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Research Article

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Association of P53 Gene and Colorectal Cancer in Iran

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Abstract

Background: *Colorectal cancer (CRC) is one of the leading causes of malignancies globally with a high mortality rate. Therefore, investigating the underlying mechanism and different risk factors contributing to this neoplasm is imperative, since P53 protein is a tumor suppressor protein, that has inhibiting effect on tumorigenesis, this study aims to assess the association of P53 gene and CRC within specific population, as geography is believed to be a risk factor for CRC.*

Materials and Methods: *In this retrospective case-control study, 90 patients aged 18 years old and above, with CRC were enrolled. They were all scrutinized before and after their surgery. Data were extracted from their medical record, laboratory test results, standard questionnaire, imaging reports, physical exam, related symptoms and colonoscopy and biopsy reports. Data analysis was performed using SPSS software and the chi-square method.*

Results: *90 patients participated in this study, with the mean age of 71.57 years. There was no statistically significant association between age of patients and P53 mutation. Similarly, no correlation between any of the symptoms and P53 gene mutation was found. However, the risk of P53 mutation increased with larger tumor size ($p<0.021$), deeper tissue invasion ($p<0.041$) and higher levels of lymph node involvement ($p<0.031$).*

Conclusion: *this study investigates the relationship between P53 mutation and various clinical and pathological parameters. A significant association was observed between P53 mutation and tumor characteristics, including larger tumor size, deeper tissue invasion, and increased lymph node involvement. These findings suggest that P53 mutation may serve as a potential biomarker for assessing tumor aggressiveness and prognosis in colorectal cancer.*

Keywords: Neoplasm – Colorectal Neoplasm – Tumor Suppressor Protein P53 _ Colorectal Cancer_ P53

1. Introduction

Colorectal cancer (CRC) ranks among the leading types of neoplasm and cause of death globally [1]. Age stands out as a major risk factor, and the chance of developing CRC increases after the age of 50 [2]. Alongside age, family history (first-degree family) hence genetics, sedentary lifestyle, diet (consumption of red and processed meat), inflammatory bowel disease, smoking, even geographic location variations, race, and ethnic background can be other associated risk factors for developing colorectal cancer in the long run [2,3]. The mortality of metastatic cases can soar as high as 14%, therefore it is essential to identify underlying mechanisms of its development. This knowledge not only enables the identification of individuals at risk, but also facilitates timely intervention in case of developing the disorder. Moreover, understanding the mechanism is necessary to identify targets to develop future therapeutic strategies [4].

A benign adenomatous colonic polyp can gradually progress into a high-grade dysplastic tissue. This slow and overtime progression is called "multistep tumorigenesis" which is associated with genetic mutation of tumor suppressors or oncogenes.

The most frequently mutated gene in humans is the one that codes p53 protein (TP53, known as the guardian of the genome) [5]. P53 regulates cellular response by inducing DNA repair, cell death, senescence, and cell cycle arrest (either permanent or transient) [6].

Long noncoding RNAs (lncRNAs) play an important role in maintaining the stemness of cancer cells. On the other hand, wild type P53 is an essential transcription factor for lncRNAs transcription in response to DNA damage or UV stress [7]. So, a single allele alteration in its corresponding gene (TP53) can

result in either loss of tumor-suppressing function or, gaining an oncogenic function, or even both [7]. Nevertheless, it can contribute to the acceleration of cancer development [8].

In this study, our objective is to evaluate the association of gene P53 and colorectal cancer within a specific population in Iran, given that geography and ethnicity are known to be a risk factor for colorectal cancer.

2. Material and Methods

This is a retrospective case-control study to assess the association between the gene p53 variants and the odds of developing colorectal cancer and was performed between March 2020 and February 2021 in the general surgery department of Shariati Hospital, Tehran, Iran. The study was approved by the ethics committee members of Tehran university of medical sciences.

2.1 Patients

The target population were male and female patients above 18 years old admitted to the Shariati hospital colorectal cancer and underwent colorectal surgeries based on tumor size and location. Each individual underwent pre- and post-surgery examination by a general surgery resident. Data were collected from their medical records, laboratory tests, standard questionnaire, imaging report, physical exam and symptoms (fatigue, abdominal discomfort, melena, change in bowel habit, incomplete defecation, weight loss), and colonoscopy and biopsy report. Patients above 18 years old who fulfilled the selection criteria were included in the study.

