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In Gastric Cancer, PCR-SSCP Is Being Performed to Recognize P53 Gene Mutations

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Abstract- Gastric adenocarcinoma is the second most common cancer in the world after lung cancer. About 500,755 cases per year new is identified. Approximately 12% of all cancer deaths are due to gastric cancer. One of the most important the genes that are important in preventing cancer are the P53 gene, which is involved in regulating the cell cycle. In some of Up to 50% of cancers are mutated in the P53 gene, of which about 87% of mutations are in exons 5 to 8. are. This study was performed to determine p53 gene mutations in gastric cancer by SSCP-PCR. Materials and Methods: During 2002, 44 biopsy specimens of gastric cancer patients were prepared from three hospital centers in Tehran. Demographic characteristics of patients and samples of patients by SSCP-PCR method to determine mutations in P53 gene was studied. Results, in this study, out of 44 patients with gastric cancer, 31 were male and 13 were female with a mean age of 60.8 years (from 34 years). Up to 84 years). Based on the type of cancer, 36 cases were intestinal (81.8%) and 5 (11.4%) were diffuse. Three cases were not included in either of the two groups with the information available. After performing SSCP-PCR in 9 cases Electrophoretic bands of patients' samples were different from normal samples. One mutation in exon 5 (11.1%), 2 mutations in Exons (22.2%), 3 mutations in exon (33.3%) and 3 mutations in exon (33.3%) were observed. Of these 9 mutations, 7 were related to intestinal cancer and 2 were diffuse. Significant relationship between age and type of cancer Not observed. There is also a significant relationship between the type of cancer and sex, between sex and the location of the mutation, as well as the type of cancer the mutation site was not seen. In the diffuse type, 2 mutations were observed in exons 6 and 8 and no mutations were observed in exons 5 and 7. was not observed. Mutations were seen in the intestinal type in all four exons studied Conclusion in this study, the incidence of mutation in P53 gene in gastric cancer was 20.5% which indicates the frequency he means mutation of p53 gene is in the study population.

Keywords: Gastric cancer, p53 gene, Mutation, SSCP-PCR, Intestinal.

Introduction

The genomes of all living organisms by internal chemical processes the body and by external factors such as chemicals and radiation the ionizer is always vulnerable to damage. There is a living by repairing DNA from the mutagenic effects of these factors and occur Cancer is protected. Whenever there is a defect in the connection with the system Inherited repair causes cancer to develop and progress. One of the most important of these genes is p53, which regulates the cycle Cellular involvement (p53 activation causes induction or inhibition) from another 150 genes (2). Lenin and Crawford first introduced p53 in 1979.Cell protein that binds to SV40 virus T antigen10 years later Finali et al Growth inhibitors and grade 1 showed that wild-type p53It is a cell (chromosomal locus of the p53 gene by Miller In 1986, the p53 gene on the short arm was identified Chromosome 17 is present. This gene has 11 exons and 10 introns Composed. In some cancers, such as colorectal cancer, it is almost about 50% of cases are mutated in the p53 gene, which is about 87% of cases of mutations are in exons 5 to 8 (5). 3 mutations, penetration of 2 p53 mutations, mainly by deletion It happens. More than 90% of p53 mutations in 4 points between codons 307-110 in the region that contains the exons It is 5-8. They occur (6. In most cancers, it is most common. Mutations occur in a specific region of the p53 gene. For example, in Hepatocellular carcinoma mutations are often found in codons 249 of the exon 7 The p53 gene is observed. While in gastric cancer No such situation has been observed so far (6) P53 is an important pre-advertising factor. It has a mutation in the progressive p53 gene and the patient has a long length Shorter lifespan than patients with mutations in the p53 gene Has not been, is (7). Gastric adenocarcinoma is the second most common cancer after cancer Lungs in the world. It is estimated to be 5005,755 per year New to be identified. The second half of the twentieth century with the world Reduce the incidence of gastric cancer and mortality due to this type Cancer has been associated (about 870,000 deaths the effect of gastric cancer occurred in 2000 It accounts for approximately 12% of all cancer deaths Becomes (9).Occurrence of this disease in some parts of the world as a result of correction Diet and food preparation methods are declining Although the incidence of gastric cancer in the late twentieth century Has decreased. But this reduction is limited to cancer the lower parts of the stomach. Number of new cases of cancer Proximal area of the stomach and adenocarcinoma at the junction of the esophagus and the stomach has been growing significantly since the mid-1980s Gives (11). In 1965 Lauren was of two types in terms of histology and described the second type 5 gastric adenocarcinoma. One river Which published a better model for understanding the etiology of this disease Can provide (12).Approximately 90% of gastric cancers are adenocarcinomas (13).The incidence of gastric cancer increases with age. Start The disease in most patients is between 50 and 70 years old. Less than 30 years are very rare. Noncardiac cancer in men It is almost twice as many as women (14). Areas at risk for gastric cancer It includes China and much of the southwestern United States The highest incidence of gastric cancer in Japan, USA Southern, Eastern Europe and races are found in the Middle East. At Chile and Costa Rica 40 percent

of gastric cancer deaths It is one hundred thousand people. While in areas with low incidence New Zealand and Australia have a mortality rate of 10 per cent (16. (The highest rate reported in Iran related to Ardabil Is Gastric cancer accounts for one-third of all cancers in this region Includes Ardabil (17).This study was performed to determine mutations in P53 gene in patients with gastric cancer referred to three hospitals in Tehran Done.

