

Association of colorectal cancer type and p53, pten and mlh I genes in northern Saudi Arabia

Abstract

Background: Colorectal cancer is a major cause of morbidity and mortality throughout the world. About 5 to 10 percent of all colorectal cancers are caused by a heritable mutation. This means that the majority of colon cancer cases are sporadic (with personalized mutation). Therefore, the objective of the present study was to assess the association of colorectal cancer type and P53, PTEN and MLH1 genes in Northern Kingdom of Saudi Arabia (KSA).

Methodology: A retrospective cohort study was performed out over a five year period in several referral hospitals. In this study 130 files were retrieved from departments of Surgery from different hospitals in Northern KSA.

Results: P53 and MLH1 positive expressions were identified in 33/130(25.4%) and 35/130(27%) CRC patients. Loss of PTEN expression was identified in 42/130(32.3%).

Conclusion: P53, PTEN, and MLH1 genes mutation might be associated with a number of CRC in Saudi Arabia. More efforts towards CRC prevention and control are deemed important.

Keywords: colorectal cancer, Saudi Arabia, adenocarcinoma, colon

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Introduction

Colorectal cancer (CRC) is a major cause of morbidity and mortality throughout the world, being the third most common cancer in the world and the fourth most common cause of death.¹⁻³ Globally, CRC represents 9.4% of all incident cancer in men and 10.1% in women. CRC, however, is not uniformly common throughout the world.⁴ The incidence rate varies up to 10-fold between countries with the highest rates and those with the lowest rates.^{1,5}

One of the fundamental processes driving the initiation and progression of CRC is the accumulation of a variety of genetic and epigenetic changes in colon epithelial cells.⁶ Gene mutations have long been known to be important in cancer formation.⁷ The MLH1 gene provides instructions for making a protein that plays an essential role in DNA repair. This protein helps fix mistakes that are made when DNA is copied (DNA replication) in preparation for cell division.⁸ Since the first report of *MLH1* promoter methylation in sporadic colon tumors,⁹ the frequency of *MLH1* promoter methylation in sporadic CRC varied from 0.0%¹⁰ to 66.9%.¹¹

The tumor suppressor p53 is widely known for its potential to induce cell death or cell cycle arrest and thereby prevent neoplastic progression.^{12,13} Deficiency or mutation of p53 commonly occurs in approximately half of all human cancers and contributes to tumor progression.^{14,15} Recently, p53 is shown to be associated with the process of Epithelial-mesenchymal transition (EMT).¹⁶

Three-dimensional (3D) colorectal gland formation is regulated by phosphatase and tensin homologue deleted on chromosome 10 (PTEN) coupling of cell division cycle 42 (*cdc42*) to atypical protein kinase C (*apKC*).¹⁷ PTEN is a tumor suppressor which dominates the PTEN/AKT/PI3K pathway. Loss of PTEN and activation of AKT has been reported in many types of cancers, including hepatocellular carcinoma, prostate adenoma and colorectal cancer.¹⁸ It was found that PTEN was a target of miR-17-5p in the colon cancer cells, and

their context-specific interactions were responsible for multiple drug-resistance.¹⁹

Materials and methods

This is a descriptive retrospective study conducted in Northern Saudi Arabia. Sample size represents a full coverage of the available cases with completed required data (including full histopathology report, age, sex etc.). Any patient underwent colonoscopy or/and biopsy due to the presence of colon lesion for the purpose of diagnosis was included. Of the 353 retrieved files, 130 were found to be referring to colonoscopy or/and biopsy. All information of the patients were retrieved from the concerned hospitals. All 130 patients were confirmed by conventional histopathology. Conventional histopathology was re-assessed. The histological examination of biopsy specimens were done to achieve the assessment process role, by giving a pathology category classification (types of malignant lesions).

Statistical analyses

Data managing was completed by using the Statistical Package for Social Sciences (SPSS version 16; SPSS Inc, Chicago, IL). SPSS was used for analysis and to do Fisher exact test for statistical significance (P value < 0.05 was considered significant). The 95% confidence level and confidence intervals were applied.