2.2 Immunohistochemistry (IHC)

The paraffin embedded tissue samples underwent IHC to detect responsible antigens of P53 gene. Harris Hematoxylin staining was applied for background coloration. XPO1 expression less than 5% was considered negative, while overexpression of XPO1 (above 5%) was considered positive.

2.3 DNA Extraction

Tumor tissue (25 mg) and adjacent normal tissue samples (25 mg) were dissected in operating room and placed in sterile 1.5 ml Eppendorf tubes, and sent to the pathology lab post-surgery. DNA extraction was performed Using 50X TEA buffer containing 10 mmol of proteinase K enzyme, 2 M Tris hydrochloric acid 0.5 mol EDTA, 0.1 M NaCl, 1 M sodium dodecyl sulfate 2%, PH close to 8.5. the tissue was incubated in temperature 55 °C for 3 hours to facilitate tissue digestion. We used PCR-SSCP to identify the mutations and immunohistochemistry to study the antigens in situ.

2.4 Data Analysis

patients were divided into two non-random groups based on p53 gene mutation status. Variables such as age, sex, axonal mutation, maximum tumor diameter, depth of tissue invasion, spindle apparatus, distant metastasis, lymph node invasion, primary symptoms such as abdominal discomfort, change in bowel movement, melena, fatigue, weight loss, incomplete defecation were evaluated. Patients under 18 years old and those with Inflammatory bowel disease, celiac disease, previous colorectal surgery, insufficient tissue sample for pathology, incomplete medical record and those who did not sign the consent form were excluded from the study.

Statistical analysis was performed using IBM SPSS version 22 software. The chi-square method assessed the association of DNA sequencing with SSCP or clinical-pathological variables. Descriptive and Frequency statistical tests determined case prevalence, while Independent T test compared the prevalence of colorectal cancer risk factors. Multivariate Logistic Regression statistical test analyzed each factors effectiveness as an independent factor for colorectal cancer incidence. All reported cited p values were two-sided and values less than 5% were considered as statistically significant.

3. Result

This is a retrospective case-control study aimed to assess the mutation of P53 gene and its association with colorectal cancer. Ninety patients diagnosed with colorectal cancer underwent surgery in Shariati hospital between 2020 and 2021 and their clinical and laboratory results were evaluated.

Of the 90 patients included in the study, 53 were male (58.9%) and 37 were female (41.1%). The age distribution is as presented in table-1.

P-value	Std. dev	Mean	Max	Min	Frequency	
0.605	3.06	71.43	79	62	53	Male
0.605	3.20	71.43	79	65	37	Female
0.605	3.10	71.57	79	62	90	Total

Table 1: Age Distribution

There was no significant age difference observed between male and female patients diagnosed with colorectal cancer.

Clinical features of the patients are illustrated in Figure-1.

Among the common clinical signs, 42 patients presented with abdominal pain, 42 patients experienced changes in bowel movements, 38 patients exhibited blood in their stool, 20 patients reported weakness and had fatigue, 10 patients had episode of incomplete defecation, and 5 patients reported weight loss. Among

these clinical features, abdominal pain (46.7%) and change in bowel habit (46.7%) were the most common.

Right colon was found to be the most frequent site of cancer occurrence (45.6%) among other locations such as sigmoid colon (31.1%), left colon (10%), transverse colon (8.9%), and unknown (4.4%).

There is no significant difference observed between abdominal pain and different locations of tumor (P-value=0.741). Similarly, no statistically significant difference was found between changes in bowel movement and tumor location (P-value=0.242).

According to Table-2, statistically significant relationship was observed between location of tumor and bleeding per rectum (P-value=0.006). However, no statistical relationship was found between the location of tumor and weakness, incomplete defecation, or weight loss (P-value= 0.867, 0.141, 0.793 respectively).

location	negative	positive	total
Right colon	**15**	**26**	**41**
Transverse colon	**7**	**1**	**8**
Left colon	**6**	**3**	**9**
Sigmoid colon	**21**	**7**	**28**
Unknown	**3**	**1**	**4**

Table 2: Melena or Hematochezia and Location of Tumor

According to analyzes patients with colorectal cancer had changing in their bowel movements (P-value=0.30), there is also a significant relation between presence of hematochezia and colorectal cancer (P-value=0.021).

