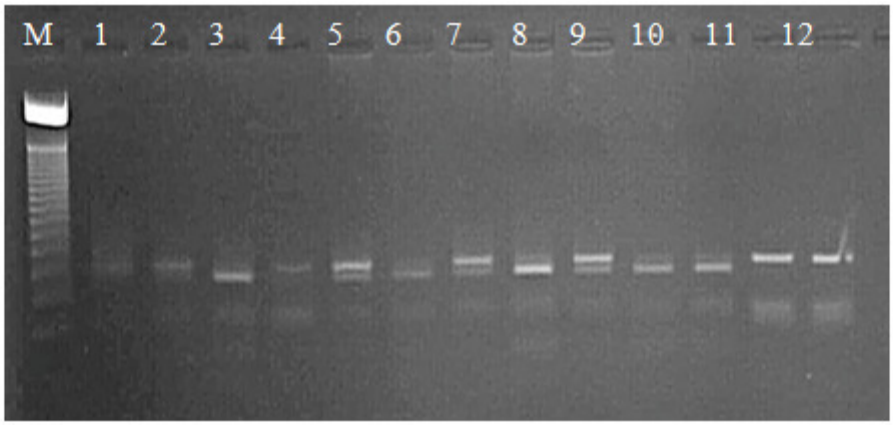

Fig. 2 : Agarose gel electrophoresis of patients and healthy subjects allelotyping (rs11614913 SNP) using MspI enzyme by PCR-RFLP method.

DISCUSSION

In the current study, the study of MIR196A2 T > C (rs11614913) and control samples of the patients included microRNA-gene polymorphism; in the current research, the findings show that the MicroRNA-gene polymorphism of control and control groups was shown in Table 1. The highest genotype was in the control group is the heterozygous TT genotype (56.6%) followed by the heterozygous TC genotype (30%) the homozygous CC genotype (13.3%) and in the patient group, the highest genotype was TC heterozygote (50%). The genotyping distribution indicated that the normal TT homozygous was more than the CC heterozygous mutant, which reached (36.6%), (13.3%), respectively.

Hepatitis B is one of the primary causes of hebatic diseases such as chronic hepatitis and HBV-infected cirrhosis (Ren *et al*, 2012). Additional factors may also lead to infection with the hepatitis B virus., *e.g.* genetic mutations of microRNA (Gao *et al*, 2011; Lu *et al*, 2012), both of which are currently under study and on a large scale. An association between the genetic diversity in MicroRNA and susceptibility to viral hepatitis B infection was also investigated, as Gao *et al* (2011) found that the deregulation of miRNA gene expression has a significant role in the development of hepatitis B virus-associated liver cancer and accumulates with advanced and different stages of liver cancer, which may lead to disease progression.

Many studies include the interaction between the genetic variants of MicroRNA and hepatitis B with the number of samples used or often conflicting in such studies. Thus, such studies may not be sufficiently robust to identify the minor effects of hepatitis B polymorphism of microRNA. The results of the statistical analysis of samples study attempted to understand the relationships between microRNA polymorphism and the risk of infection with hepatitis B. It was investigated several targeted genetic variants in the study by miR-196a-2, miR-146a, in which the presence of microRNA binding strength was revealed, adversely affecting target gene regulation and the susceptibility of people to hepatitis B infection (Liu *et al*, 2012; Xiang *et al*, 2012).

Important linkages between miR-196a-2 polymorphism, static analysis results from ten independent studies suggest that mication RNAs play an integral role in various hepatitis B processes including immune response and tumorigenesis. This is also seen in the results of ten independent studies. MicroRNA mutations can not only influence the interactions of hepatitis B and the host immune system; they can also affect many stages of malignant processes such as carcinogenesis, development and invasion of tumours and our findings indicate the promising target of immunotherapy for microRNA, early detection and hepatitis B intervention.

Mir–196a2C > T was shown to be associated with HCC risk in this analysis. Multiple cancer types, including colorectal, breast, pancreatic, gastrointestinal, and pulmonary cancer, have been found to play an significant part in T polymorphisms (Yuan *et al*, 2013). Further Chinese research has shown patients with HCC, with TT genotype miR 196a2 and T alone, that the HCC risk has been significantly increased (Hao *et al*, 2013). A recent study has also shown that the polymorphism of miR 196a2C > T is non-susceptible to HCC, but can cause HBV mutations. The findings of different studies may be very conflicting due to the varying frequencies in the development of liver cancer at different stages (HCC) among the ethnic groups used in the studies and the miR 196a2 C > T Genotype may be broad and different depending on the groups used to study it (Han *et al*, 2012).

CONCLUSION

We appear to assess critical differences between

HBV patients and controls using P=0.043, OR = 0.46(0.21-1.02) within the miR-196a2 genotype range.

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