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Title the Effect of P53 Codon 72 Polymorphism on Esophageal Cancer in North of Iran

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Abstract: The p53 gene is one of the most extensively studied human genes because of its role as a tumor suppressor gene. Its diverts functions include DNA binding, cell cycle control, DNA repair, differentiation, genomic plasticity and apoptosis.A common polymorphism of the p53 gene at codon 72 has been associated with human cancer susceptibility and prognosis. P53 mutations are seen in all kinds of hystologic cancers like colon cancer (%60), stomach cancer (%60), breast cancer (%20), lung cancer (%20), brain cancer (%40),esophagus cancer (%60) [12].In this study, we investigated codon 72 polymorphism in 40 esophagus cancer cases and 40 healthy individual in northern Iran. AS-PCR method was applied for determination of codon 72 polymorphism.From 40 pations, 4 (%10) cases have been homozygote Arg/Arg,22 (%55) cases have been heterozygote Arg/pro, 14 (%35) cases have been homozygote prolin.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 different (P=0.001). According to the result the Pro allel has an elavated frequency among patients.The different distribution of codon 72 genotypes in patients is a result of biochemical difference of two form of p53 (one with Arg at codon 72 and the other with pro). Arg form of P53 is a stronger apoptosis inducer; however this form is more vulnerable to proteasomic degradation. On the other hand,that Pro form of P53 is a strong transcriptional factor, it is a weaker apoptosis unducer. Generally according to the examined population of patients, Allele pro codon 72 had more frequency,that represents it's likely role in induction esophageal cancer in northern Iran.

Key word: P53 codon 72 · Esophageal cancer · North of Iran.

INTRODUCTION

Cancer of the esophagus is the eighth most common cancer worldwide with more than 400.000 case per year incidence [1,2].The 2 main type of esophageal cancer are squamous cell carcinoma and adenocarcinoma [3]. The cause of esophageal cancer is unknown, How ever, epidemiologic studies in several areas of the world suggest a relation ship with Alcohol, Tobacco, Nitrosamine, Vitamin deficiencies, Aflatoxin, Candidal and viral infectious [4,5,6].The incidence of esophageal cancer shows certain geographic variation South African countries, Iran china, India, Ceylon and Puerto- Rico are high incidence areas [7, 8, 9].Early esophageal cancer may not cause [10]. Diagnosis in people without symptom is rare and usually accidental (because of tests done to check other medical problems). Unfortunately, most esophageal cancer does not cause symptoms until they have reached an advanced stage, when acure is less likely. Many risk factors play a role in the etiology of esophageal cancer, although these vary with geographic region. For example, betel chewing and oral consumption of opium are factors primarily found in southeast Asia and the Caspian sea area. P53 as transcription factor, has a role of diagnosing inner and outer signals and indeed has the role of keeping stability of hereditary material. Generally each factor that causes changes in DNA, causes the cell cycle arrest and reparation of the damage or apoptosis. Destroying the activity of p53 is very current in carcinogen process and it seems that it is

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regarded as a prerequisite for this stage. Making mutation in p53 is observed in more than 50% of all human cancer [10].Today there are more than 2000 scientific essay that introduce mutation in p53 in wide groups of cancers as the most current genetic changes [11]. This gene has 10 known polymorphism. One of these polymorphism that has been studied a lot, exists in codon 72. This polymorphism causes producing two forms of p53 molecule which are in 72 codon have Arg or pro [12]. frequency of allele gene p53 in codon 72 in different population and in different geographical areas is variable[13].The aim of this study was to examine P53 codon 72 polymorphism on esophageal cancer in north of Iran.

MATERIAL AND METHOD

The total of 40 tumor biopsies plus 40 samples of the control groups have been analysed in the north of Iran.biopsy specimens were collected from operation theatre of gastro endoscopies in the internal department of chahid Rajai tonekabon hospital. This samples were collected from 2008 to 2011. Tissue samples were stored in -20 -70.

