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Association between COX-2 Gene Polymorphism and Susceptibility to Colon and Rectal Cancer

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ABSTRACT

Background: Intestinal cancer the third most prevalent malignancy and leading cause of cancer mortality worldwide, is colorectal cancer (CRC). More than one-third of colorectal cancer cases in Egypt include people under the age of 40 who are found to have the disease at an advanced stage. Due to its function in human tumours, cyclooxygenase (COX), a crucial enzyme in the prostanoid biosynthesis pathway, has drawn a lot of interest. COX-2 regulates cell proliferation, cell transformation, tumour growth, metastasis, and invasion, and so plays an important role in the origin and development of metaplastic and dysplastic tissues, as well as the beginning and progression of cancer. Increased COX-2 expression has been linked to a variety of epithelial-based premalignant and malignant lesions in the gastrointestinal system, including the colorectal area.

Objectives: To investigate the probable link between COX-2 gene polymorphism and colorectal cancer risk.

Methods: This is a case-control study with 100 participants. Were selected 50 with colorectal cancer (case group) from Inpatient and Outpatient Oncology Clinic, Faculty of Medicine, Menoufia University and 50 participants without colorectal neoplasia (control group) matched in age and gender with case group. With the use of the usual literature approach, the polymorphism -765G/C COX2 gene was being identified using molecular genetic analysis. Clinical and pathological data from the patient were also examined. The findings demonstrated a link between the existence of the COX2 gene polymorphism and susceptibility to colorectal cancer in this pattern, with a significant incidence of GC and CC genotype in those with colorectal cancer. Additionally, there were variations in allele frequencies between the groups. There was a greater incidence of polymorphism in the left colon when cancer patients were divided based on the location of their tumours.

Results: The comparison between the two studied groups indicated the genotype distribution of COX 2 gene polymorphisms in CRC patients that was 42%, 50%, and 8% with GG, GC, and CC respectively whereas in control group, it was 76% with GG, 22% with GC, and 2% with CC .The genotypic distribution revealed statistical difference (p=0.003). The allelic frequencies were 67% who had the wild allele G and the remaining 33% had the variant allele C in CRC group while in control group there were 87% with G and 13% with C allele. The difference was statistically significant (p=0.001). The GC genotype revealed a significant risk of CRC as compared to GG genotype. Subjects carrying the C allele had a significant risk of CRC compared to those carrying the allele G

Conclusion: The COX2 gene polymorphism is linked to an increased risk of colorectal cancer, particularly rectosigmoid tumours.

Keywords: Gene polymorphism, Colorectal cancer, Cyclooxygenase enzyme, Restriction fragment length polymorphism.

INTRODUCTION

A kind of gastrointestinal cancer that can start in the colon or the rectum is called colorectal cancer (CRC). The majority of instances of colorectal cancer (up to 95%) are adenocarcinomas [(1)]. CRC is the third most prevalent malignant illness in the globe, only lung and breast cancers occurring more frequently. In addition, it is the second most prevalent disease in women after breast cancer and the third most frequent cancer in men after lung and prostate cancer [(2)].

Unfortunately, CRC may be unnoticed for a very long time in a lot of people, at least until it significantly develops and spreads, which negatively affects the prognosis [(3)]. CRC risk rises with age and is more prevalent in those over the age of 50. More than one-third of CRC cases in Egypt involve people under the age of 40, and they are typically discovered at an advanced stage [(4)]. The incidence of CRC has decreased in nations that have embraced preventative initiatives. Therefore, early detection by screening is essential for lowering patient death from CRC, and awareness initiatives also encourage screening [(5)].

