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Research Article

Genetic Analysis in Exon2 of TP53 Gene Among Colorectal Cancer Patients in Najaf City

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Abstract:
Colorectal cancer (CRC), which is also known as cancer of the rectum or colon, is a significant contributor to cancer-related mortality on a global scale. Age, having polyps, having inflammatory bowel disease, lifestyle, having certain genes, and having a family medical history are all things that have been shown to put people at risk for CRC. The significance of the present study is in examining the mutations occurring in exon 2 of the TP53 gene, which acts as a tumor suppressor gene. The study aims to determine the presence of mutations that are linked to colorectal cancer and their impact on the effectiveness of chemotherapy treatment.
As well as to know the relationship between diagnosed mutations and between each (health status - tumor size – stage). Blood samples were collected from 25 patients with breast cancer from the collected by the Laboratory of Center of Oncology in AL-Najaf as they were compared with 10 who were apparently healthy. The diagnosis of mutation in both patients and healthy subjects has been studied and compared to see the effect of mutations on any of them.
As a conclusion the study showed that all eight mutations in exon2 of the TP53 gene in colon cancer could be diagnosed. DNA from blood samples was taken out several times, and the mutations were found by sequencing the DNA after it was amplified by PCR technology .The single nucleotide polymorphism (SNP) rs, identified in patients with colorectal cancer, has potential as a reliable predictive tool or genetic prediction test for colorectal cancer, particularly if it yields positive results in a larger sample size.

Keywords: *TP53* Gene, *P53*, Colorectal Cancer, Multiple Sequence Alignment (MSA), Oncogenes, Cancer in Najaf city.

Introduction

Colorectal cancer is a significant worldwide health issue, affecting around 1.7 million people each year. (Kamangar et al., 2006). Approximately 6.3 million individuals worldwide are affected by colorectal cancer, making it the second most often diagnosed cancer. It is predicted that there were over 1.8 million new cases of colorectal cancer in 2018 (Bray et al., 2018). There is also a high rate of death and loss

of good life years linked to this disease. In 2017, a total of 4856 individuals in Denmark received a diagnosis of colorectal cancer, Fand out of those, 3649 patients had surgical procedures. (Sharkas et al., 2017)

Some people with the disease has severe symptoms because of holes, blockages, or bleeding. About 10 to 30 percent of people who have surgery for colon cancer have severe symptoms of the disease, which is a life-threatening condition (Pilleron et al., 2018). The mortality rate following acute colorectal cancer surgery ranges from 6% to 22%. (Heriot et al, 2008). The death rates within 30 days and 90 days after acute surgery for colorectal cancer in Denmark were 11% and 21% correspondingly in 2016. Compared with elective surgery, the 30-day and 90-day postoperative mortality was only 1.4% and 3% ((Sharkas et al., 2017)), Postoperative complications are more likely to occur after acute surgery than to elective surgery, and medical issues following acute surgery are strongly associated with postoperative death (Schootman et al,.2014). From a long-term point of view, acute surgery is linked to lower overall survival, disease-free survival, and a higher chance of cancer coming back. Prior research has determined that those with a lower socioeconomic class face an increased likelihood of undergoing emergency surgery rather than elective treatment for colorectal cancer (Zhao, et al., 2019).

The TP53 gene is crucial for regulating the cell cycle, initiating programmed cell death (apoptosis), and repairing damaged DNA. The presence of the Arg/Pro polymorphism at codon 72 of the TP53 gene modifies the capacity of TP53 to initiate apoptosis, impacts the characteristics of mutant TP53, diminishes the ability to repair DNA, and maybe correlates with a heightened susceptibility to cancer (Hu et al., 2005, Zhao et al., 2018).

Aims of Study

The objective of the study is to do an analysis screen of mutations on exon 2 of the TP35 gene in Colorectal cancer, and then compare these alterations with those found in healthy individuals. Additionally, need to determine if there is a connection between the number of mutations and patient-specific variables including age, tumor grade, stage, and metastasis.

Materials and Methods

Sample collection

This study was conducted to examine 25 instances of colorectal cancer. The patients' ages spanned from 22 to 80 years. The clinical features of the patients were assessed, and the average age was found to be 49.6 ± 10.9 years (mean ± standard deviation), 76% (19 cases) of these are men and 24% (6 cases) are women.

A control group consisting of 10 male and female subjects who were in good health was included in the investigation. A total of individuals aged 22 to 78 years, with a mean age of 47.1 ± 14.9 years (mean ± standard deviation), who were asymptomatic

and showed no evidence of chronic diseases such as diabetes mellitus, heart diseases, renal diseases, or other conditions, were chosen to take part in this study.

