The Association between The DNA Repair Genes Variants XRCC1 c. 1196 A>C and MLH1 −93G>A & c. 655A>G with Colorectal Cancer Risk

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ABSTRACT

Background: Colorectal cancer (CRC) is the third most common malignancy worldwide. DNA damage that contributes to carcinogenesis is normally corrected by the specific DNA repair pathways which reduces genomic instability and carcinogenesis risk, through removal of damaged DNA. The aim of the study was to assess the Association of DNA repair gene variants XRCC1 c. 1196 A>C and MLH1 −93G>A & c. 655A>G with CRC susceptibility in a sample of Egyptian patients. **Methods**: Eighty CRC patients and 80 apparently healthy subjects were tested for the DNA repair gene variants XRCC1 c. 1196 A>C and MLH1 −93G>A & c. 655A>G by Taqman Real-Time PCR. **The results**: No statistically significant association was found in the genotype distribution of the studied three variants (XRCC1 c. 1196 A>C and MLH1 −93G>A & c. 655A>G) between the CRC cases and the control group. A statistically significant association between the MLH1 −93G>A genotype and both the site of the tumor and the lymph node staging (N), a part of TNM staging, has been demonstrated with a P-value of (0.025 and 0.016), respectively. **Conclusion**: The results of this study suggest that the DNA repair genes variants XRCC1 c. 1196 A>C and MLH1 −93G>A & c. 655A>G have no statistically significant association with CRC in Egyptian patients.

Key words: Gene; DNA repair genes; MLH1 gene; XRCC1 gene; variants.

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INTRODUCTION

Colorectal cancer (CRC) is the third most common malignancy worldwide. Over 95% of CRC cases are adenocarcinomas, and approximately half of them develop local recurrence or distant metastasis during the course of their illness. There was increased incidence of CRC among adults younger than 50 years, that was mostly attributed to a combination of genetic and environmental factors **(1)**.

DNA damages are estimated to be over 20,000 lesions per day, normally corrected by the specific DNA repair pathways **(2)**. DNA repair pathway plays a role in reducing genomic instability and carcinogenesis risk, through removal of damaged DNA **(3)**. The base excision repair (BER) pathway is responsible for repairing single-strand breaks in DNA **(4),** while the mismatch repair (MMR) pathway corrects for inappropriate nucleotide insertions, deletions, and single nucleotide mismatched incorporations **(5)**.

XRCC1 was the first in a series of cloned DNA repair genes from the BER pathway. XRCC1 c. 1196A>C (rs 25487) variant, presents as a substitution in exon 10. It may alter the DNA repair activity and lead to accumulation of genetic errors in the genome, which promotes tumorigenesis **(8) (9)**.

The studied variants were extensively investigated in the CRC with controversial results **(6).**

The MLH1 gene, is the major gene in MMR pathway and the most frequently mutated MMR gene in both sporadic and hereditary cancers, The MLH1 −93G>A (rs1800734) variant is the most well-studied intron SNP in the MLH1 core promoter area near the transcription start site **(6)**. Although the specific method by which rs1800734 blocks *MLH1* function is not well known yet, numerous possibilities have been discussed like ; inhibition of the transcription, hypermethylation of the promotor area and epigenetic mechanism. It has been described as a pathogenic variant that raises the risk of many cancers, including sporadic CRC and endometrial cancer **(7).**

The *MLH1* gene variant c. 655 A>G (rs1799977) is another well-studied SNP in the *MLH1* gene. It may lead to *MLH1* gene silencing, so promote carcinogenesis, and is associated with a higher risk of a variety of cancers **(6).**

***This study aimed to detect the prevalence of* DNA repair genes variants XRCC1 c. 1196 A>C and MLH1 −93G>A & c. 655A>G in a sample of Egyptian CRC patients and evaluate their possible association.**

Methods

Study population included 160 subjects, divided into two groups; eighty colorectal carcinoma patients and eighty apparently healthy individuals served as a control group. Both groups were age matched. Ethical committee approval was taken for this study, code: MD-11-2020, Date: 13-2-2020.

Study subjects were divided into two groups:

Group I (colorectal carcinoma group): included eighty patients diagnosed with CRC with no other cancer.