A crucial enzyme in the biosynthesis of proteinoid molecules is cyclooxygenase (COX). The development of metaplastic and dysplastic tissues, the onset and course of cancer, and the regulation of cell division, cell transformation, tumour growth, tumour metastasis, and invasion are all impacted by COX-2 [(6)]. In several parts of the gastrointestinal system, epithelial-based premalignant and malignant lesions have been linked to increased COX-2 expression [(7)]. It is hypothesised that COX-2 polymorphisms may change the enzyme's activity by regulating COX-2 production differently [(8)]. Differential COX-2 expression may affect the likelihood that gastrointestinal adenocarcinomas, such as CRC, may

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develop [9]. Since specialised COX-2 inhibitors, such as different non-steroidal anti-inflammatory medications (NSAIDs), COX-2 is of special relevance since it has been created or is being produced and may have a role in the chemoprevention of gastrointestinal neoplasms [10].

The current study intended to quantify the allele and genotype frequencies of the COX-2 gene polymorphism, In order to assess any potential associations between these frequencies and CRC susceptibility.

PATIENTS AND METHOD

This case-control study was carried out at Laboratory Medicine Department, National Liver Institute, Menoufia University, Egypt from January 2022 to July 2022. This study included 100 subjects, and were divided into 2 groups, the first group (Case group) included 50 patients with CRC. Those CRC patients were selected from Clinical Oncology Department, Faculty of Medicine, Menoufia University. The second group (Control Group) included 50 healthy subjects without colorectal neoplasia matched by gender and age with case group.

Ethical consent:

The National Liver Institute at Menoufia University and its Cancer Clinic approved the research protocol. After outlining the purpose and potential drawbacks of the study, informed consents were obtained from both the patients and the volunteers in the control group. The Declaration of Helsinki for human beings, which is the international medical association's code of ethics, was followed during the conduction of this study.

The clinical data of the patients were collected from the files, demographic data was collected, laboratory data included hemoglobin level, absolute lymphocytic count, calcium, transferrin, albumin and carcinoembryonic antigen were registered.

The clinico-pathological data regarding tumor grade, tumor stage, surgery done, and treatment received were registered. Preoperative rectosigmoidoscopies/colonoscopies were analyzed, including size of lesion, location and sidedness of the tumor. Seven ml of venous blood were withdrawn from the cubital vein: 2 ml were gathered for PCR-RFLP molecular testing of polymorphism in vacutainer tubes containing Ethylene Diamine Tetra Acetic acid (EDTA). The second 2 ml were obtained for a full blood count using vacutainer tubes containing EDTA (Sysmex XT1800i Automated Hematology Analyzer, Kobe, Japan)). The third part: the remaining volume was collected in plain vacutainer without additives used for measurement of Ca level, iron profile (Cobas 6000 (c 501 module) analyzer, Roche Diagnostics, Mannheim, Baden-Württemberg, Germany), CA19.9 and CEA level using Cobas 6000 (e 601 module), Roche Diagnostics, Mannheim, Baden-Württemberg, Germany).

DNA extraction and genotyping:

PCR-RFLP was used to do molecular testing for COX 2 gene polymorphism. Using the Gene JET Genomic DNA Purification Kit from Thermo Scientific, total DNA was recovered from an EDTA-treated blood sample. The Lysis Solution's Proteinase K breaks down whole blood samples. Following the addition of ethanol, the lysate is poured into the purification column, where the DNA adheres to the silica membrane. By using the ready-made wash buffers to wash the column, impurities are successfully eliminated. The Elution Buffer is then used to elute genomic DNA under low ionic strength conditions. Following DNA extraction from the samples, the COX-2 gene's polymorphism -765G/C region was analysed using the PCR-RFLP technique. The primer for the PCR was chosen based on the genome database (region sequence -765G/C: Forward" 5'-ATT CTG GCC ATC GCC GCT TC-3' and "Reverse," 5'-CTC CTT CTT TCT TGG AAA GAG CG-3').

The following temperatures and periods were used to establish the amplification conditions: 94° C for -3 min; 94° C, 59° C, and 72° C for 60 s each, with 35 cycles; and 72° C for 5 min. The following parameters were utilised for the proline allele detection: 94° C for -3 min, followed by 35 cycles of 94°, 57°, and 72° C for 30 s each, then 72° C for 5 min.

