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Cyclooxygenase-2 (COX2) gene polymorphisms and the risk of sporadic colorectal cancer and polyps among Jordanian population

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Maha Shomaf[1], AL-Motassem Yousef[2], Nidaa Ababna[3], Yahya Bobali[4]

[1]Department of Histopathology, The University of Jordan, Amman, Jordan
[2]Department of Biopharmaceutics and Clinical Pharmacy, College of Pharmacy, The University of Jordan, Amman, Jordan
[3]Department of Cell Theraphy, The University of Jordan, Cell Therapy Center, Amman, Jordan
[4]Medical School, The University of Jordan, Amman, Jordan

Original Article

ABSTRACT

Background/Aims: Cyclooxygenase-2 (COX2) is a crucial enzyme involved in the metabolism of Prostaglandins and it has been implicated in several processes. This study was done to investigate the associations of polymorphisms in COX2 gene with the risk of colorectal cancer or polyps development among Jordanian population and to correlate with other ethnicities.

Materials and Methods: One hundred and thirty five cases (135) of colorectal carcinoma were studied for COX2 –A1195G polymorphisms employing PCR-RFLP technique, in addition to 104 cases of adenomatous polyps and 115 matched controls taken from the general population.

Results: Sixty eight colorectal cancer patients were males and 67 of patients were females with a median age of (58.0±13.9 year). Sixty six (66) of polyp cases were males and 38 were females with a median age of (58.1±14.16).

The A-1195G AA carriers were 3.1 times less likely to develop CRC (95% CI: 1.8-5.3, p<0.0001), and 1.8 times less likely to develop polyps (95% CI: 0.99-3.2, p=0.056).The A-1195G AG carriers were at higher risk to develop cancer in a dose dependent manner. The AG carriers were 2.9 time more likely to develop CRC and two times more likely to develop polyps when compared to controls.

The A allele was more predominant in controls than in polyps or CRC cases. Carriers of the A allele were 1.6 times less likely to develop polyps and 2.6 times less likely to develop CRC.

Conclusion: The presence of the COX-2 -1195AA genotype may protect against risk of developing colorectal cancer.

Keywords: Colorectal cancer risk, COX2 polymorphisms, allele, genotype, frequencies, Jordan

INTRODUCTION

Colorectal cancer (CRC) is the second most common cancer in Jordanian adults and it is the leading cancer incidence, accounting for 14.3% of all male cancers (1). It is thought to result from a combination of environmental factors and accumulation of specific genetic alterations, and consequently mainly affects older patients. Recent studies showed strong evidence between CRC and Cyclooxygenase (COX) gene polymorphisms (2). COX enzyme, also known as prostaglandin end peroxide H synthase((5*Z*,8*Z*,11*Z*,14*Z*)-icosa-5,8,11,14-tetraenoate,hydrogen-donor:oxygen oxidoreductase, EC 1.14.99.1) is a key enzyme in the conversion of arachidonic acid into prostaglandins and belongs to a family of two isozymes: COX-1 and COX-2 (3). COX-1 is expressed in most cell types and is involved in the homeostasis of various physiological functions, while COX-2 is an inducible form and its expression can be induced by mitogenic and proinflammatory stimuli (3). Increased expression of COX-2 is observed in many other types of cancers of stomach, breast, skin (4,5,6) and others. COX-2 overexpression has been associated with an inhibition of apoptosis (7), an increased metastatic potential (8) and neoangiogenesis (9). Single nucleotide polymorphisms (SNPs) in the COX-2 may alter the function of the enzyme & modulate the risk for breast cancer (10), gastric

Address for Correspondence: Maha Shomaf, Department of Histopathology, The University of Jordan, Amman, Jordan
E-mail: mshomaf@ju.edu.jo
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adenocarcinoma (11), prostate cancer (12) and colorectal adenoma (13). Different polymorphic sites in the COX-2 gene, as polymorphisms rs20417 (-765G>C) and rs5275 (8473T>C) were extensively studied in relation to cancer risk (14).

The purpose of our study was to determine the prevalence of a common COX-2 polymorphisms rs689465 (-1195A>G) among Jordanian population with sporadic CRC and adenomatous polyps and their role on CRC development and progression and to compare our results to other ethnicities.

MATERIALS AND METHODS

Study population

A total of 138 colorectal cancer patients & 106 cases with adenomatous polyps retrieved from the archives of histopathology department at Jordan university hospital between Jan/2009 and Dec/2011. However, three CRC cases and two polyp samples were excluded, either because the sample was consumed or because of the failure of PCR or RFLP, and thus 135 CRC cases, 104 adenomatous polyps cases and 115 normal controls were included in our study. All the cases of cancer were adenocarcinoma of colon or rectum. None of the cases was of familial adenomatous polyposis (FAP) or non-polyposis colorectal cancer (HNPCC). The 104 cases with polyps consisted of cases diagnosed with adenomatous polyps and none of them was of polyposis syndromes. The control samples were collected from patients from the same geographical area whom their colonic biopsies revealed no significant pathological changes. Prior to DNA extraction, patient samples were verified by a histopathologist for the presence of tumor cells. Informed consent was obtained from each patient and the study was approved by the ethics committee in Jordan university hospital (institutional review board) in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration.