Blood sampling

Blood samples were collected by the Laboratory of Department of Oncology in AL-Najaf. The Center of Oncology's Institutional Ethics Committee gave its permission for the samples to be collected, and all participants signed an informed consent form. The samples were then sent to the Molecular Biology Laboratory in the Department of Biology at the University of Kufa's College of Science, from October 2020 to January 2021. Each sample was 1 milliliter of blood that was put in a tube with EDTA and used to get the DNA.

Genotyping

DNA extraction

DNA extraction was optimized with the adoption of the variant using Wizard® Genomic DNA purification kit promega USA of whole blood protocol.

Isolating Genomic DNA from Whole Blood (300µl Sample Volume).

Polymerase chain reaction

The DNA under investigation was amplified using PCR technique. Utilizing the designated primer listed in table (1) for the target gene *(*Alzeydi et al., 2020).

Table 1. Primer sequences used to amplify exon2 of *TP53* gene (Alzeydi et al., 2020)

Gene	Primer sequences	
TP35	Forward primer	(5’ CTAGAGCCACCGTCCAGGGAG 3’)
Exon2	Reverse primer	(5’ AGTTTCATAGGTCTGAAAAT 3’.)

I also use a Thermocycler program of amplification The PCR thermocycler program that gave the best results of amplification of exon 2 as the fallowing in Table 2

Table 2. Thermocycler program for PCR amplification of *P35* gene.

Type of Cycle	Temperature	Time	No. of Cycles
Initial denaturation	95	5 min	1
Denaturation	95	1 min	35
Annealing	60	1 min	
Extension	72	1.2 min	
Final extension	72	5 min	1

Analysis sequencing

The polymerase chain reaction (PCR) products A total of 35 samples, consisting of 25 from patients and 10 from healthy individuals, were shipped to the company MacroGen in South Korea. The samples were packed in an ice bag and sent via DHL. The results of the DNA sequencing were obtained via email using the AB DNA sequencing system. The Migax64 software and SLC Sequence Viewer software were utilized for the analysis of the sequencing data in order to identify any mutations in the samples. This was achieved by comparing the observed DNA sequences of the local samples with the retrieved DNA sequences.

Statistical analysis

For statistical analyses, the Statistical Package for the Social Sciences (SPSS) (Version 22; SPSS, Inc., Chicago, IL, USA) program was utilized. The Chi-square test was employed to evaluate the correlation between mutation status and other factors. Significance was attributed to P values less than 0.05.

Results & Discussion

Results showed that out of 8 mutations identified, the one with the highest recurrence rate in patients was the M7 mutation Rs1418778734 SNP, which occurred in 26% of cases; the one with the lowest recurrence rate was in the M2 (rs786203938) gene, which did not contain any mutations and had a recurrence rate of zero in each case. In healthy subjects for exon2 the highest recurrence of mutations was absent as shown the table (3), figure 1.

