Meta Gene 16 (2018) 139–142

Contents lists available at ScienceDirect

Meta Gene

journal homepage: www.elsevier.com/locate/mgene

Association of MicroRNA related single nucleotide polymorphisms 196A-2 and 499 with the risk of hepatocellular carcinoma in Egyptian patients

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ARTICLE INFO

Keywords:
MicroRNA
Polymorphisms
Hepatocellular carcinoma
MiR- SNP
PCR-RFLP

ABSTRACT

Background: Many studies revealed that several micro RNA-SNPs (MIR-SNPs) act as genetic risk factors in development of hepatocellular carcinoma (HCC) in different populations either alone or with environmental risk factors.
Objective: To study the role of two hsa-miR (-196a-2 C > T rs11614913 and -499 A > G rs3746444) polymorphisms individually, with each other and with some environmental risk factors in HCC Egyptian patients.
Subjects and methods: This study is a retrospective study that was conducted on 2 groups; HCC group and control group each included 50 subjects. The polymerase chain reaction-restriction fragment length polymorphism technique (PCR-RFLP) was performed for genotyping of the two pre MIR-SNPs. The relationships of polymorphisms with the HCC risk were estimated by conditional logistic regression analysis.
Results: HCC patients who carried the MIR-499 GG genotype had significantly increased HCC risk (*p*-value = 0.004) while, there was no significant association between MIR-196A2 gene polymorphism and HCC risk (*p*-value = 0.49). Moreover, there was a significant correlation between the interaction of MIR-SNPs-196A-2 and infection with HCV (*p*-value = 0.004).
Conclusions: MIR-SNP-499 might affect the susceptibility of Egyptians to HCC while, MIR-SNP-196A-2 may affect the HCC risk in Egyptians with HCV infection.

1. Introduction

Hepatocellular carcinoma (HCC) is the most widely recognized primary liver tumor (Balogh et al., 2016). The most recognized HCC environmental risk factors are hepatitis B virus (HBV) or/and hepatitis C virus (HCV) chronic infections, cirrhosis, aflatoxin B1 and excessive alcohol consumbtion (Mittal and El-Serag, 2013). However, only 10% of the exposed persons may suffer from HCC throughout their life. Consequently, genetic factors may have a significant role in the HCC development. The interaction between different risk factors, genetic and environmental, might be the chief reason for HCC development (Yu and Yuan, 2004).

Micro RNAs (MIR/MIRNAs) are endogenous non-coding RNAs that control expression of genes by either preventing translation or diminishing mRNA stability (Valencia-Sanchez et al., 2006). MIRNAs play important roles in development, apoptosis, propagation and discrimination of eukaryotic cells of different organisms (Li et al., 2009).

A number of previous studies in different populations have been studied the correlation between genetic variation in MIRs genes and the vulnerability and development of diverse malignancy (Ryan et al., 2010) including HCC (P1 et al., 2010; Xiang et al., 2012). In particular, SNP in MIR196A2 present in mature location and MIR499 present in seed location are likely connected with HCC hazard in other populations (Xiang et al., 2012; Qi et al., 2010; Pipan et al., 2015). Collectively, we performed this case control study to investigate the correlation between each of MIR-SNP-196A-2C > T and -499 A > G and risk of HCC. Add to that, to explore the role of their interaction with HCV infection as an environmental risk factor and risk of HCC in Egyptian patients.

2. Materials and methods

This study is a case control study that was conducted on one hundred subjects who attended to National Cancer Institute of Egypt in the period from October 2014 to December 2014. A written agreement was gained from all contributors and the institution ethical committee permitted the study. The subjects were divided into two groups: fifty HCC patients were diagnosed by triphasic spiral CT and fifty control

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https://doi.org/10.1016/j.mgene.2018.02.007
Received 29 October 2017; Received in revised form 15 February 2018; Accepted 19 February 2018
Available online 21 February 2018
2214-5400/

subjects (healthy blood donors negative for anti-HCV and HBsAg). Any subject has a history of tumor, liver disease, renal disease, coronary artery disease or other metabolic disorders was excluded (Hao et al., 2014). All participants were subjected to full clinical examination, assessment of viral markers for HCV and HBV and determination of Alpha fetoprotein (AFP) using Pointe Scientific ELISA kit (Cat. No. TM 1009). Also, abdominal triphasic spiral CT and tumor-node-metastasis (TNM) classification were done only for HCC group.

2.1. DNA extraction and genotyping

Five ml blood sample was obtained from every participant then divided into 2 parts. Two ml blood sample was taken into EDTA tube for DNA and RNA extraction to be used for qualitative polymerase chain reaction (qPCR) for HCV assessment and genotyping for both MIR-SNPs using the PCR-RFLP method. The remaining blood sample was centrifuged and the serum separated then kept at −80 °C until estimation of serological markers and AFP.

DNA was obtained from whole blood samples by the QIA amp® DNA Mini kit (Qiagen, Catalog No. 51106, Santa Clarita, USA) according to the manufacturer's instructions. PCR-RFLP was done to determine the MIR196A2C > T and 499 A > G genotypes. The primers and products of MIR-196A-2 C > T and -499 A > G were used as defined before (Jang et al., 2011).