Materials and Methods

During 2002, 44 gastric biopsy specimens from patients undergoing Stomach cancer has been proven in them from three hospitals Hazrat Rasool (PBUH), Firoozgoo Hospital Dr. Shariati of Tehran was collected. From paraffin molds of these samples by device the microtome was prepared in two sections with a thickness of 5 µm. The parts were paraffinized by xylol. Then brshaha with Absolute ethanol was washed. 5ml / 0 solution per sample Lubricant containing 100 mM HCl-tris, 4 mM Microliter 20 and X100 triton 0.1%, (PH = 8) EDTA Proteinase K was added at a concentration of 15 ml / mg. Samples overnight It was set at 56 degrees Celsius. After this period Samples were placed at 95 ° C for 10 minutes. In front of 1 in PCR microtube, 1 l of both primers Inverse 2 DNA polymerases from 0.5 µ l, 50 pmol concentration with 5 unit / 3l 3 Concentration with deoxy nucleotides from 0.2‹ l, DNA, MgCl2 containing 10 X PCR, 5 l, 10 mmol Sample with a concentration of 100ng and up to a volume of 50 l of sterile distilled water Was dumped.Set the heat cycle device for 35 cycles in this order For Examples 6 and 7, one minute at 94 degrees, one Minutes at 60 degrees and one minute at 72 degrees, for Exon 8One minute at 94 degrees, one minute at 58 degrees and one minute At 72 degrees and for Exon 5 one minute at 94 degrees, one minute at 55 degrees and one minute at 72 degrees. To perform SSCP 5 µl of PCR product with 20 µl From Solution containing 95% format in water, 20mmol/ l Na2EDTA, 0.5 l/ g bromophenol blue was mixed and left for 5 minutes 96 degrees was placed. After this period to prevent Reconnection of two strands of DNA immediately in an ice container were placed, Electrophoresis at room temperature on acrylamide gel with Concentration of 12.5% was performed for 8 hours at a voltage of 70. DNA strands by silver nitrate method are dyed as follows Were. 5 minutes in ethanol-acid-acetic solution-water (relative 20 minutes, 1 g / l soluble silver nitrate in 40 minutes, (60-10-30 In 15 l / g sodium hydroxide solution and 1.5 l / g formaldehyde in Finish for stopping and holding the gel in 5% solution Acetic acid was inserted. In this study, 10-SPSS statistical software and experiments T-test, chi-square and Fisher's exact test were used. The reliability of the study was 95% ($\alpha = 0.05$).

Results

Out of 44 patients with gastric cancer, 31 died (70.5%) And 13 were women (29.5%). The age of the male group is 34 years Up to 84 years (mean 59.6 14 14) and age group of women from 50 to 76 Year (mean 63.7 8 8.5). The lowest patient was 34 years old and the oldest patient was 84 years old. Mean age of all patients It was 60.8 12 12.7 years. Based on the

type of cancer, 36 cases of the type Intestines (81.8%) and 5 cases (11.4%) of published type They were. We could not find three samples with the available information in two groups Classify above. The mean age of intestinal patients was 61.7 12 12.7 and the mean the age of patients was 55.4 ± 16.04. During the test No significant relationship was observed between age and type of cancer. Out of 28 men, 2 were of the diffuse type (7.2%) and 26 were of the type the intestines (92.8%) were closed. 3 out of 13 people Published (23.1%) and 10 intestinal types (76.9%) they had. According to Fisher's exact test, there is a significant relationship between these types Cancer and sex were not observed. Has 9 samples after electrophoresis the bands were different from the other samples and the sample was natural. PCR and SSCP was repeated for all 9 cases and the previous results were confirmed. One mutation in exon 5, two mutations in exon 6, three mutations Exon 7 (Figure 1) and three mutations were observed in Exon 8. Of these 9 mutations, 7 are related to intestinal cancer and 2 The items were of the published type. Two mutations propagated in exons 6 and 8 Was observed and no mutations were observed in exons 5 and 7. At Intestinal type was seen in all four exons. age average Individuals whose mutations were observed in four exons 5-8, It was 54.6 17 17.6 years. The most concentrated person in whom mutation Was observed to be 35 years old and the oldest of them was 82 years old. According to the Chi-square test, there is a significant relationship between the sexes and the location of the mutation is also the type of cancer and the location of the mutant.

