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The Effect of Polymorphism and Human Papilomavirus on Esophageal Cancer

Maryam Taghi Zoghi and Anna Boyajyan

Institute of Molecular Biology of the National Academy of Sciences of Armenia

Abstract: The p53 gene is one of the most extensively studied human genes because of its role as a tumor suppressor. A common polymorphism of the p53 gene at codon 72 has been associated with human cancer susceptibility and prognosis. Human papilloma virus (HPV) has also been suggested to be involved in the pathogenesis of esophageal cancers. In this study, we investigated codon 72 polymorphism in 40 esophagus cancer cases and 40 control samples in northern Iran. AS-PCR method was applied for determination of codon 72 polymorphism in the way that two pairs of primers have been used for identifying allels arginine and proline. Distribution of genotypes in esophagus cancer cases and controls were different (P=0.001). From 40 patients, 4 (%10) cases have been homozygote Arg/Arg, 22 (%55) cases have been heterozygote Arg/pro and 14 (%35) cases have been homozygote prolin. In none of the samples the HPV virus was observed that is in harmony with genotype results. In conclusion, this result can be considered as a probable reason in observing difference in frequency of different genotypes codon 72 in geographical regions.

Key words: P53 Codon 72 · HPV · Esophageal Cancer

INTRODUCTION

Esophageal carcinoma is one of the six most common malignant diseases in the world with a remarkable geographical distribution [1]. Environmental exposures such as tobacco, alcohol, chronic mucosal irritation and ethnic background increase the risk of developing. Moreover, genetic change affecting the p53 tumor suppressor such as different mutations, loss of heterozygosity (LOH) and high-risk human papillomavirus (HPV) infection are important for carcinogenesis in the esophagus [2]. The p53 gene has a common sequence polymorphism resulting in either proline (CCC) or arginine (CGC) at amino-acid position 72.This polymorphism occurs in the proline-rich domain of the protein which is necessary to induce apoptosis the Arg72 variant induce apoptosis more effectively than does the pro 72 variant [3]. In smokers, several studies have suggested an increased risk of lung cancer associated with the pro/pro genotype [4, 5]. In contrast, Arg/Arg homozygotes are frequently found in non-smoking patients with lung cancer [5, 6]. Allelic deletions detected as LOH have been proved useful for mapping regions of DNA that contain tumor suppressor genes [7]. Cancer lesions show a high frequency of LOH in the p53 tumor suppressor gene locus on chromosome 17p13.1 measured by repetitive DNA sequences (microsatellites),scattered widely within the genome [8]. The p53 codon 72 polymorphism is also affected by LOH in tumors [9]. Either arginine or proline could be lost by this mechanism. With the progression of (pre-) cancerous lesions in the esophagus, the rate of LOH at 17p13.1 raising the possibility that these changes may be one of the important mechanism driving precancerous lesions to esophageal carcinoma [1]. It was found previously that the p53 codon 72 arginine genotype is a high-risk factor for development of HPV associated cervical carcinoma [10]. The arginine allele was found to be more susceptible to degradation by HPV E6 protein than the proline allele, resulting in a high frequency of cervical SCC in individuals homozygous for arginine at the codon [9]. This raises the possibility that homozygous for codon Arg 72 may lead to an increased susceptibility

Corresponding Author: Maryam Taghi Zoghi, Institute of Molecular Biology of the National Academy of Sciences of Armenia, Tel: +98-911-393-53-79, Fax: +98-192-42-10-521.

to other type of HPV related cancers as well. HPV infection occurs infrequently in association with esophageal carcinoma in patients from North America [11, 12] but regularly in high-incidence areas like P.R. China. The p53 codon Arg72 homozygous genotype is one of the high-risk genetic factors for HPV associated ESCC among the Chinese population [13]. Especially HPV 16 and 18 encode two major oncoproteins, E6 and E7 and are implicated in the pathogenesis of carcinoma by deactivating the p53 tumor suppressor [14]. The aim of this study was to examine P53 codon 72 polymorphism and human papilomavirus on esophageal cancer in north Iran.

MATERIALS AND METHODS

This was a case control study conducted in north Iran. The total of 40 tumor biopsies and 40 other samples of the control group have been analysed. Biopsy specimens were collected from operation theatre of gastro endoscopies in the internal department of Shahid Rajai Tonekabon hospital. These samples were collected from 2008 to 2011. Tissue samples were stored in -20 -70 Degree centigrade (-20 -70°C).