Inclusion criteria for Group I: Patients diagnosed with colorectal cancer by, radiological investigations, including ultrasound or CT and histopathological examination of core biopsies. Denovo cases not receiving any treatment or surgical intervention.

Exclusion criteria for Group I: Benign tumors of the colon and other malignancies or patients who received any treatment.

Group II (healthy Control group): included eighty apparently healthy subjects

Both groups were age matched ranging from 32 to 60 years.

Both groups were subjected to the following: Full history, including lifestyle, special habits of medical importance like smoking and family history of colorectal cancer. Clinical assessment both general and local clinical examination for CRC cases. Investigations: Radiological: abdominal CT and Endoscopic examination and biopsy for CRC cases group.

Detection of the DNA Repair Gene variants: DNA repair gene variants XRCC1 c. 1196A>C (rs 25487) and MLH1 −93G>A (rs1800734) & c. 655A>G (rs1799977) was done by by Real time TaqMan PCR. After PCR amplification, endpoint plate reads were performed using StepOne™ Plus v2.3, Real-Time PCR System. The Sequence Detection System (SDS) Software used the fluorescence measurements to plot fluorescence signals that indicated which alleles were in each sample, plot fluorescence values based on the signals from each well. **Figure (1) & Figures (2)** show the allelic discrimination and the multicomponent plots, respectively, of a positive case for *MLH1*c. 655A>G (rs.1799977).

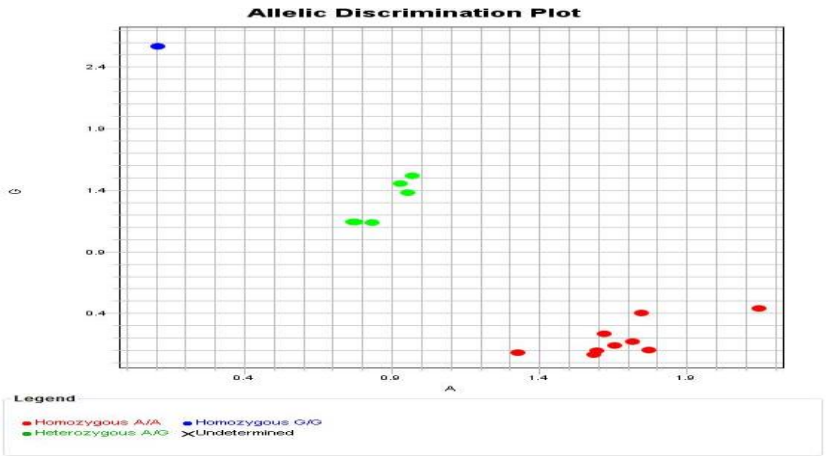

Fig. (1): Allelic discrimination using StepOne™ Plus v2.3, Real-Time PCR System

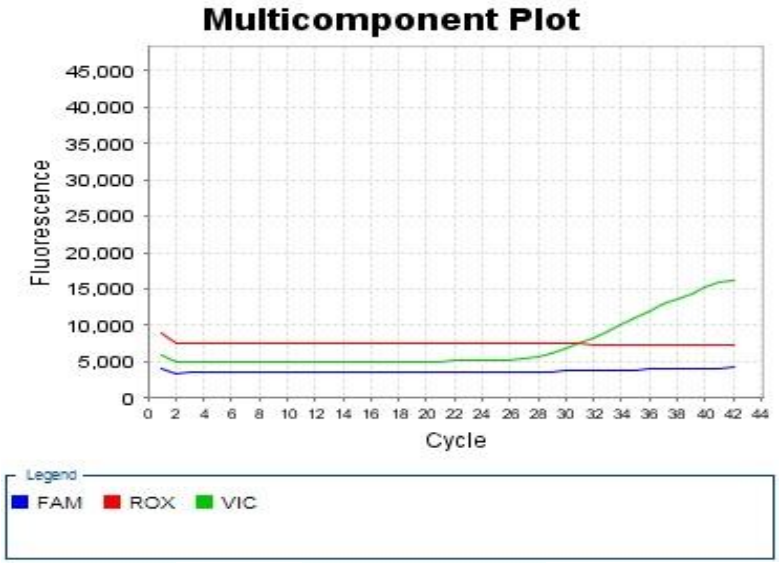

Fig. (2): Show a case of *MLH1*c. 655A>G (rs.1799977), using *StepOne*™ Plus v2.3, Real-Time PCR System, VIC was positive and FAM was negative