Electrophoresis and 2% agarose gel were used to separate the amplified products. Then, electrophoresis was done for 20 minutes at 100 volts. Detection of positive bands is confirmed by detecting specific bands, which corresponds to ladder specific band of 157 bp for COX-2 gene. After amplification, the samples were digested with restriction enzyme XmnI. Five minutes at 37°C were spent incubating the digesting mixture. After adding restriction enzyme, the PCR products were electrophoresed for electrophoresis onto a 3% agarose gel that had been prepared and stained with ethidium bromide. Electrophoresis was performed at room temperature for 60 min. The gel was visualized by on a 302 nm ultra-violet transillumination.

134 and 23 base pair (bp) amplified segments suggested homozygosity for the wild-type allele (-765GG). For the C allele, homozygosis was indicated by a single fragment of 157 base pairs (-765CC), whereas heterozygosis was indicated by the presence of three fragments of 157, 134, and 23 base pairs (-765GC) (Figures 1 and 2).

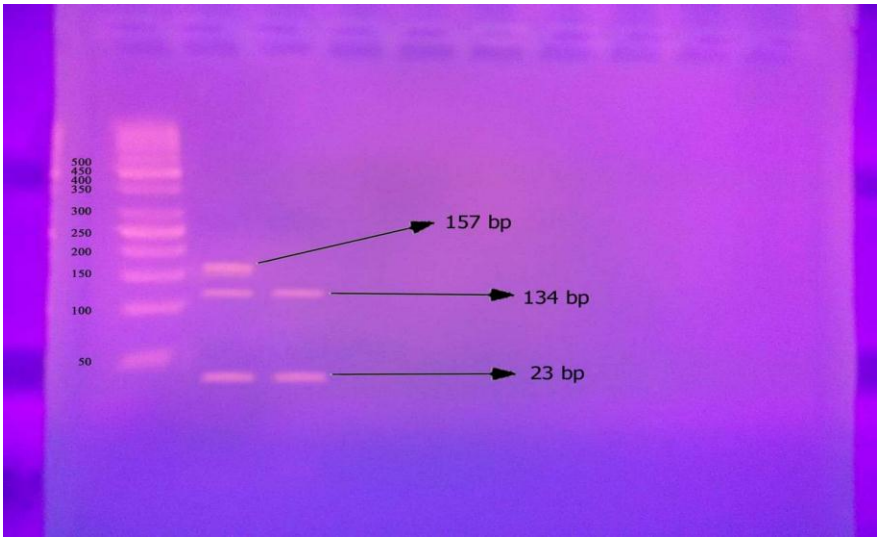

Figure (1): A representative agarose gel picture showing PCR-RFLP analysis of COX 2 genotypes in genomic DNA of study subjects with restriction endonuclease enzyme MseI. M 50-bp DNA ladder, lane 1, C/C homozygous (157 bp).