DNA Extraction

Genomic DNA was extracted from paraffin-embedded tissue using the QIAmp DNA FFPE kit (Qiagen), according to the manufacturer's protocol. The DNA obtained was eluted in 100 µL of sterile Nuclease free water, and the extracted DNA was stored at -20°C until used.

COX2 Genotyping

COX2 (-1195 A>G)

Polymerase chain reaction-restriction fragment length polymorphism analysis was performed to determine the genotypes of the -1195A>G polymorphisms of COX-2 gene, with the following primers F: 5' CCC TGA GCA CTA CCC ATG AT 3' (NT_004487.19 from 38139445 to 38139426) and R: 5' GCC CTT CAT AGG AGA TAC TGG 3' (NT_004487.19 from 38139173 to 38139193). The 20 µL PCR mixture contained approximately 50 ng DNA, 12.5 pmol of each primer, 0.1mM of each dNTP, 1X PCR buffer, and 1U Taq polymerase. The following PCR cycling conditions were used: an initial melting step of 5 min at 95°C; 35 cycles of 30 seconds at 95°C, 40 seconds at 61°C and 45 seconds at 72°C, and final elongation step of 10 min at 72°C. After confirmation of successful PCR amplification by 1.5% agarose gel electrophoresis, restriction enzymes PvuII (New England BioLabs, Beverly, MA) was used to distinguish the -1195A>G polymorphism. Not all samples were successfully analyzed for the two SNPs.

Statistical analysis

Allele and genotype frequencies for different alleles among Jordanian population were estimated from the results of the PCR-RFLP test. Genotype and allele frequencies were analyzed for concordance to the Hardy-Weinberg equilibrium. Differences in allele/genotype frequencies between controls and CRC cases or polyps were assessed using chi square test or Fisher exact test as appropriate. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated as a measure of association between *COX* genotypes/alleles and CRC. The Mantel-Haenszel statistics were used to estimate common odds ratios, their 95% CI and p values. A *p*-value below 0.05 was considered statistically significant throughout the population comparisons.

RESULTS

Sixty eight CRC patients were males and 67 of patients were females with a median age of (58.0±13.9 year). Sixty six (66) of polyp cases were males and 38 were females with a median age of (58.1±14.16). Fifty seven of the healthy controls were males and fifty eight were females with a median age of (46.10±18.32) as shown in Table 1.The difference in frequency of the A-1195G AA wild genotype between controls, polyp cases and CRC patients were 75.7%, 63.5% and 50.4% respectively. The AA carriers were 3.1 times less likely to develop CRC (OR=3.1, 95% CI: 1.8-5.3, p<0.0001), and 1.8 times less likely to develop polyps (OR=1.8, 95% CI: 0.99-3.2, p=0.056).The frequency of the heterozygous A-1195G AG genotype in CRC patients (45.2%) was higher compared to the control group (23.5%) and polyp cases (36.5%). The AG carriers were at higher risk to develop cancer in a dose dependent manner. The AG carriers were 2.9 time more likely to develop CRC (OR=0.35, 95% CI=0.2-0.6, p=0.0003) and two times more likely to develop polyps (OR=0.5, 95% CI 0.3-0.96, p=0.039) when compared to controls. The A allele was more predominant in controls than in polyps or CRC cases (Controls: 87.8%; Polyps:

Table 1. Age and gender characteristics of cases and controls

Parameter	Number (%) Average±SD			
Gender	Colorectal cancer (N=135)	Polyps (N=104)	Controls (N=115)	p value
Male	68 (50.4%)	66 (63.5%)	57 (49.6%)	0.04
Female	67 (49.6%)	38 (36.5%)	58 (50.4%)	
Median Age (years)	58.00±13.90	58.10±14.16	46.10±18.32	<0.0001

SD: standard deviation
N: number of the cases

81.73; CRC: 73.3%). Carriers of the A allele were 1.6 times less likely to develop polyps (95% CI: 0.7-3.4, p=0.26) and 2.6 times less likely to develop CRC (95% CI: 1.3-5.2, p=0.004). Distributions of all genotype frequencies are summarized in (Table 2).

DISCUSSION

In this case–control study, we assessed the role of -1195A>G, polymorphisms in CRC development. Given its location in the 5'UTR of COX-2 gene, the -1195A>G polymorphism is a potential candidate to modulate the genetic predisposition for CRC.

According to our results the the difference in frequency of the A-1195G AA wild genotype between controls, polyp cases and CRC patients found in our study suggests that this genotype has a protective effect against polyp formation & CRC since the AA carriers were 3.1 times less likely to develop CRC (95% CI: 1.8-5.3, p<0.0001), and 1.8 times less likely to develop polyps (95% CI: 0.99-3.2, p=0.056).