Results

In the present study we investigated 130 CRC patients their ages ranging from 20 to 90 years with a mean age of 62.9 years old. Out of the 130 patients 69/130(53.1%) were males and 61/130(46.9%) were females giving males' females' ratio of 1.13: 1.00. Most of the study population were found in age range 61-70 demonstrating 37/130(28.5%) followed by age ranges 51-60, 71-80, 41-50, 81+ and < 40 years constituting 22/130(16.9%), 21/130(16.2%), 18/130(13.8%), 17/130(13.1%) and 15/130(11.5%), respectively

as indicated in Table 1 & Figure 1. The distribution of males and females in each age group was relatively varied. High variations were encountered in age group <40 years and 61-70 years. In age range < 40years, the proportions of males and females were 4/15(26.7%) and 11/15(73.3%) in this order. In age group 61-70, the proportions of males and females were 24/37(65%) and 13/37(35%) in this order. The remaining age groups, however, showed relatively similar proportions, as shown in Figure 1. With regard to the site of the tumor, most of them were raised in the colon constituting 75/130(57.7%), followed by Recto-sigmoid, and Rectum representing 33/130(25.4%) and 13/130(10%) respectively, as indicated in Table 2. Out of the 75 presented with colonic lesions, 39/75 (52%) were females and 36/75(48%) were males. Of the 33/130(25.4%) identified with recto-sigmoid tumors, 19/33(58%) were males and 14/33(42%) were females, as indicated in Table 2 & Figure 2.

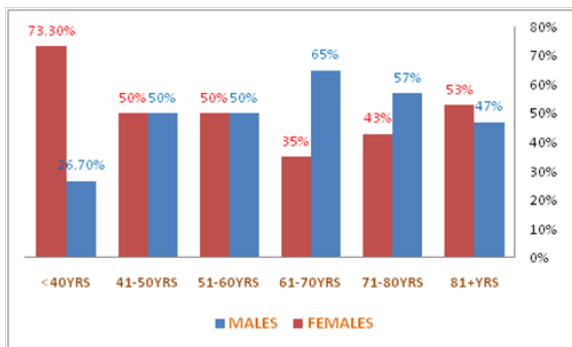


Figure 1 Description of the study subjects by age and sex.

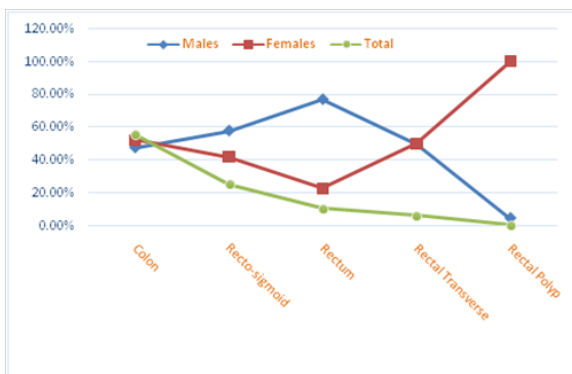


Figure 2 Description of the study subjects by sex and Tumor site.

Table 1 Distribution of the study subjects by sex and age

Age Group	Males	Females	Total
<40 years	4	11	15
41-50	9	9	18
51-60	11	11	22
61-70	24	13	37
71-80	12	9	21
81+	9	8	17
Total	69	61	130

Furthermore, Adenocarcinoma (AD) was diagnosed in 115/130(88.5%) of the patients, of whom 63/115(54.8%) were males and 52/115(45.2%) were females. Papillary AD was also diagnosed

in 7/130(5.4%), of whom 4/7(57%) were males and 3/7(43%) were females, as indicated in Table 2 & Figure 3. With regard to tumor differentiation, the great majority of patients were categorized with moderately differentiated carcinoma representing 81/130(62.3%), followed by well differentiated and poorly differentiated carcinomas constituting 38/130(29.2%) and 10/130(7.7%), respectively. Out of the 81 patients with moderately differentiated carcinomas, 41/81(50.6%) were males and 40/81(49.4%) were females. Out of the 38 patients with well differentiated, 23/38(60.5%) were males and 15/38(39.5%) were females, as shown in Table 2, Figure 4.

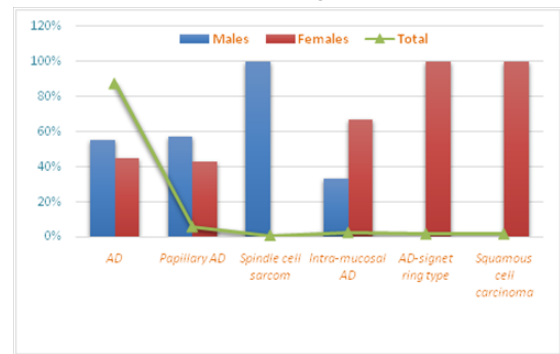


Figure 3 Description of the study subjects by Sex and Diagnosis.