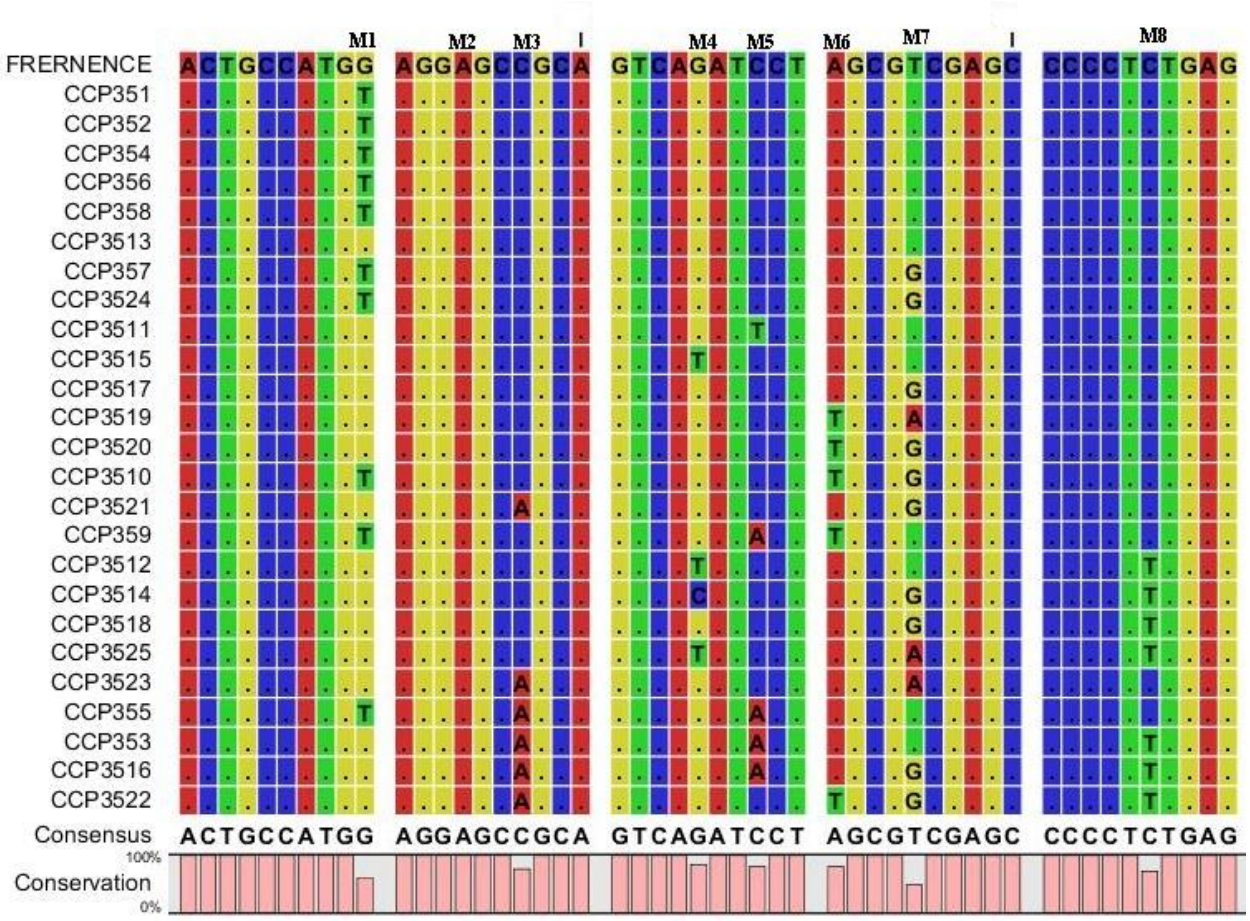

Figure 1. Showing multiple sequence *alignments* of a number of sequences showing mutations in exon 2 of TP35 gene in patints of colorectal cancer CC (25 Samples) and the reference according of NCBI site, the (M) termed to location of mutation in sequences of gene.

The results of the statistical analysis showed that there was a correlation between some mutations that were stronger between M1(rs 769884991) and each of M3(rs 878854064), M5 (rs1597376589) and M6 (rs1555527017) as well as a correlation between M2 and M3 as well as between M3 and M4(rs 587782646) and between M6 and M5 and each of (M8)(rs1567558112) as well as between M8 and M7 as shown in table 3.

As shown in our study covariance rs1567558112 missense variant or called L/V or (lysine / lysine) appeared in Seven patients (14.2%) and not in healthy people, and rs878854064 SNP called this variable appeared during this study with a frequency of in six patients (12.2%) and this study did not appear in healthy people and is considered relatively large.

We show in our study covariance in other frequency of missense variant in rs1597376589 was present in Five patients (10.2%) and the SNP rs 587782646 M4 and in four patients (8.1%) also same percentage in M6 rs1555527017 SNP in four patients (8.1%).

The mutation in second frequency (M2) called rs786203938 SNP, was absent in all 25 samples of colorectal cancer that is may lead to this frequency is rare in colorectal cancer Although Budhathoki mentioned it in celine of colorectal cancer cell (Budhathoki, K.et al. ,2016).

The study indicated the diagnosis of 8 mutations that were the highest recurrence in mutations in patients was the Rs1418778734 SNP(M7) mutation by 26.5% and the lowest recurrence was in the rs1567558112 (M8) absent.

In healthy subjects for exon2 the highest recurrence of mutations was M5 rs1597376589 30% and the lowest iterations were M3 (rs 878854064) SNP, Rs1418778734 SNP(M7) and rs1567558112 (M8), absent.

The study also showed that there was a correlation between some mutations that were

Table 3. descript all mutations frequency in exon2 of P35 gene of colorectal cancer (M)= mutation frequency

No.Of Lacation Of Mutation	Typ.Of Mut.	Sample	No.Of Mutations	**Persentage %**
M1	G/T	1,2,4,5,6,7,8,9,10,24	10	**20.4%**
M2	A	……..	0	**0**
M3	C/A	3,5,16,21,22,23	6	**12.2%**
M4	G/T/C	12,14,15,25	4	**8%**
M5	C/A/T	3,5,9,11,16	5	**10.2%**
M6	A/T	10,19,20,22	4	**8%**
M7	T/G/A	7,10,14,16,17,18,19,20,21,22,23,24,25	13	**26.5%**
M8	C/T	3,12,13,16,18,22,25	7	**14.2%**
Tatal			49	**100%**

stronger between rs 769884991 SNP (M1) and each of rs786203938 SNP (M2) and Rs1418778734 SNP (M7) Table 4.