The PCR cycling settings that were utilized: melting stage for five minutes at 95 °C then 35 series of denaturation at 94 °C for 30 s then annealing at 64 °C for 30 s with a last extension at 72 °C for ten minutes. Reproducibility was confirmed by duplicate examination of arbitrarily nominated subcategory of 10% of the contributors. *Hsa-mir-196A-2* rs11614913 (C > T polymorphism was processed by Thermo Scientific Fast Digest® *Msp*I (Cat. no. FD0544) while *Hsa-mir-499* A > G polymorphism rs3746444 was processed by Thermo Scientific Fast Digest® *BCLI* (Cat. no. FD0544).

Statistical analysis of results was done utilizing SPSS program version 20.0 (SPSS, Inc., Chicago, IL, USA) for Windows. Continuous data are displayed as the mean ± Standard error of the mean (SEM) and nominal data are exhibited as frequencies and percentages (%). Variation in the dispersion of demographic features between the HCC group and control group were examined by $\chi 2$ test for nominal variable and Student's *t*-test for continuous variables. The $\chi 2$ test was utilized for evaluating the Hardy-Weinberg equilibrium of the genotype frequencies of MIR196A2 C > T and MIR499 A > G in the controls. The correlation between MIR196A2 C > T or MIR499 A > G polymorphisms and the HCC hazard was assessed by using odds ratios (ORs) and its 95% confidence intervals (CIs) that was tested by conditional logistic regression analyses. A homozygous genotype was considered as a reference for calculating ORs. All *P*-values were two sided and $P < 0.05$ was considered as a statistically significant (see Table 3).

3. Discussion

In the current study, demographic data of the two studied groups was represented to show that the two groups were matched in base line characters to avoid selection bias. This came in agreement with (Vandenbroucke and Pearce, 2012) (Vandenbroucke and Pearce, 2012). Environmental risk factors for HCC included HCV and HBV infections and there was a significant variation between the two studied groups in prevalence of HCV but there was no significant variation among them in HBV prevalence. These results are in accordance with that revealed in Egypt as HCV infection is considered an important risk factor in liver cancer etiology (Omar et al., 2013). Furthermore, AFP serum levels were significantly higher in HCC patients in relation to controls. These results are similar to a study done by Shaheen et al. (2015). This elevation can be explained by alternation of hepatocyte-hepatocyte communication and the damage of normal architectural arrangements (Xu et al., 2014).

Table 1
Clinicopathological characteristics of the studied groups.

Demographic feature	Controls (50 subjects)	HCC group (50 patients)	*P*-value
Age (years)			0.4
Range	40–74	38–76	
Mean ± SD	54.4 ± 8.5	55.82 ± 8.3	
Sex			0.26*
Male N(%)	35 (70%)	39 (78%)	
Female N(%)	15 (30%)	11 (22%)	
Smoking habits			0.64**
Smoke N(%)	17 (34%)	15 (34%)	
Non-smoke N(%)	33 (66%)	35 (66%)	
HCV Ab			< 0.0001***
Positive N(%)	0	46 (92%)	
Negative N (%)	50 (100%)	4 (8%)	
HCV PCR			
Positive N (%)	0	44 (88%)	
Negative N (%)	50 (100%)	6 (12%)	
HBs Ag			0.118
Positive N (%)	0	3 (6%)	
Negative N (%)	50 (100%)	47 (94%)	
HBcAb			
Positive N (%)	31 (62%)	38 (76%)	
Negative N (%)	19 (38%)	12 (24%)	
Both negative (PCR HCV and HBsAg)			
N (%)	50 (100%)	4 (8%)	
Both positive (PCR HCV and HbsAg)			
N (%)	0	1 (2%)	
AFP (ng/ml)			< 0.0001***
Rang	2–8	10.8–1059	
Mean ± SD	4.86 ± 1.49	518.4 ± 476.3	
TNM stage			
I N (%)		12 (24%)	
II N (%)		19 (38%)	
III N (%)		6 (12%)	
IV N (%)		13 (26%)	

The Demographic data; some HCC environmental risk factors and clinical characters of all subjects are presented in Table 1. No significant variation was found between the two studied groups in age, sex and smoking (P-value = 0.4, 0.26 and 0.64, respectively). In addition, HCV infection (88%) is more predominant than HBV (6%) in HCC group. For the clinical characteristics, 54% of HCC patients were TNM stage I and II, while 28% were in stage III and IV and 24% couldn't be assessed. The serum level of AFP was significantly increased in HCC group than controls.

* =p < 0.05.
** =$p < 0.001$.
*** =$p < 0.000$.

The present study revealed that the allele and genotype distributions of MIR196A2 C > T and MIR499 A > G in the control group in Hardy-Weinberg equilibrium ($P > 0.05$). This suggests that there was no population stratification and no selection bias (Li et al., 2015). Contrary to our expectation, distribution of MIR196A2 genotype was not significantly different between HCC cases and controls. Therefore, no significant association between HCC susceptibility and miR-SNP-196A-2 in our study population. This finding is a diverse from previous data that was showed by meta-analysis done by Peng et al. (2014) but this result is in harmony with the results of previous studies done by Li et al. (2015) and Han et al. (2013).

There was a significant difference in MIR499 genotypes and allelic frequency between the two studied groups which came in agreement for Chinese population and in Taiwan (Qi et al., 2014; Chu et al., 2014). Furthermore, meta-analysis done by Xu et al. (2015) found an association between MIR499 and HCC risk. However, these results disagree with studies done by Akkiz et al., (2011) for Turkish Population, Li et al., (2015) for Chinese population and Hu et al., (2013) for Asian population and one meta-analysis done by Zhu et al., (2016). Additionally, cases carrying MIR499 G allele had an increased risk for HCC. These results are in the same line with Zou and Zhao, (2013) who