Statistical Method: Data management and analysis were performed using the Statistical Package for Social Sciences (SPSS) version22. Comparisons between the two groups for normally distributed numeric variables were done using the Student's t-test while for non-normally distributed numeric variables were made by Mann-Whitney U test. Chisquare or Fisher's tests were used to compare between the groups with respect to categorical data, as appropriate. All tests were two-sided. P-values < 0.05 were considered statistically significant.

RESULTS:

Descriptive and clinical data of CRC cases are presented as frequencies and percentages in **Tables (1)**

Table (1): Descriptive data of CRC patients

		Cases (n=80)
Site	Rectum	37/80(46.2%)
	Colon	43/80(53.8%)
Differentiation degree	Low	16/80(20%)
	Moderate	61/80(76.2%)
	High	3/80(3.8%)
Histological type	Mucinous adenocarcinoma	25/80(31.2%)
	Adenocarcinoma	55/80(68.8%)
Stage	Invasive	58/80(72.5%)
	Localized	22/80(27.5%)
Primary tumor	T1	2/80(2.4%)
	T2	7/80(8.8%)
	T3	52/80(65%)
	T4	19/80(23.8%)
Lymph node	N0	23/80(28.7%)
	N1	19/80(23.8%)
	N2	27/80(33.7%)
	N3	11/80(13.8%)
Metastasis	M0	66/80(82.5%)
	M1	14/80(17.5%)

Data are presented as frequencies and percentages.

Frequency distribution of alleles in the two studied groups is illustrated in **Table (2).** They showed no statistically significant difference in the genotype distribution of the studied variants between the 2 studied groups.

Table (2): Frequency distribution of alleles in the two studied groups

	Cases (n=80)	**Controls (n=80)**	**P-value**
rs 25487			
A allele (Wild) C allele (Variant)	118/160 (73.8%) 42/160 (26.3%)	116/160 (72.5%) 44/160 (27.5%)	0.801
rs 1800734			
G allele (Wild) A allele (Variant)	52/160 (32.5%) 108/160 (67.5%) 47/160 (29.4%)	47/160(29.4%) 113/160 (70.6%)	0.545
rs 1799977			
A allele (Wild) G allele (Variant)	122/160 (76.3%) 38/160 (23.8%)	129/160 (80.6%) 31/160 (19.4%)	0.341

Data are presented as number (percent).
P value <0.05 is considered significant.

Regarding clinical and demographic data and their association with the three studied variants. A statistically significant association has been demonstrated between Rs 1800734 and the site of the tumor and the lymph node staging (part of TNM staging) with a P-value of (0.025) and (0.016) respectively as shown in **Table (3).**

Table (3): Demographic and clinical data and rs 1800734 in CRC patients.

