Association of Mir-146aG>C Polymorphism with Gastric Cancer Susceptibility in an Iranian Population

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ABSTRACT

Background: Gastric cancer is one of the most prevalent types of cancer in the world, and the performance of some miRNAs as oncogene and tumor suppressor in carcinogenesis has been recently on focus. Furthermore, SNPs in miRNA specifically mir-146aG>C have been under investigation and numerous studies have been reported the relationship between this polymorphism and its potential to become a tumor in many types of cancer, especially those related to the digestive system such as gastric and liver cancer. Materials and Methods: Mir-146aG>C polymorphism was genotyped in 100 blood samples including 50 patients suffered cancer and 50 healthy individuals, using PCR-RFLP method. Results: No statistical significant association was found for the mir-146a G/C genotypes with susceptibility to gastric cancer risk. Conclusion: In the present study demonstrate that the mir-146aG>C polymorphisms was not association with gastric cancer risk, at least in the sample size studied here.

INTRODUCTION

Despite of decreasing gastric cancer in the past 50 years, it ranks the second in East Asia and third in the world, respectively, with regard to death rate and incidence[1, 2]. Although many developments have been reported in early diagnosis and treatment of gastric cancer, the interaction among host, environment and bacterial factors effect gastric cancer spread and there is still little chance of survival in advanced gastric cancer Patients[3, 4]. Moreover, many oncogenes and tumor suppressors have been reported to be effective in gastric cancer development. Nonetheless, molecular mechanisms effecting gastric carcinogenesis metastasis have not been fullyexplored yet[5]. In cancer carcinogenesis, some of miRNAs play important roles as oncogene and tumor suppressor[6-8]. miRNA is a category of endogenous single strand RNA, non-coding and appropriately 22 nucleotides, which act as gene regulator expression by pairing with 3 untranslated regions mRNA of target genes [9]. The miRNAs participate in adjusting many cell processes including reproduction, migration, differentiation and apoptosis[9-11].Single nucleotide polymorphisms have been found in most genesin recent years and lots of attention has been directed to investigate SNPs in miRNA. Studies showed that there is a relationship between SNP in miRNAs and gene expression change or miRNA function and risk of cancer. Also, several researches have been conducted to study polymorphisms especially mir-146aG>C which seems to be associated with many types of cancer. Nevertheless, the results have been in general contradictory[12-17]. Extensive researches suggested the effect of this polymorphism in tumorigenesis most cancers, especially in digestive system [18, 19]. This G/C SNP have alteredbase pairs: G to the C: U due to the mismatch inthe stemstructure of the precursor of miR-146a[20].Therefore, the purpose of the present study is to shed more light on the relationship between mir-146aG>C [rs2910164] and risk of gastric cancer in Iran.

MATERIALS AND METHODS

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Population:
This case study included 50 gastric cancer patients randomly selected from Iranian patients. 50 individuals without any sign of gastric diseases were selected from a referral to Fatemeh Zahra private laboratory. Blood samples were collected from October 2013 until June 2014. The disease was diagnosed using histopathology. All patients in terms of age and sex matched control individuals. Patients' consent was obtained with regard to using their blood sample for the purpose of the study.

DNA extraction:
1) 2 ml whole blood was collected from each participant into tubes containing EDTA-K and kept in -20°C until DNA extraction. Then, DNA genome was extracted from 100 µL of blood based on DNG\textsuperscript{15}. Plus solution protocol [SinaClon, Iran]. Appropriate concentration of DNA with higher OD than 1.6 were chosen for PCR and stored in two separate vial aliquots. One put for genotyping in -20°C and the rest in -70°C.

Genotyping assay:
Rs2910164 SNP situated in mir146a gene was selected for genotyping. Extracted DNA was genotyped by polymerase chain reaction restriction fragment length polymorphism [PCR-RFLP] assay. PCR were performed in total volume of 20 µl containing 100 ng DNA, 10 µL of 2x PCR Master Mix [Sina Clon, Iran] and 10 pmol from primers sense and antisense. The selected primers, after final confirmation with miRdsnp [http://mirdsnp.ccr.buffalo.edu] and BLAST website for DNA reproduction 147bp in PCR were as following: the sense primer CATGGGTGTGTACAGTGCAGCT and the antisense one TGCCCTTGCTGCTCCAGTCTTCCCAA. PCR reaction was optimized at 95 °C for 2 min, 35 cycles at 95°C for 30 seconds, 60°C for 30 and 72°C for 30 . finally proceed at 72°C for 5 min. For more validity, a negative control without DNA was PCR. The PCR products were run on a 2% agarose gel and stained by DNA Green Viewer. For RFLP analysis, PCR products were digested by FastDigest SacI, 1 µL of enzyme and 17 µL Water. Then, digestion was incubated at 37°C for 30 min and at 65°C for 5 min in [inactive mode]. Then, the digestion product was visualized by running on 2.5% agarose gel. PCR product with total length of 147 bp created 122 and 25 bp fragments for CC genotype and 147, 122 and 25 bps for GC genotype after digestion. The validity of the results was obtained by DNA sequencing of 10% of all PCR products randomly selected.