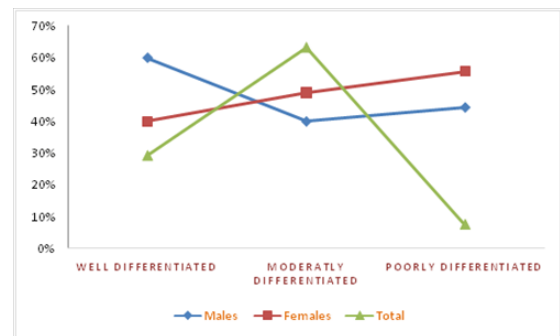


Figure 4 Description of the study subjects by Sex and Grade of differentiation of carcinoma.

Table 3 summarizes the distribution of the study subjects by pathology and P53, PTEN and MLH1 genes expression. With regard to the tumor site, the overall positive P53 expression was found in 33/130(25.4%). Out of 33 positive cases, 19/33(57.6%), 10/33(30.3%), 1/33(3%) and 3/33(9%) were found colon, recto-sigmoid, rectum and rectal transverse, respectively. The overall loss of PTEN expression (negative) was identified in 42/130(32.3%). Out of the 42 negative cases, 24/42(57%), 10/42(23.8%), 7/42(16.7%), and 1/42(2.4%), of colon, recto-sigmoid, rectum and rectal transverse, respectively. The overall positive MLH1 was found in 35/130(27%). Out of 35 positive cases, 21/35(60%), 7/35(20%), 6/35(17%), 0/35(0%) and 1/35(3%) were found colon, recto-sigmoid, rectum, rectal transverse and rectal polyp, respectively, as shown in Figure 5.

With regard to the tumor diagnosis, out of 33 P53 positive cases, 31/33(94%), 1/33(3%), and 1/33(3%) were found among AD, AD-signet ring type and spindle cell sarcoma, respectively. Out of the 42 negative PTEN cases, 37/42(88%), 3/42(7.2%), 1/42(2.4%), and 1/42(2.4%), were found among AD, Papillary AD, spindle cell sarcoma and intra-mucosal AD, respectively. Out of 35 positive MLH1 cases, 30/35(85.7%), 1/35(2.8%), 1/35(2.8%), 2/35(5.7%) and 1/35(2.8%) were found among AD, Papillary AD, spindle cell sarcoma, AD-signet ring type and SCC, respectively, as shown in Figure 6.

Table 2 Distribution of the study subjects by sex and pathology

Category	Variable	Males	Females	Total
Tumor presentation	Colon	36	39	75
	Recto-sigmoid	19	14	33
	Rectum	10	3	13
	Rectal transverse	4	4	8
	Rectal polyp	0	1	1
	Total	69	61	130
Diagnosis	Adenocarcinoma(AD)	63	52	115
	Papillary AD	4	3	7
	Spindle cell sarcoma	1	0	1
	Intra-mucosal AD	1	2	3
	AD signet ring type	0	2	2
	Squamous cell Carcinoma	0	2	2
	Total	69	61	130
Tumor Differentiation	Well differentiated	23	15	38
	Moderately differentiated	41	40	81
	Poorly differentiated	5	5	10
Total	69	61	130	

Table 3 Distribution of the study subjects by pathology and P53, PTEN and MLH1 genes expression

Category	Variable	P53		PTEN		MLH1	
		+ve	-ve	+ve	-ve	+ve	-ve
Tumor presentation	Colon	19	56	51	24	21	54
	Recto-sigmoid	10	23	23	10	7	26
	Rectum	1	12	6	7	6	7
	Rectal transverse	3	5	7	1	0	8
	Rectal polyp	0	1	1	0	1	0
	Total	33	97	88	42	35	95
Diagnosis	Adenocarcinoma(AD)	31	84	78	37	30	85
	Papillary AD	0	7	4	3	1	6
	Spindle cell sarcoma	1	0	0	1	1	0
	Intra-mucosal AD	0	3	2	1	0	3
	AD signet ring type	1	1	2	0	2	0
	SCC	0	2	2	0	1	1
	Total	33	97	88	42	35	95
Tumor Differentiation	Well differentiated	9	29	27	11	11	27
	Moderately differentiated	23	58	52	29	20	61
	Poorly differentiated	1	9	9	1	3	7
	Total	33	96	88	42	34	95

With regard to the tumor differentiation, out of 33 P53 positive cases, 9/33(27%), 23/33(70%), and 1/33(3%) were found among well differentiated, moderately differentiated and poorly differentiated, respectively. Out of the 42 negative PTEN cases, 11/42(26.2%), 29/42(69%), and 1/42(2.4%), were found among well differentiated,

moderately differentiated and poorly differentiated, respectively. Out of 35 positive MLH1 cases, 11/35(31.4%), 20/35(57%), and 3/35(8.6%) were found among AD, Papillary AD, spindle cell sarcoma, AD-signet ring type and SCC, respectively, as shown in Figure 7.