	GG (n=7)	AG (n=38)	AA (n=35)	P-value
Smoking				
Smoker	2/7(28.6%)	7/38(18.4%)	8(22.9%)	
Non- smoker	5/7(71.4%)	31/38(81.6%)	27/35(77.1%)	0.777
Site				
Rectum	1/7(14.3%)	23/38(60.5%)	13/35(37.1%)	
Colon	6/7(85.7%)	15/38(39.5%)	22/35(62.9%)	**0.025**
Family history				
Positive	1/7(14.3%)	3/38(7.9%)	2/35(5.7%)	
Negative	6/7(85.7%)	35/38(92.1%)	33/35(94.3%)	1
Differentiation degree				
Low	2/7(28.6%)	9/38(23.7%)	5/35(14.3%)	
Moderate	4/7(57.1%)	28/38(73.7%)	29/35(82.9%)	0.444
High	1/7(14.3%)	1/38(2.6%)	1/35(2.9%)	
Histological type				
Mucinous	0/7(0%)	16/38(42.1%)	9/35(25.7%)	
Adenocarcinoma	7/7(100%)	22/38(57.9%)	26/35(74.3%)	0.073
Stage				
Invasive	6/7(85.7%)	29/38(76.3%)	23/35(65.7%)	
Localized	1/7(14.3%)	9/38(23.7%)	12/35(34.3%)	0.457
Tumor				
T1	0/7(0%)	1/38(2.6%)	1/35(2.9%)	
T2	1/7(14.3%)	2/38(5.3%)	4/35(11.4%)	
T3	4/7(57.1%)	29/38(76.3%)	19/35(54.3%)	0.615
T4	2/7(28.6%)	6/38(15.8 %)	11/35(31.4%)	
	GG (n=7)	**AG (n=38)**	**AA (n=35)**	**P-value**
Lymph node				
N0	1/7(14.3%)	10/38(26.3%)	12/35(34.3%)	
N1	5/7(71.4%)	7/38(18.4%)	7/35(20%)	
N2	0/7(0%)	18/38(47.4%)	9/35(25.7%)	**0.016**
N3	1/7(14.3%)	3/38(7.9%)	7/35(20%)	
Metastasis				
M0	6/7(85.7%)	31/38(81.6%)	29/35(82.9%)	
M1	1/7(14.3%)	7/38(18.4%)	6/35(17.1%)	1

* P value <0.05 is considered significant.
† Data presented as number (percent)
No statistically significant associations were found between Demographic and clinical data and both rs 1799977 & rs 25487 in CRC patients.

Table (4): Association between the CRC group who have single variant and the CRC group who have more than one variant with clinical data of the CRC cases

	CRC patients with single variant (n=19)	CRC patients with more than one variant (n=57)	P-value
Site			
Rectum	9/19(47.4%)	27/57(47.4%)	1
Colon	10/19(52.6%)	30/57(52.6%)	
Histological type			
Mucous	7/19(36.8%)	18/57(31.6%)	0.672
Adenoma	12/19(63.2%)	39/57(68.4%)	
Adenocarcinoma			

	CRC patients with single variant (n=19)	CRC patients with more than one variant (n=57)	P-value
Stage			
Invasive Localized	15/19(78.9%) 4/19(21.1%)	39/57(68.4%) 18/57(31.6%)	0.381
T			
T1 T2 T3 T4	1/19 (5.3%) 2/19 (10.5%) 11/19 (57.9%) 5/19 (26.3%)	1/57 (1.8%) 5/57 (8.8%) 38/57 (66.7%) 13/57 (22.8%)	0.881
N			
N0 N1 N2 N3	5/19 (26.3%) 4/19 (21.1%) 8/19 (42.1%) 2/19 (10.5%)	18/57 (31.6%) 11/57 (19.3%) 19/57 (33.3%) 9/57 (15.8%)	0.901
M			
M0 M1	15/19 (78.9%) 4/19 (21.1%)	48/57 (84.2%) 9/57 (15.8%)	0.726

P value <0.05 is considered significant.

DISCUSSION

Different factors are involved in the pathogenesis of CRC, some of them are modifiable factors like (environmental, lifestyle, type of food and drugs) and the others are non-modifiable factors like (genetic, precancerous lesions and family history) **(10).**

The present work investigated the defects in 2 DNA repair pathways, the MMR and BER pathways, by studying two variants in the MLH1 gene, which is a part of the MMR pathway, −93G>A (rs1800734) and c. 655A>G (rs1799977) and one variant in the XRCC1 gene, which is a part of the BER pathway, c. 1196 A>C (rs 25487), by using Real Time Taqman PCR.

The MMR pathway is one of the principle pathways in the DNA repair process **(5)**. The MLH1 gene is the most often mutated gene in both sporadic and hereditary malignancies. MLH1 gene mutations represent the most prevalent cause of an inherited form of CRC, hereditary non-polyposis colorectal cancer (HNPCC) **(11)**. The human MLH1 gene is located on chromosome 3 at the p22. 2 and it has 19 exons with 57360 base pairs long regions**.** It contains 15,721 variants; however, only 49 variants were studied **(6)**. The encoded protein heterodimarizes with other MMR proteins in the MMR repair pathway.