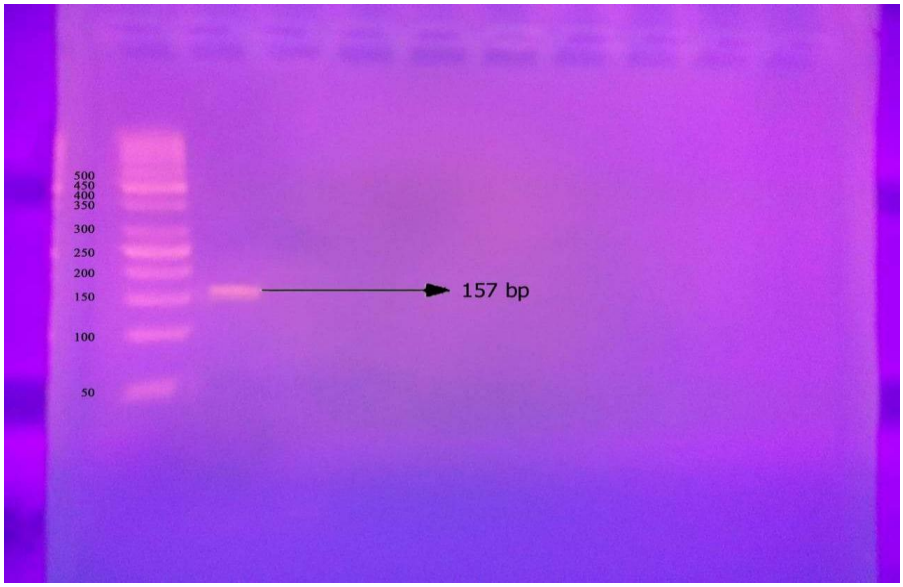

Figure (2): A representative agarose gel picture showing PCR-RFLP analysis of COX 2 genotypes in genomic DNA of study subjects with restriction endonuclease enzyme MseI. M 50-bp DNA ladder, lane 1, G/C heterozygous (23, 134 and 157 bp bands), lane 2, G/G homozygous (23 and 134 bp bands).

Statistical analysis

The IBM SPSS software tool, version 20.0, was used to enter data into the computer and analyse it (IBM Corp., New York, Armonk). Number and percentage were utilised to describe qualitative data. If the distribution was normal, it passed the Shapiro-Wilk test. The range (minimum and maximum), mean, standard deviation, median, and interquartile range were used to characterise quantitative data (IQR). The 5% level was chosen to determine the importance of the results. $P \leq 0.05$ was regarded as significant.

RESULTS

1-Biochemical parameters of the studied groups:

By statistical analysis regarding biochemical parameters in the two studied groups (in table 1), the comparison revealed significant difference between the 2 groups ($p < 0.001$) regarding total plasma iron concentration, TIBC, transferrin saturation and ferritin. There was significant increase ($p<0.001$) regarding CEA and CA 19,9. On the other hand, there was no significant difference between the two groups regarding total serum calcium and albumin.

Table (1): Comparison between the two studied groups according to biochemical parameters

	Control (n = 50)	CRC group (n = 50)	Test of Sig.	P
Total serum calcium (mg/dl) Mean ± SD.	9.20 ± 0.55	9.14 ± 0.54	t= 0.549	0.584
Total plasma iron concentration (mcg/dl) Mean ± SD.	109.0 ± 10.38	66.66 ± 5.32	t= 25.642*	<0.001*
TIBC (mcg/dl) Mean ± SD.	358.6 ± 32.42	271.9 ± 28.48	t= 14.201*	<0.001*
Transferrin saturation (%) Mean ± SD.	30.62 ± 3.96	24.77 ± 3.12	t= 8.207*	<0.001*
Ferritin (mcg/L) Mean ± SD.	212.5 ± 17.74	243.1 ± 19.66	t= 8.160*	<0.001*
Albumin (g/dl) Mean ± SD.	3.81 ± 0.30	3.85 ± 0.36	t= 0.517	0.606
CEA (ug/L) Min. – Max. Median (IQR)	0.52 – 3.29 1.78 (1.37 – 2.22)	0.49 – 1550.0 3.18 (1.50 – 24.38)	U= 780.5*	0.001*
CA 19.9 (U/ml) Min. – Max. Median (IQR)	0.59 – 5.10 2.67 (1.78 – 3.34)	0.20 – 1190.0 6.10 (2.0 – 23.56)	U= 658.5*	<0.001*

Median (IQR) and Range: non-parametric test *: Statistically significant at $p \leq 0.05$

2-Hematological parameters of the studied groups:

The comparison between the two studied groups regarding hematological parameters (as shown in table 2) revealed a significant decrease ($p<0.001$) in CRC patients regarding hemoglobin. Whereas WBCs and platelets showed no statistical difference between the two groups.