Table 4 Distribution of the study subjects by gender, age and P53, PTEN and MLH1 genes expression

Category	Variable	P53		PTEN		MLH1	
		+ve	-ve	+ve	-ve	+ve	-ve
Gender	Males	20	49	40	29	20	49
	Females	13	48	48	13	15	46
	Total	33	97	88	42	35	95
Age	<40 years	3	12	9	6	5	10
	41-50	4	14	13	5	3	15
	51-60	4	18	13	9	8	14
	61-70	10	27	28	9	9	28
	71-80	6	15	13	8	5	16
	81+	6	11	12	5	5	12
	Total	33	97	88	42	35	95

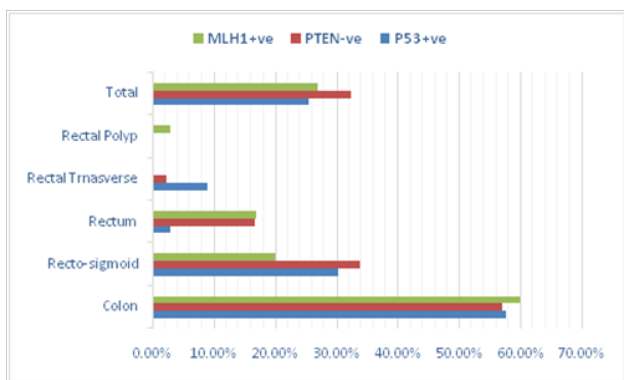


Figure 5 Description of the study subjects by tumor site and P53, PTEN, MLH1 expression.

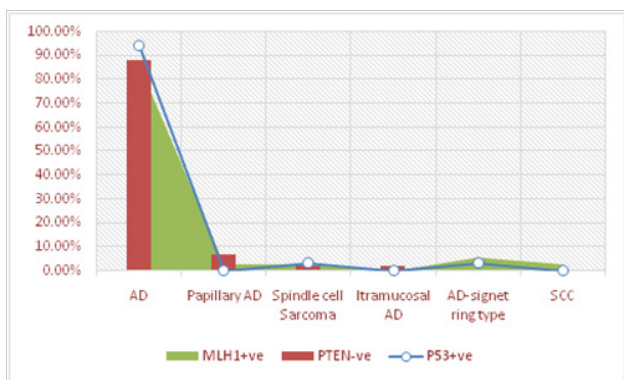
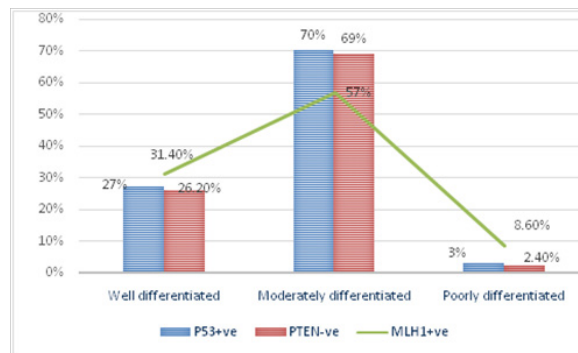


Figure 6 Description of the study subjects by tumor diagnosis and P53, PTEN, MLH1 expression.

Discussion

This study assessed the association of colorectal cancer type and P53, PTEN and MLH1 genes in Northern KSA. Males were found more than females in the present study, which was previously reported in many settings [20]. Most of patients in the current study presented with colonic site lesions followed recto-sigmoid sites. Right colon tumors spread to local and distant sites in 90% of autopsies, and to

distant sites alone in 10%. Rectal tumors spread locally only in 25% of cases, to distant site alone in 25%, and to both in 50%. Regardless of the origin of the primary tumor, the liver is the most common site of metastasis, followed by the regional lymph nodes and the lungs. Two-thirds of the patients with right colon lesions died of liver metastases, and three-quarters of those with rectal tumors succumbed



to disseminated disease.²¹

Figure 7 Description of the study subjects by tumor differentiation and P53, PTEN, MLH1 expression.