The MLH1 −93G>A (rs1800734) variant is the most well-studied intron SNP in the MLH1 core promoter area 93 bases upstream of the transcription start site. It has been described as a pathogenic variant that raises the risk of many cancers, including sporadic CRC and endometrial cancer **(7).**

The present study compared the frequency of distribution of the MLH1 −93G>A (rs1800734) variant in CRC cases and control groups, showing no statistically significant difference in the distribution of the genotypes between CRC cases and control groups.

In harmony with the present work, two studies were performed by **Raptis et al., (12)** and **Campbell et al., (13)**, both showed no statistically significant association between the MLH1 −93G>A (rs1800734) variant and CRC risk in Canadians and Americans respectively.

Similarly a meta-analysis study, which was conducted by **Chen et al., (14)** did not find any statistically significant association between the presence of MLH1 −93G>A (rs1800734) variant and CRC risk.

Again, in agreement with the current work, a meta-analysis by **Zare et al., (15)**, failed to reveal a statistically significant association between MLH1 −93G>A (rs1800734) variant and CRC risk (OR = 1.101, 95% CI 0.638-1.901, P >0.001).

On the other hand, a study by **Nizam et al., (16)** which reported that the heterozygote variant (AG) was associated with increased risk of sporadic CRC in a group of Malaysian population (OR of 2.273, 95%CI: 1.133-4.558 and p-value=0.021). Also**, Martinez-Uruena et al., (17)**, found a statistically significant association between MLH1 −93G>A (rs1800734) homozygous variant (AA) and increased CRC risk (OR = 3.35; 95 % CI = 1.16–9.27; p = 0.019).

While, a study by **Mik et al., (18)**, reported that the wild genotype (GG) of the –93G>AMLH1 gene was associated with an increased the risk of sporadic CRC (OR = 2.07; 95% CI: 1.11–3.83; p < 0.02).

In the present study, a statistically significant association was found between the genotype distribution of the MLH1 −93G>A (rs1800734) variant and the tumor site (rectum or colon) of CRC with a P-value of (0.025).

However, a meta-analysis of **Pardini et al., (19)**, showed that the MLH1 −93G>A (rs1800734) homozygote variant (AA) was mainly associated with proximal colon tumors (OR = 1.13, 95% CI = 1.07–1.18).

The current research studied the association between the MLH1 −93G>A (rs1800734) variant and the tumor TNM staging. A statistically significant association with the lymph node metastasis (N) was detected with a P-value of (0.016). Regarding the tumor size (T) and metastasis (M), no statistically significant associations between them and the MLH1 −93G>A (rs1800734) variant in CRC cases were detected, with P-value of (0.615 and 1) respectively.

Regarding the other MLH1 variant studied in this work c. 655A>G (rs1799977), no significant difference in variant distribution was observed between cases and control group. In parallel with the present work, a case-control study by **Peng et al., (20),** showed no statistically significant association between the MLH1 c. 655A>G (rs1799977) variant and the risk of sporadic CRC with a P-value of (0.29).

In contrast to the present work results, a pilot study done by **Picelli et al., (21)**, on the MLH1 c. 655A>G (rs1799977) variant. It reported increased risk in both colon and rectum patients with the presence of homozygous variant (GG) (OR: 1.28, CI = 1.02–1.60) and (OR: 1.34, CI = 1.05–1.72), respectively.

In the present study, the frequency of distribution of the alleles in the two studied groups was done regarding the MLH1 c. 655A>G (rs1799977) variant, showing no statistically significant difference between CRC cases and control groups with a P-value of (0.341)

In contrast to the current results, **Nejda et al., (22)**, on MLH1 c. 655A>G (rs1799977) variant, revealed that the mutant allele (G) carriers (AG or GG genotype) displayed a high risk of CRC, AG (OR = 2.55, 95% CI = 1.48–4.39; P = 0.01) and GG (OR = 2.48, 95% CI = 1.20–5.11; P = 0.01).

Again, a meta-analysis by **Li et al., (23)**, revealed a statistically significant association between colorectal cancer risk and the presence of the mutant allele (G) (OR = 1.21, 95%CI = 1.03-1.42, P = 0.023)**.**

Also, in the subgroup analysis (by ethnicity) of a study by **Zare et al., (15)** a statistically significant association between the mutant allele (G) of the MLH1 c. 655A>G (rs1799977) variant and the risk of CRC in Asians was detected (OR = 2.251, 95% CI 1.7582.884, P < 0.001), while no such association was observed among Caucasians.