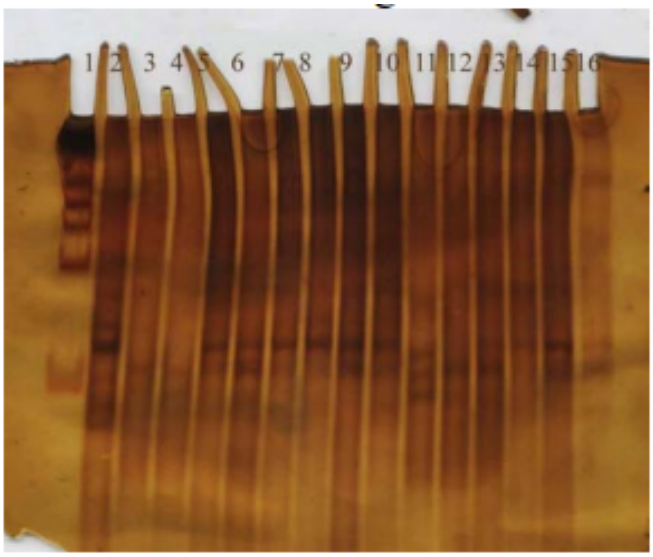

Figure 1.Electrophoresis of SSCP Exon 7 products

Wells: 16-15-13-11-10-9-8-6-5

- Normal electrophoretic motion

Well 11: Exon 7 PCR product with abnormal electrophoretic motion

Well 1: Molecular weight marker

Well 2: Un denatured sample

Wells 3-4-14: Repeated

(12.5% acrylamide, 15 ° C, 8 hours at 70 volts).

Conclusion

Cancer is a multi-step process that involves Genetic changes in cancer-causing genes and genes. It also suppresses cancer. The most common changes in an association with cancer has been observed to be associated with the p53 gene in particular Is in gastric cancer (18. (Gastric cancer is one of the most common types of cancer in Iran. Like most parts of the world, it is thought to be the second most common cancer It is common in Iran. In some parts of Iran, such as Ardabil Gastric cancer is the most common type of cancer (17. (Also, in Southeast of the Caspian Sea (Golestan province) Very esophageal cancer It is common (19.) These areas can be found in terms of the causes of the two the type of cancer is very important. According to reports Increased risk of gastric cardiac cancer (20). Golestan province is very important in terms of future studies Is. In the present study, out of 44 samples, 9 mutations (20.5%) seen. This rate is almost identical to the results published by Some researchers, such as Wang et al., Conducted in Taiwan (Yamada et al. In 19 cases of cancer the primary stomach did not show any mutation in p53 (22 One study found a 25% p53 mutation in primary gastric cancer in advanced gastric cancer, it was 42% (23. (but Levinti et al. Increase p53 mutation rate even in cancer Advanced stomach, did not get more than 26% (24. (a Report from Italy High rate of mutation in p53 gene (66.7%) shows (25). Reports of p53 gene mutations not only in cancer It is different in stomach but also in other cancers. In a study that In Iran, 54 cases were performed on p53 gene in esophageal cancer Mutations were observed in 74 samples (65%) (26). Et al., 61% of mutations in exons 5-8 of the p53 gene Primary breast cancer has been reported (Jasm et al.) This rate was reported in 29% of lung cancers Considering the frequency of mutations by each exon in Jasem's study Values were obtained, 33%, weight 5, 22% Exon 6, 16% Exon 7 and 29% Exon 8. Difference The extent of p53 mutations in various studies can be due to: Histological differences, sample source and diverse geographical areas Be. On the other hand, the sensitivity of the SSCP method is extremely dependent It is tested according to environmental conditions and skills. The combination of these factors can cause differences in the results. However, there is a statistically significant difference between age and type There was no cancer, but the average age of the patients was diffuse the type of lower intestines. This point is made by studying Parkin and et al. (14) who believed that the published type was more genetic and the type of intestines is more dependent on environmental factors. Because mainly disorders of hereditary origin at a younger age are updated. Mean age of men (59.6) compared to women (63.7) is lower. Justification of this result in addition to genetic issues. This may be because men are more at risk Are located. Due to the small number of mutations, perhaps the relationship between mutation status and

age is not very reliable. But in comparison, it can be said that the average age of people who in exon 7 they have mutations from other exons Is lower. This point needs more work. According to this Exon 7 has hotspots in terms of mutation and communication Mutation in codon 249 in hepatocellular carcinoma and Aflatox in has been shown to be effective (6, if Further research is likely to find one of the major causes of cancer the stomach is present in some parts of Iran.

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