Table (2): Comparison between the two studied groups according to hematological parameters

	Control (n = 50)	CRC group (n = 50)	t	p
Hb Mean ± SD.	13.52 ± 1.10	11.04 ± 1.86	8.093*	<0.001*
PLT Mean ± SD.	249.9 ± 58.48	248.0 ± 60.64	0.131	0.896
TLC Mean ± SD.	6.70 ± 1.60	7.20 ± 1.81	0.977	0.331

*: Statistically significant at $p \leq 0.05$

3- COX2 genotypes and alleles distribution among study subjects:

Table (3) indicated the genotype distribution of COX 2 gene polymorphisms in CRC patients that was 42%, 50%, and 8% with GG, GC, and CC respectively, whereas in control group, it was 76% with GG, 22% with GC, and 2% with CC. The genotypic distribution revealed statistical difference ($p=0.003$) and with adjustment for age and gender ($p = 0.002$). The allelic frequencies were 67% who had the wild allele G and the remaining 33% had the variant allele C in CRC group, while in control group there were 87% with G and 13% with C allele. The difference was statistically significant ($p=0.001$). The GC genotype revealed a significant risk of CRC as compared to GG genotype. Subjects carrying the C allele had a significant risk of CRC compared to those carrying the allele G.

Table (3): Comparison between the two studied groups according to COX-2 gene polymorphism

COX-2 gene polymorphism	Control® (n = 50)		CRC group (n = 50)		χ^2 (p)	Univariate	
	No.	%	No.	%		p	OR (LL – UL 95%C.I)
Genotype						0.003*	
GG®	38	76.0	21	42.0	11.982*		1.0
GC	11	22.0	25	50.0	MCp=	0.002*	4.11 (1.69 – 9.99)
CC	1	2.0	4	8.0	0.002*	0.085	7.24 (0.76 – 69.03)
$^{HW}\chi^2$	0.038		0.854				
^{HW}p	0.846		0.355				
Dominant							
GG®	38	76.0	21	42.0	11.947*		1.0
GC + CC	12	24.0	29	58.0	(0.001*)	0.001*	4.373(1.854 – 10.316)
Recessive							
GG + GC®	49	98.0	46	92.0	1.895		1.0
CC	1	2.0	4	8.0	FEp=0.362	0.202	4.261(0.459 – 39.544)
Allele	(n = 100)		(n = 100)				
G®	87	87.0	67	67.0	11.293*		1.0
C	13	13.0	33	33.0	(0.001*)	0.001*	3.296 (1.61 – 6.748)

$^{HW}\chi^2$**:** Chi square for goodness of fit for Hardy-Weinberg equilibrium (If P < 0.05 - not consistent with HWE.)
MC: **Monte Carlo** ®: **Reference group** OR: **Odd`s ratio** C. I: Confidence interval
LL: Lower limit UL: Upper Limit *: Statistically significant at $p \leq 0.05$.

4- Relation between COX-2 gene polymorphism and different parameters in CRC group:

The present study verified significantly statistical differences between different genotype subgroups of COX2 polymorphism regarding some of tumor characteristics. CRC patients with CC genotype have significantly advanced tumor stage and positive LN metastasis (p<0.001). While, the distant metastasis, tumor side missed the statistical difference as CC was compared to GG (table 4).

Table (4): Relation between COX-2 gene polymorphism and different parameters in CRC group

	COX-2 gene polymorphism						Test of Sig.	p
	GG (n = 21)		GC (n = 25)		CC (n = 4)			
	No.	%	No.	%	No.	%		
Side of tumor								
Right	4	19.0	4	16.0	4	19.0		
Left	15	71.4	19	76.0	15	71.4	χ^2=	MCp=
Total colon	0	0.0	2	8.0	0	0.0	5.082	0.605
Transverse colon	2	9.5	0	0.0	2	9.5		
TNM stage								
Stage I	12	57.1	0	0.0	0	0.0		
Stage II	3	14.3	0	0.0	0	0.0	χ^2=	MCp
Stage III	2	9.5	15	60.0	2	50.0	30.786*	<0.001*
Stage IV	4	19.0	10	40.0	2	50.0		
L.N metastasis								
Negative	15	71.4	0	0.0	0	0.0	χ^2=	MCp
Positive	6	28.6	25	100.0	4	100.0	30.789*	<0.001*
Distant metastasis								
Negative	15	71.4	11	44.0	2	50.0	χ^2=	MCp=
Positive	6	28.6	14	56.0	2	50.0	3.615	0.145