The present work failed to detect a statistically significant association in the distribution of the genotypes between the MLH1 c. 655A>G (rs1799977) variant and the site of colorectal cancer with a p-value of (0.37).

Also, no statistically significant association between the MLH1 c. 655A>G (rs1799977) variant and the tumor TNM staging was found, with a p-value of (0.174, 0.218 and 0.842) respectively.

The other pathway that may be involved in CRC pathophysiology is the BER pathway . XRCC1 was the first in a series of cloned DNA repair genes in the BER pathway. It is located on19q13. 31 with an Exon count of 17 encoding 633 amino acid proteins **(24).**

The present study compared the frequency of distribution of the XRCC1 c. 1196 A>C (rs 25487) variant between CRC cases and control groups, showing no statistically significant difference in the distribution of the genotypes between CRC cases and control groups, with P-value of (1, 0.751 and 0.746) respectively.

Similarly, a meta-analysis conducted by **Liu et al., (25)**, could not find a statistically significant association between the XRCC1 c. 1196 A>C (rs 25487) variant and colorectal risk.

In contrast to the present work, a meta-analysis performed by **Zeng et al., (8)**, detected a statistically significant association between the mutant allele (C) of the XRCC1 c. 1196 A>C (rs 25487) variant and CRC risk.

In the present work, the CRC cases group (80) was further subdivided into three separate groups according to the presence of the three variant combination, cases with wild genotype (4 cases), cases with a single variant (19 cases) and cases with more than one variant (double and triple variants) (57 cases). No statistically significant association was reported between the CRC group who have single variant and the CRC group who have more than one variant with a family history of cases, site, histological type and TNM staging of the tumor with a P-value of (1, 1, 0.672, 0.881, 0.901 and 0.726) respectively. As shown in **table (4)**.

CONCLUSION

In conclusion, our study showed no statistically significant association between the 3 studied variants (rs1800734, rs1799977 and rs 25487) and the risk of CRC in the studied group of Egyptian patients, but showed a statistically significant association between rs 1800734 variant and both the site of tumor and the lymph node staging (N) a part of (TNM staging) with a P-value of (0.025 and 0.016) respectively. However, the studies are extensive, controversial and new evidence is rising all the time. The variant classification is continuously updated with new research as the ongoing battle against cancer continues, and the potential use of rs 25487, rs1800734 and rs1799977 variants as predictive markers for colorectal cancer risk needs more investigations.

List of Abbreviations

A	**:**	**Adenine**
BER	**:**	**Base excision repair.**
C	**:**	**Cytosine**
CRC	**:**	**Colorectal Carcinoma.**
CT	**:**	**Computed tomography**
DNA	**:**	**Deoxyribonucleic Acid**
G	**:**	**Guanine**
MLH1	**:**	**MutL homolog 1**
MMR	**:**	**Mismatch Repair.**
OR	**:**	**Odds Ratio.**
P- value	**:**	**Probability Value.**
PCR	**:**	**Polymerase Chain Reaction.**
SPSS	**:**	**Statistical Package for Social Science.**
SSBR	**:**	**Single strand break repair.**
T	**:**	**Thymine**
TNM	**:**	**Tumor, Node, and Metastasis classification.**
XRCC1	**:**	**X-ray repair cross complementing 1**

DECLARATIONS:

Consent for publication: not applicable

Ethical committee approval and consent to participate was taken for this study (Cairo university, Faculty of medicine, Research Ethics Committee)
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Code availability: not applicable

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Authors' contributions:

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Dr. Dina Farouk Ahmed El-Gayar
Made substantial contributions to the conception and revised the manuscript critically

Dr. Ahmed Mustafa Ahmed
Interpretation of data and revised the manuscript critically

Dr. Naglaa Fathy El-Salawy
Drafted the work and substantively revised the manuscript

Dr. Reham Assem Zeyada
Made substantial contributions to the conception, design the work, revised the manuscript critically and reviewing the scientific material

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