Filtering Dna from Samples: (by fermentase kit):First 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.Ancubation was carried out for 5miniutes in the temperature of 55c.washing buffer was added to the settle and Then vortex was performed.the quick centrifusion 3times, 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)

RESULT

In this project 40 esophageal cancer pations and 40 Normal Biopsys for control group have been sampled. The samples were kept in -20 °C- 70 °C. All the samples as mentioned in the last part, have been DNA extracted and then the result has been analysed 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. As mentioned already in part 2 pairs of primers for doing PCR, were used.2 pairs of primers that are designed for Allels Arg/pro, just in the case of existence of sequence with the related Allel, make the production of required segment. For prolin it is equals 177 bp and for Allel Arg it equals to 141 bp (Figure 2). From 40 pations, 4 (%10) cases have been homozygote Arg/Arg,22 (%55) cases have been heterozygote Arg/pro,

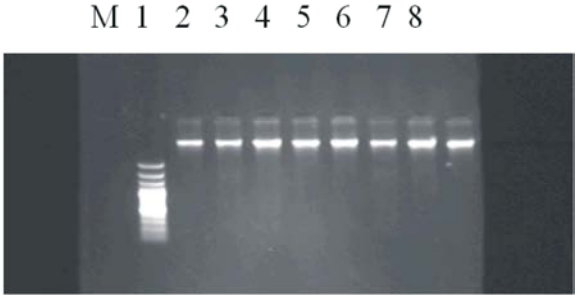

Fig. 1: Extracted DNA in gel Agarose %0.8 column M, in related to molecular weight marker and the other columns with numbers 1-8 are related to samples.

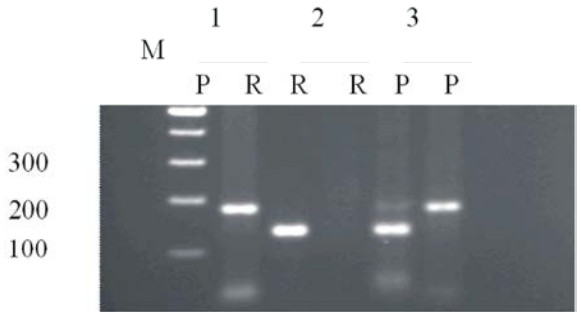

Fig. 2: PCR codon 72 production in Agarose gel 2% M column is for molecular weight marker. Each sample was examined once for Arg and again for pro so that the genotype of each sample to be specified, as it is with R and P for the samples 1 to 3. So the sample 1 has the genotype of RP and the sample 2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

14 (%35) cases have been homozygote prolin.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 different (P=0.001).

DISCUSSION

Cancer of esophagus is the eighth most common cancer world wide with more than 400000 case per year incidence[1,2]. The cause of esophageal cancer in unknown, However epidemiologic studies in several areas of the world suggest a relationship with alcohol, tobacco,nitrosamine,vitamin deficiencies aflatoxin, candidal and viral infections [4]. Esophageal cancer incidence increase with age – specific rate were very low below 50 year of age.conspared with women man have a 3 fold higher rate of esophageal cancer.Diet high fruits and vegetables diet is linked to a lower risk of esophageal cancer about %15 of esophageal cancer can be linked to a diet poor in fruits and vegetables Other than environmental factors,genetic factors have also an important role.Genetic changes cause increasing sensitivity to some environmental factors. Among the genes having the main role in induction cancer,P53 can be refered.Generally each factor that cause changes in DNA,cause activation of P53 and cell cycle arrest and reparation of damage or apoptosis.Destroying the activity of P53 is very current in carcinogen process and seems that it is regarded as a prerequisite for this stage.Making mutation in p53 is observed in more than %50 of all human cancer [14].P53 mutations are seem in all kinds of histological cancers like colon cancer (%60),stomach cancer (%60)lung cancer (%20),brain cancer (%48)esophagus cancer (%60) [15]. One of these polymorphism that has been studied a lot, exists in codon 72. This polymorphism causes producing two forms of p53 molecule which are in 72 codon have Arg or pro [12]. frequency of allele gene p53 in codon 72 in different population and in different geographical areas is variable[13].There is a lot of information regarding P53 in malignant esophagus tumor.Several studies have been carried out regarding the effect of P53 on esophageal cancer.Including the studies done by Prof. Ru Gang zhang Academy of medical science, Beiging, china - Dr. P.W. Lucas, 1997,Proff. Alfred K. Yalmy and colleagues from Honk Kong special administrative region - Mehmet Gundogdu Ataturk university of Erzurumturkey turkey 2001. According to the results of this study, the various rate between Alleles Arg and pro showed,prolin allele has higher frequency in esophageal cancer in northern Iran.And they all represent the effect of this protein on esophageal cancer.

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