Our results were rather unexpected as the findings were in contrast to a recent meta-analysis of COX-2 -1195G>A polymorphism and the risk of digestive system cancers (15). In that study a total of 47 case-control studies were included in the meta-analysis which revealed that the genotypes GA/AA of -1195G>A were associated with a significantly increased cancer risk of the digestive system (e.g. colorectal, gastric, esophageal, oral, biliary tract, gallbladder, and pancreatic).

It should be noted that this association was evident among Asians than Caucasians. Additionally, only four case-control studies examined cases with colorectal cancer. None of the three Caucasian studies done, two in Netherlands (16,17) and one in USA (18), reported statistically significant association. Only one study done in china was consistent with the main findings of the meta-analysis (19).

The protective role of the A allele has also been reported by others. Peters WH et al investigated the -1195G>A genetic polymorphisms in patients with familial adenomatous polyposis (FAP) in The Netherlands. Logistic regression analysis revealed an overrepresentation of the -1195GG genotype compared to the -1195AA genotype in patients with FAP (OR=2.81; 95% CI=1.00-7.91, p=0.042) (20).

Pereira C et al conducted a hospital-based case-control study involving 117 CRC patients in Portugal and found that -1195A>G polymorphism was associated with a 1.73-fold increased predisposition to CRC onset (21).

An abundance of evidence supports a role for COX2 in colon cancer risk (22). It is known that COX2 is not constitutively expressed in the colon mucosa. However, different studies showed that COX2 is over expressed in colon carcinomas and in adenomas indicating the role of COX2 in colorectal tumorigenesis (23). The COX-2 protein was detected in 70% of all colorectal cancer tissues while in adjacent normal colorectal tissue in the same slide the COX-2 protein was not observed. These results suggest that increased expression of COX-2 is associated with CRC (24). The absence of COX2 inhibits the development of colorectal polyps and the use of Celecoxib and other specific inhibitor of COX2 in clinical trials showed a reduction in the number and size of colorectal polyps (25).

A recent publication offers a biological explanation for the involvement of this polymorphism in CRC. They characterized the influence of the -1195A>G promoter region polymorphism on COX-2 transcription activity in colon cancer cell lines (26). Luciferase reporter assays were performed to assess whether the -1195A/G alleles influenced COX-2 transcription. The COX-2 promoter's region containing either the -1195A or -1195G alleles was cloned into pGL3-basic reporter vector. The reporter vectors were transiently co-transfected with the pGL4.73 control plasmid to HCT-116 and HCA-7 colon cancer cell lines. The levels of reporter gene expression driven by the -1195G allele-containing COX-2 promoter were significantly higher in both colon cancer cell lines. A 2.2-fold increase in promoter activity was observed in the HCT-116 cell line (p<0.001). This overexpression was even more noticeable in the HCA-7 COX-2 ex-

Table 2. COX2 A1195G genotypes and allele type and risk of colorectal cancer

Parameter	Controls (N=115)	CRC (N=135)	OR1 (95%CI)	p value	Polyps (N=104)	OR2 (95%CI)	p value
AA	87 (75.7%)	68 (50.4%)	3.1 (1.8-5.3)	<0.0001	66 (63.5%)	1.8 (0.99-3.2)	0.056
AG	27 (23.5%)	61 (45.2%)	0.35 (0.2-0.6)	0.0003	38 (36.5%)	0.5 (0.3-0.96)	0.039
GG	1 (0.9%)	6 (4.4%)	0.19 (0.02-1.6)	0.13	0 (0.0%)	Not calculable	1
AA+AG	114 (99.1%)	129 (95.6%)	5.3 (0.6-44.7)	0.13		Not calculable	1
GG	1 (0.9%)	6 (4.4%)			0 (0.0%)		
A	101 (87.8%)	99 (73.3%)	2.6 (1.3-5.2)	0.004	85 (81.73%)	1.6 (0.7-3.4)	0.26
G	14 (12.2%)	36 (26.7%)			19 (18.26%)		

Data are reported as number (N= actual numbers) with percent in parentheses.
[1]Odds ratio was calculated against CRC
[2]Odds ratio was calculated against polyps

pressing cell line with a threefold higher transcriptional activity (p=0.001). The -1195G allele appeared to enhance COX-2 transcription, providing a molecular basis underlying the increased susceptibility for CRC and potentially a new mechanism for COX-2 overexpression (26).

These findings opposes the molecular mechanism suggested by Zhang et al. (27). It was reported that -1195 change created a transcriptional factor c-myeloblastosis oncogene binding site, and the-1195A allele displayed higher transcription activity and mRNA expression compared with the -1195G allele.

Limitations of the study

The findings of the current study should be read carefully with the following limitations:

Unfortunately we did not measure the COX-2 mRNA expression in -1195 genotypes. A major limitation is the missing information on BMI, smoking status, and the use of NSAIDs. Additionally, selection bias cannot be excluded as the study was conducted retrospectively. The influence of age difference between CRC patients and controls on current findings should not be underestimated. Finally, the sample size of our population could be insufficient to allow conclusive findings.

Ethics Committee Approval: Ethics committee approval was received for this study from Institutional Review Board of Jordan University Hospital.

Informed Consent: N/A.

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