*: Statistically significant at $p \leq 0.05$

DISCUSSION

The process by which arachidonic acid is changed into prostaglandin is carried out by the enzyme COX-2. Numerous mediators involved in the inflammatory process might cause it to behave. Studies have revealed a substantial rise in COX-2 expression in a variety of neoplasms, including the CRC [(11)]. The COX2 gene's single nucleotide polymorphism, which involves the substitution of a guanine for a cytosine at position -765, has been identified as a risk factor for the development of CRC [(12)]. In this study, regarding biochemical parameters there was significant increase in CEA level and CA 19,9 level in CRC group than in control group. The study of **Rao *et al.*[(13)]** agrees with our findings as regards CEA and CA 19,9 levels. They suggested that serum CEA and CA19-9 are useful markers for estimating the likelihood of colorectal cancer.

Regarding COX 2 gene polymorphism, the present study showed statistically significant difference in distribution of the genotypes and allele frequencies between the two studied groups. In the present study, the distribution of COX2 genotypes GG, GC, and CC in control and CRC groups revealed a significant difference. This difference postulated an association between the COX2 genotypes and increased vulnerability to colorectal cancer. The allelic frequencies were 87% who had the wild allele G and the remaining 13% had the variant allele C in control group. While, in CRC group there were 67% with G and 33% with C alleles. The findings suggest that the variant G allele is the major allele in the Egyptian population. **Cossiolo *et al.*** [(14)] reported in their study that G allele is the predominant allele in populations that they studied. The comparison of genotypes distribution and allele frequencies of COX 2 gene polymorphism between control and CRC groups showed that CC genotypes were associated with a significant risk of developing CRC than GG genotype, also GC genotypes were associated significantly with the risk of CRC compared with GG genotype. In agreement with our findings, **Cossiolo *et al.*** [(14)] reported in their study that G allele is the predominant allele in their studied populations, whereas, this polymorphism, which occurs in the promoter region of the gene, may lead to an increase in the COX-2 protein, an enzyme implicated in the stimulation of cell proliferation and angiogenesis, the prevention of apoptosis, and immune suppression, all of which have the potential to cause cancer. The C allele showed a significant risk of CRC.

In contrast to this result, **Khorshidi *et al.*[(15)]** reported that in an Iranian population, there was no association between COX-2 polymorphism genotype frequencies and sporadic CRC risk and decreased risk of CRC with the CC genotype. The present study verified significantly statistical differences between different genotype subgroups of COX2 polymorphism regarding some of tumor characteristics. CRC patients with CC genotype have significantly advanced tumor stage and positive regional LN metastasis. While, the distant metastasis, tumor side missed the statistical difference as CC was compared to GG. In their investigation, **Khorshidi *et al.*** [(15)] validated our findings by finding no connection between the COX2 polymorphism and tumour site or distant metastasis in an Iranian population. On the other hand, **Cossiolo *et al.*** [(14)] hypothesized that CC genotype was more prevalent in left colon tumour patients than right colon tumour instances.

This study aimed to characterise the relationship between the rectosigmoid lesions, which are significantly more common in the Egyptian population, and the COX2 gene polymorphism and CRC. Future developments in polymorphism research might be crucial because they will make it possible to pinpoint the patients who are most at risk for developing malignancies and personalise their treatment. For the treatment of colorectal neoplasm, polymorphic individuals with the C allele were likely candidates, particularly in cases of colorectal carcinoma.

CONCLUSION

The current study revealed that the COX2 gene's single nucleotide polymorphism is linked to a higher risk of developing colorectal cancer, particularly in tumours of the rectosigmoid segment. CRC in patients carrying risk allele C might have more invasive behavior especially to regional lymph nodes.

RECOMMENDATIONS

At the end of this study, we recommend that: Further studies on a high number of CRC patients are wanted to confirm the association between COX2 polymorphism and CRC risk. patients who have COX2 polymorphism should be investigated in future studies for distant metastasis with large sample size. Also, we recommend identifying COX2 genotype in early diagnosis to prevent CRC progression and invasion. Further studies are recommended on the effect of COX2 inhibitors on tumor progression and invasiveness.

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