Filtering DNA from Samples(by Fermentase Kit): Prior to DNA extraction, the sample should be digested for a night with digestion buffer 100ml and 2.5ml proteinase k. Mixing binding solution with tissue samples has been carried out with the ratio 1 to 3 (100ml to 300ml) and then 5ml of silica was added. Incubation was carried out for 5 minutes in the temperature of 55°C. Washing buffer was added to the settle and then vortex was performed. The quick centrifusion 3 times, for 5-10 seconds was performed and then DNA was extracted during this process and the result was analysed in Agarose gel 0.8 %.

p53 Polymorphism Analysis: Exon 4 of the p53 Gene containing the poly morphic sequence variant at codon 72 was analysed using direct genomic sequencing. The following primers have been used:

- Arg- primer F:5' –TCCCCCTTGCCGTCCCAA-3' (25 Pmol).
- Arg- primer R:5' – CTGGTGCAGGGGCCACGC-3' (25 Pmol).
- Pro- Primer F: 5'- GCCAGAGGCTGCTCCCCC- 3' (25- Pmol).
- Pro- Primer R: 5'- GCCAGAGGCTGCTCCCCC- 3' (25 Pmol.

Primer Name	PCR Temperature Profile				
Arg- Primer F	94°C (5')	94°C (30")	60°C (30")	72°C (30")	72°C (5')
Arg- Primer R					
Pro- Primer F	94°C (5')	94°C (30")	54°C (30")	72°C (30")	72°C (5')
Pro- Primer R					

HPV Detection and Identification: This step was also done the same as previous treatment with this difference that GP5+/GP6+Primers were used. The following primers have been used: Gp5+: 5'TTGGA TCCTTTGTTTACTGTGGTAGATACTAC_3',GP6+:5'_T TGGATCCGAAAAATAAACTGTAAATCATATTC_3'.

Primer Name	PCR Temperature Profile				
GP5	94°C (5')	94°C (30")	40°C (30")	72°C (30")	72°C (5')
GP6					

RESULTS

In this study, 40 esophageal cancer pations and 40 normal biopsys as control group have been sampled. The samples were kept in -20°C - 70°C. All the samples have been DNA extracted and then the result has been analyzed in gel Agaroz 0.8%.

The use of molecule weight marker, was due to making sure of the suitable quality of extracted DNA for PCR. As observed in Figure 1, the amount of extracted DNA fragility is little. Detection of codon 72 genotype, was done by AS-PCR method. Two pairs of primers for doing PCR, were used. The primers are designed for allels Arg/pro, just in the case of existence of sequence with the related allele, make the production of required segment. For prolin it is equals 177 bp and for allele Arg it equals to 141 bp (Figure 2).

Our findings showed that from 40 patients, 4 (%10) cases have been homozygote Arg/Arg, 22 (%55) cases have been heterozygote Arg/pro, 14 (%35) cases have been homozygote prolin. In contrast, in control group from 40 samples, 8 (%20) cases have been homozygote Arg/Arg, 24 (%60) cases hetrozygote prolin Arg and 8 (%20) cases have been hemozygote prolin. The distribution of genotypes in esophagus cancer cases and controls were significantly different (P=0.001).

The use of molecule weight marker, was due to making sure of the suitable quality of extracted DNA for PCR. As observed in Figure 3, the amount of extracted DNA fragility was little.

Analysis of HPV virus in esophageal cancer samples, revealing of the existence of HPV virus in esophagus cancer samples was done by PCR method. In this regard

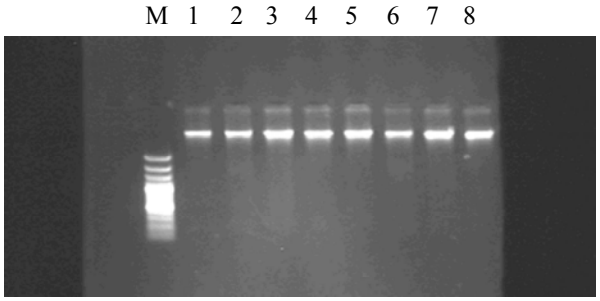

Fig. 1: Extracted DNA in gel Agarose %0.8 column M, in related to molecular weight marker define the marker and the other columns with numbers 1-8 are related to samples