Tumor size	Abdominal pain	Change in bowel habit	melena	weakness	Incomplete defecation	Weight loss
0-2 cm	7	2	3	0	0	0
2.1-4 cm	15	14	10	5	7	0
4.1-6 cm	9	15	14	8	2	1
>6 cm	11	11	11	7	2	4

Table 3: Prevalence of Clinical Features in Relation to Tumor Size

Histology study about tumor depth presents that 6.7% are T1, 15.6% are T2, 60% are T3, 13.3% are T4a and 4.4% are T4b.

In 61.1% of cases there was no lymph node involvement and the rest are presented on table-4.

Lymph node involvement	frequency	Percent
N0	55	61.1
N1a	11	12.2
N1b	11	12.2
N2a	9	10.0
N2b	4	4.4

Table 4: Lymph Node Involvement

No metastases were found in the patients. The study indicates a significant relationship between tumor invasion depth and lymph node involvement (P-value=0.00). Additionally, a relationship was found between the depth of tumor invasion in colon and the size of tumor, with a Spearman correlation coefficient of 0.490. Furthermore, it was stablished that there is a significant relationship between lymph node involvement and tumor size, with a Spearman correlation coefficient of 0.565.

Among population, 42 patients had normal P53 genes and 48 patients had mutated P53 genes. As it is illustrated in Table-5.

total	female	male		P53
42	18	24	Normal	
48	19	29	mutated	

Table 5: Demographic Features

No significant difference was found between male or female sex regarding P53 mutation occurrence. Similarly, no relationship was found between patient age and P53 mutation (P-value=0.722). Also, there is no statistically significant difference between tumor location, changing in bowel habit, abdominal pain, melena, weakness, incomplete defecation, weight loss, and P53 mutation (P-value=0.254, 0.187, 0.094, 0,371, 0.337, 0.595 and 0.564 respectively). However, a significant association was noted between tumor size and P53 mutation occurrence (P-value=0.021), as well as between deeper tumor invasion and P53 gene mutation (P-value=0.041).

According to table-6, patients with higher levels of lymph node involvement exhibited a higher frequency of P53 gene mutation.

N						
P-value	N2b	N2a	N1b	N1a	N0	
0.036	0	2	5	3	32	normal
	4	7	6	8	23	mutated

Table 6: Lymph Node Involvement and P53 Gene Mutation

4. Discussion

Colorectal cancer is one of the most common malignancies in gastrointestinal system and its etiology is not fully discovered and has a high mortality rate. while lifestyle factors, particularly Western dietary habits, contribute to its rising incidence [9]. CRC is a complex disease with genetic and biochemical background [10]. Among the key players, the tumor suppressor protein P53 plays a pivotal role in cell cycle, DNA repairment, apoptosis and senescence [11]. Recent genome-wide analysis mutation in APC, KRAS, SMAD4 and P53 genes are identified in colorectal cancer and about 80% of advanced CRC are associated with P53 gene mutation (APC mutation is the most frequent mutation observed in colorectal cancer specifically missense-type P53 mutations are significantly correlated with poor diagnosis in CRC [12,13]. An in-depth understanding of CRC pathophysiology and pathogenesis can help us enhance patient survival rates [14].

In our retrospective case-control study, we examined 90 CRC patients undergoing surgery, 53 of the patients were male and 37 of them were female with mean age of 71.57 years old. Our sample size aligns with comparable studies by Eman A.E. et al., and Arantza Farina Sarasqueta et al ensuring its reliability. The study reveals no significant gender-based or age-related association with CRC. Comparable patient demographics across existing literature, including studies by Antonio Russo et. al., Cao DZ et. al., and Samowitz et. al.and underscore the robustness of our findings. Variations in female predominance noted in study by Sukamal Saha et al. deemed negligible [15-20].