Data analysis:
Data analysis was performed by using SPSS Version 18.0 software. Fisher's exact test was used to investigate the relationship between mir146a G/C and gastric cancer. Individual genotype and allele frequency was measured using counting. Hardy-Weinberg equilibrium was used for comparing genotype observed frequency in case and control subjects. The level of significance was set to 95% confidence interval. The validity of GG genotype was considered as the reference [21, 22].

Results:
Genotypes and allele frequency:
The frequency of genotype and allele of mir146a and its relationship with gastric cancer susceptibility is summarized in Table 1.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cases [%]</th>
<th>Controls [%]</th>
<th>OR [95% CI]</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>38 [76]</td>
<td>36 [72]</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>CG</td>
<td>9 [18]</td>
<td>8 [16]</td>
<td>1.06 [0.3-3.06]</td>
<td>0.9</td>
</tr>
<tr>
<td>CC</td>
<td>3 [6]</td>
<td>6 [12]</td>
<td>0.4 [0.1-2.03]</td>
<td>0.4</td>
</tr>
<tr>
<td>GC + CC</td>
<td>12 [24]</td>
<td>14 [28]</td>
<td>0.8 [0.3-1.9]</td>
<td>0.6</td>
</tr>
<tr>
<td>G-allele</td>
<td>56 [85]</td>
<td>52 [80]</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>C-allele</td>
<td>44 [15]</td>
<td>48 [20]</td>
<td>0.8 [0.4-1.4]</td>
<td>0.5</td>
</tr>
</tbody>
</table>

As it is apparent, significant deviation is seen in Hardy-Weinberg equilibrium in the control group. In the analysis of the relationship between this SNP and cancer risk [Table2], it is GC genotypes has increased risk of gastric cancer [OR=1.06; 95% CI=0.3-3.06, P=0.9]. This trend was predicted in genotype frequency in Table 1.

Nevertheless, G allele frequency is more in old men suffering from the disease but notstatistical results confirm the relationship between polymorphism G allele in pre-mir146a and the risk of gastric cancer.

Discussion:
SNPs in miRNA, as a new category of applied polymorphism in human genome, play a significant role in cancer development [23]. SNP rs2910164 [C>G] was identified by Shen and his colleagues [20]. They discovered that this variant polymorphism is a potential cause of familial breast cancer and ovarian. In
the present study, the potential role of SNP rs2910164 in mir146a associated with gastric cancer susceptibility, for the first time, was investigated in Iran. The results showed that there was no statistically significant association between pre-miRNA-146a rs2910164 polymorphism and risk of gastric cancer, at least in the sample size studied here. However, to ensure the validity of the study should be further assessed with a larger sample and more accurate analysis. Numerous studies confirmed that there is a relationship between rs2910164 and many discrepancies such as prostate, liver, thyroid, and papillary thyroid carcinoma[22, 24-26]. Nonetheless, obtained results, regarding high risk of gastric cancer, have been contradictory and complicated. For instance, in a study in China, individuals with rs2910164 GG/GC have a higher risk of cancer compared to those with CC genotype[22]. However, results with small sample size are similar to the Turkish population. But a study with large sample size in Korea, indicated that there is not a significant relationship between rs2910146 and gastric cancer risk[27]. On the other hand, in a study conducted on Japanese population, gastric cancer susceptible in individuals with allele CC was higher than those with GG[17]. In general, heterogeneity of the results can be attributed to the small sample size and patients genetic and ethnic models. Since miRNAs indirectly play their biological role. SNPs in miRNA and the target genes may be effective in causing and developing gastric cancer[28]. Therefore, one of the limitations of these studies is that SNPs in the target genes of miRNAs have not been appropriately analyzed. Generally, in our study, despite the lack of statistical significant difference, it was revealed that Gallele in both control and experimental groups is more frequent than C allele. Taken together, further studies with large sample size will be necessary to validate the risk of cancer in carriers who had at least one miR-146a variant Callele.

**Conclusion:**

The present study conducted in Iran indicated that the potential of miR146a G/C polymorphism with the risk of gastric cancer needed further evaluation in large sample size. To validate the results of the study, more experiments should be done in different parts of the country and different ethnic groups.

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**REFERENCES**