With regard to colon cancer subtype, most of patients in the present study were found with adenocarcinoma followed by papillary adenocarcinoma. Adenocarcinoma of the colon is the most frequent histopathological type of colorectal cancer. Most patients present late, and have rapid tumor progression and poor outcome.^{22,23} Adenocarcinoma may be well-differentiated, frequently rising within a villous adenoma, or poorly-differentiated. The poorly-differentiated lumps (such as, adenocarcinoma -signet ring type) have a poor prognosis and likely to happen in younger patients. Most are well-differentiated adenocarcinoma and are classified according to mucin content. Mucin-secreting adenocarcinomas have less than 50% mucin production, mucinous carcinomas have more than 50% extracellular mucin, and adenocarcinoma-signet ring types have intracellular mucin that shifts the nucleus to one side.²⁴ Diagnosis of adenocarcinoma-signet ring types is made when at least 50% of the cells are of the signet ring type.²⁵

Although the age of patients is relatively comparable in all age ranges, but it was perceived that there was an escalation in the younger age group. Furthermore, though the males were more than females, but many females attend at younger age. Such results were previously reported from Saudi Arabia. Colorectal cancer has been the most common cancer among men and the third commonest among women since 2002 in Saudi Arabia. There has been a slight predominance among men with an average ratio of 116:100 over the years (range: 99:100-132:100). The overall age-standardized rate (ASR) approached a plateau of 9.6/100000 in 2010. Colorectal cancer presents at a younger age in Saudis, especially in women. This has a major implication for decisions about the threshold age for screening. The ASR has increased, but is still much lower than in developed countries.^{26,27}

The diagnosis of different carcinomas was relatively similar among both sex with exception of squamous cell carcinoma and AD signet ring type were more common among females. Tumor differentiation was relatively similar for both sex, but what is surprisingly, that most cases present in advanced stage of the disease, which might be due to the lack of screening programs as well as, the low levels of awareness. Such results were stated from diverse regions in Saudi Arabia.

Emergency CRC presentation is common in the Tabuk region. Patients tend to present at an advanced stage, which necessitates an endeavor to detect the disease in its early stages, possibly through initiation of health education programs and suitable screening projects.²⁸

In the present study P53 positive expression was identified in 33/130(25.4%) of the patients with colorectal cancer. In a study documented the incidence and role of p53 and DNA mismatch repair proteins in colorectal carcinomas, and to evaluate the relative frequency of major molecular pathways in colorectal cancers from Saudi Arabia. The p53 positivity was observed in 57.5% of tumors, and was inversely linked to expression loss of mismatch repair genes ($p=0.0102$).²⁹ This results show some variation from our findings, although they used the same methods, but their sample size was relatively lower than ours. Another study assessed colorectal cancer from Middle East, however, found relatively similar Findings (The TP53 mutations were found in 24% of the cases studied).³⁰

The overall loss of PTEN expression (negative) was identified in 42/130(32.3%). Variable results were previously reported regarding absence of PTEN expression in CRC. In one study from Saudi Arabia, PTEN was inactivated in 66.1% of the 51 CRC cases, and PTEN loss was more frequent in microsatellite stable (MSS) CRC ($P=0.043$).³¹ Another study has investigated 99 CRC cases for PTEN mutation and it was identified in 13% of the cases.³²

In the present study the overall positive MLH1 was found in 35/130(27%). Microsatellite instability (MSI) is present in more than 90% of colorectal cancers of patients with Lynch syndrome, and in study performed MSI analysis on 593 unselected CRC patients and subsequently searched for the presence of point mutations, larger genomic rearrangements and MLH1 promoter hypermethylation in patients with MSI-high tumors. MLH1 promoter methylation was detected in 56% of patients without detected germline defects and in 1 (14%) suspected Lynch syndrome.³³ In another study 17 patients underwent germline mutation analysis, 24% were found with MLH1 mutations.³⁴

Conclusion

Patients with CRC were more frequent to present with advanced stages of the disease and relatively at younger ages in Saudi Arabia. P53, PTEN, and MLH1 genes mutation might be associated with a number of CRC in Saudi Arabia. More efforts towards CRC prevention and control are deemed important. The findings of the present study may inspire the inclusion of these markers in screening and diagnostic panels of colorectal cancers in Saudi Arabia.

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Conflicts of interest

The authors declare there are no conflicts of interest related to the article.

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