As well as a there are correlation between rs786203938 SNP (M2) and rs 587782646 SNP (M4) as well as between rs 878854064 SNP (M3) and rs 587782646 SNP (M4)

and Rs1418778734 SNP and between rs1597376589 SNP (M5) and rs1555527017 SNP (M6) and each of Rs1418778734 SNP (M7), rs1567558112 SNP (M8).

Table 4. Shown coloration between Eight mutations in exon 2 fo *TP53* gene in colorectal cancer (M)= mutation frequency.

	M1	M2	M3	M4	M5	M6	M7	M8
M1	1	.479**	0.23	0.058	0.19	-.048-	0.452	0.136
M2		1	.294*	0.567	.269*	0.157	0.012	.238*
M3			1	.469**	.269*	.269*	0.433	-.018-
M4				1	0.174	.348**	.469**	0.085
M5					1	.464**	0.157	0.204
M6						1	0.157	0
M7							1	.567**
M8								1

The frequency of Mutations in the P35 gene for colorectal cancer was 23/25 (92%) and it is a large proportion when compared to previous studies where (Liu et al., 2019) showed that the percentage of P35 mutations in colorecta cancer is (0.54%) and (Baran, B et al., 2018) that the Percentage of mutations of P35 in colorecta cancer (4%) and the ratio was (56%) for a study conducted by (Joung et al., 2017) while (Dong et al., 2017) stated that the rate of mutations In P35 in colorecta cancer ranged between (16% -5%) and in a study conducted in China by (Lee et al., 2015) the rate of mutation recurrence in women with breast cancer was (76%)

The reason for the difference is due to the ethnic and geographical differences, as well as the sensitivity of the techniques for detecting the mutation (Mármol et al. ,2017), as well as the environment playing a role in influencing the mutations by increasing the carcinogenic pollutants in them such as Najaf Governorate (Alhesnawi, Alsalman and Najem, 2019) in addition to the small size of the studied samples (Zhao et al., 2019) Another reason is the lifestyle of each people and the quality of food (Mai et al., 2016; Li et al., 2017) .

The study found that there were significant differences (p = 0.042) between the number of cases of cancer and the presence of mutations. This suggests that there is a link between the presence of mutations and the number of cases of colon cancer. Also, the percentage of mutations in women with cancer showed 59%.

The statistical analysis revealed no statistically significant difference in the occurrence of mutations in relation to tumour size, metastasis, and age. How much did the study show the largest number (38 (54%) was in the T2 stage and the lowest number 4 (5,71)%) Were on stage T4, for a study conducted by Løes and her colleague (Løes et al.,2016).

As for metastasis, the study showed the largest number 16 (50%) of the patients were in the T3 stage and the lowest number 8 (zero) were in the T1 stage.

The study indicated that category (T2) had the highest percentage of mutations (44%) while the category (T4) contains the lowest percentage of mutations (4%). When compared to previous studies where by Schulz-Heddergott 2018, showed that the percentage of P35 mutations in colorecta cancer is in stage T1 highest (0.54%) while in stage T3 lowest percentage (15%) (Schulz-Heddergott et al., 2018).

DNA was extracted from the blood samples by several DNA extracts and mutations were detected by Sequencing analyzing after amplification by PCR technology.

The results of our study are consistent with the findings of the study conducted in China by (Zhao et al., 2019) and conducted with the use of cell-free plasma DNA to obtain the mutations. Similarly, it was identical to a study conducted in Iran that dealt with breast cancer and demonstrated the existence of the relationship between mutations and disease (Sanaei et al., 2017). It also agreement with the results of (Li et al., 2019). On the other hand, between (Nigro et al., 2016), through a study conducted there was no significant relationship between TP53 mutations and the appearance of a cancer tumor. These results were also identical to the study (Nakayama et al., 2019) on Caucasian residents. Also, (Russo et al., 2005) showed a slight increase in the risk of developing colorectal cancer.

The reason for the different results is due to several reasons, the quality of the samples used in such as blood or biopsy (Zhao, et al., 2019) as well as the quality of the techniques used in the examination and the size of the sample (Solomon et al. 2018). Also, multiple mutations inside the codons can lead to the biological activity of KRAS proteins changed (Zekri et al., 2016).

To summaries the study identified the presence of certain single nucleotide polymorphism (SNP) mutations in exon 2 of the TP53 gene in patients with colorectal cancer. There were notable disparities in the occurrence of mutations and the occurrence of cancer, suggesting a correlation between the existence of mutations and the occurrence of colorectal cancer.

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