Consistent with prior research, changing bowel habits and abdominal pain emerged as predominant clinical symptom in our study that mirrors established patterns reported by Knut Holtedahl et al. and Mona Abdullah Alsayed et al. [21,22]. Moreover, our identification of right colon as the primary tumor site (45.6%) resonates with trends documented in prior studies by Sukamal Saha et al. (20), and Hiroko Nakagawa-Senda et al. [23].

Notably, our analysis revealed a significant association between tumor location and certain clinical symptom, particularly noting increased rectal bleeding in right-sided colorectal cancer (P-value=0.006), however discrepancies in symptom association with tumor location, as observed in our study compared to others, underscore the complexity of CRC manifestation.

Tumor size analysis suggest tumors size predominance of 2.1-4 cm. Consistent with findings by Sukmal Saha et al. who reported that most common tumor size were between 2-4 cm. on the other hand, size and location of tumors demonstrate no correlation, a trend supported by Sukmal Saha et al.

Histological staging predominantly revealed T3 and T2 stages, in alignment with findings by Karl Bilimoria et al., Sukmal Saha et al. and, Young Jin Park et al., underscoring the aggressive nature of CRC in our study. While lymph node involvement was minimal, a statistically significant correlation between T stage and N stage was observed, similar to Sjovall et al. study [24-26].

Our study identified P53 mutation in 48 patients (53.33%), aligning with reported prevalence rates by Agneta Jansson et al., Antonio Russo et al., Reiping Tang et al, Da-Zhong et al., and Mizuho Nakayama et al. [27,28]. Notably, we observed significant relationship between P53 mutation and tumor size, depth of invasion, and lymph node involvement. P53 gene mutations were more prevalent in larger tumors and those invading deeper tissues, indicating potential role of P53 alterations in tumor progression and aggressiveness. This observation is consistent with prior studies by Victor E. Pricolo et al. and Da-Zhong et al. [29].

However, our study diverged from the findings of Tang et al., who reported a significant correlation between tumor location and P53 mutation status. This may stem from difference in sample demographics and observation methods. Conversely, our results align with those of Carmen J. Allegra et al., suggesting no significant association between tumor location and P53 mutation, highlighting the complexity of P53 involvement in CRC [30].

Regarding demographic characteristics, our study agrees with Tang et al., Carmen. J. Allegra and Agneta Jansoon et al. who found no correlation between age, sex, and P53 gene mutation status. Notably, Da-Zhong et al. reported an association between age and P53 mutation, contrasting our findings. This discrepancy may be attributed to our broader age range without categorization, unlike Da-Zhong stratification into 3 groups as less than 40 years old, 40-60 years old and above 60 years old.

Furthermore, our study revealed a positive correlation between tumor stage and p53 mutation indicating a higher prevalence of P53 mutation in advanced tumor stages, consistent with Mizuho Nakayama et al. This suggests a potential role of P53 alteration in driving tumor progression and metastasis.

Limitations of our study, including its single-center nature and reliance on paper documentation, necessitates cautious interpretation of results and underscore the need for multi-center studies and electronic data integration to enhance research efficiency and accuracy.

5. Conclusion

In conclusion because of the importance of colorectal cancer due to its high prevalence worldwide this retrospective case-control study focused on association between P53 gene mutation as a possible common mutation in CRC and primary symptoms, cancer stage, lymph node involvement, tumor size and age in the sampled population. Our findings underscore the significance of P53 mutation expression levels, particularly in larger tumor size, greater lymph node involvement and deeper tissue invasion suggesting a direct correlation with cancer stage progression. However, our study did not reveal any significant relationship between P53 mutation expression and demographic characteristics, primary disease symptoms, tumor location, or age.

We believe we can get more accurate and dependable results by broadening the scope of our investigation, ultimately informing more targeted and effective strategies for diagnosis, treatment, and patient management in future.

Availability of Data and Materials

The datasets generated and analyzed during the current study are available from corresponding author upon reasonable request.

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Conflict of Interest

The authors declare that they have no conflict of interest related to this research. This study was conducted with impartiality and integrity, and no financial or personal relationships with individuals or organizations could have influenced the research process or findings.

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