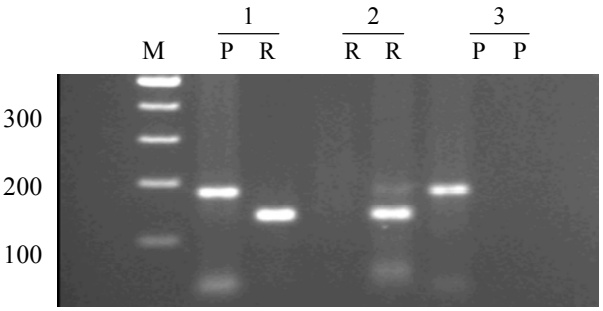

Fig. 2: PCR codon 72 production in Agarose gel 2% M column is for molecular weight marker. Each sample was examined once for Arg and again for pro so that the genotype of each sample to be specified, as it is with R and P for the samples 1 to 3. So the sample 1 has the genotype of RP and the sample 2 has the genotype of RR and the third sample has the genotype of PP

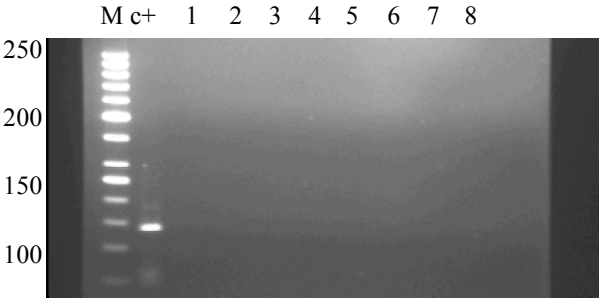

Fig. 3: PCR result of the sample with primer GP5+/GP6+. M (molecular weight) C+ is positive control and the other columns are for samples 1 to 8

a pair of primer called Gp5+ Gp6+ has been used and joined to a segment in the area ORF, gene L1 HPV virus and produced a segment 140 long. Protein L1 among the capsid virus proteins and is observed in all types HPV virus. Also primers Gp5+/Gp6+ pair, has the ability of linking to all type of HPV and is considered as a universal primer. There was no HPV affected area in esophagus cancer samples.

DISCUSSION

Several studies have revealed that in different types of cancers, one of the alleles show higher frequency, for example studying the role of codon 72 in patients affected to liver cancer showed that allele pro has higher frequency [15]. In this regard there are also some studies about the role of codon 72 p53 in lung cancer, stomach cancer breast cancer and so on [16, 17]. It is similar to the present findings that among the examined population of patients, Allele pro codon 72 had more frequency that represents its likely role in induction esophageal cancer in north Iran.

Furthermore, codon 72 has different frequency in ethnic populations and geographical situations and in this regard different studies have shown completely various results regarding the frequency of this codon. In another word, the frequency in allele p53 even in a case of esophageal cancer is extremely an important variable in different ethnics and geographical populations [18], So regarding this fact the population of caspian sea region, fails to have various races, the present research has been done and the result can be only justified as a probable reason in observing difference in frequency of different genotypes codon 72 in geographical regions but not the races.

The virus HPV is involved in some kinds of cancers and the DNA of this virus has been observed in skin, genitalia and oral tumors [19], But there is little information regarding HPV in malignant esophagus tumors. Several studies have been carried out regarding the effect HPV on esophageal cancer [20]. The results represent the existence of virus HPV in sample of esophageal cancer. In contrast with these findings, there are no results confirming the affection of HPV in the present examined patients in spite of using primers $Gp5^+$ &$Gp6^+$ which are capable of detection high risk types.

On the other hand, this fact may represent various frequency results of codon 72 in various geographical regions. According this fact that affection to virus HPV, as an environmental factors is extremely influenced by people life style and capability of this virus in induction esophageal cancer and it is possible in an Iranian setting to encounter less incidence of HPV virus because of cultural and Islamic recommendations.

Considering all the above stated it seems that there is a relation between the amount of affection to HPV and frequency of genotypes p53. In the case of high percentage of affection to HPV, the frequency of Allele Arg will be increased and in the case of non existing HPV, the frequency of Allele prolin, will be increased however it is needed more complete studies to be done in this regard.

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