Original Article

A preliminary study of cytokine gene polymorphism effects on Saudi patients with colorectal cancer

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ABSTRACT

الأهداف: تحديد الارتباطات المحتملة لتعدد الأشكال الجيني في IL–8 (rs4073 T/A)، و IL–10 (rs1800896 A/G)، و IL–22 (rs1179251C/G، rs2227458 C/T و IL–27 (rs17855750T/G و TGFβ1 (rs1800469 مع القابلية للإصابة بسرطان القولون والمستقيم لسعوديين.

المنهجية: أجريت دراسة الحالات المرضية والمجموعه الضابطة خلال الفترة من يوليو 2019م ويناير 2020م بمستشفى الملك خالد الجامعي، الرياض، المملكة العربية السعودية. وتضمنت 70 مريض بسرطان القولون و 70 فرد سليم. حددت التغيرات الفردية متعددة الأشكال للنيوكليوتيد بمناطق المحفز باستخدام مقياس التنميط الجيني TaqMan.

النتائج: وجد انخفاض بخطر الإصابه بسرطان القولون والمستقيم لحاملي الأنماط الجينية IL–10 AG (rs1800896 A/G) و IL–22 (rs1179251 C/G) أليل G و أليل G (rs17855750T / G) IL–27 و rs1800469 C / T (TGFß1) قيما كان الأنماط الجينية CT،TT و TGFß1 (rs1800469 C/T) CC، و IL–10 (rs1800896 A/G) محفزا للإصابة بالمرض. لم تحدد ارتباطات بين تعدد الأشكال الجيني لـ IL–8 (rs4073 T/A) و (rs2227485 C/T) IL–22 وخطر سرطان القولون والمستقيم.

الخلاصة: تشير النتائج لتأثير الكبير لتعدد الأشكال IL–10 (rs1800896 A/G)، و IL–22 (rs1179251 C/G)، و IL–27 (rs17855750 T/G) و TGFß1 (rs1800469 C/T) على خطر سرطان القولون والمستقيم؛ لم يترافق تعدد الأشكال IL–8 (rs4073T/A)، و IL–22 (rs2227485) C/T مع خطر سرطان القولون والمستقيم.

Objectives: To determine the possible associations of polymorphisms in interleukin (IL)-8 (*rs4073* T/A), IL-10 (*rs1800896* A/G), IL-22 (*rs1179251* C/G and *rs2227485* C/T), IL-27 (*rs17855750* T/G), and transforming growth factor beta 1 (TGFß1) (*rs1800469* C/T) with colorectal cancer (CRC) susceptibility in Saudi patients.

Methods: The case-control study was carried out between July 2019 and January 2020 in King Khaled University Hospital, Riyadh, Saudi Arabia. A total of 70 patients with CRC and 70 healthy controls were included in the study. Single nucleotide polymorphisms of promoter regions were determined using TaqMan genotyping assays.

Results: A statistically significant reduction in CRC risk was identified for carriers of the IL-10 (*rs1800896* A/G) AG genotype, IL-22 (*rs1179251* C/G) G allele, IL-27 (*rs17855750* T/G) G allele and TGFß1 (*rs1800469* C/T) CT and TT genotype. While IL-10 (*rs1800896* A/G) AA genotype and TGFß1 (*rs1800469* C/T) CC genotype were significantly associated with increased susceptibility to CRC. No significant associations were identified between the cytokine polymorphisms of IL-8 (*rs4073* T/A) and IL-22 (*rs2227485* C/T), and CRC risk.

Conclusion: Our findings indicate a significant impact of IL-10 (*rs1800896* A/G), IL-22 (*rs1179251* C/G), IL-27 (*rs17855750* T/G) and TGF-ß1 (*rs1800469* C/T) polymorphisms on risk of CRC; while the IL-8 (*rs4073* T/A) and IL-22 (*rs2227485* C/T) and polymorphisms were not associated with CRC risk.

Keywords: colorectal cancer, polymorphism, cytokines, IL-8, IL-10, IL-22, IL-27, TGFß1

Saudi Med J 2020; Vol. 41 (12):1292-1300
doi: 10.15537/smj.2020.12.25543

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Received 16th August 2020. Accepted 1st November 2020.

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Cancer is the world's second leading cause of death: it was responsible for 9.6 million deaths in 2018.[1] In the United States, 1,806,590 new cases of cancer and 606,520 cancer deaths are estimated to occur in 2020.[2] Cancer also exerts a powerful influence over the social and economic circumstances of the affected people, and it has become a major problem for the healthcare systems of many countries.[3]

Colorectal cancer (CRC) is one of the most common types of cancer and the most common cause of cancer-related deaths in developed countries.[4] Worldwide, CRC is the second most frequently identified cancer in men and the third most frequently identified cancer in women. In men, it is the fourth most common cause of cancer-related deaths and in women it is the third most common cause of cancer-related deaths.[5] It is projected that approximately 147,950 people will be diagnosed with CRC and 53,200 will die from this disease in 2020.[6]

In Saudi Arabia, CRC is the most common cancer among men (19.6% of 2405 new cases in 2018) and the third most common cancer among women (9.5% of 1159 new cases in 2018).[1] Since 2002, CRC has been recognized as the most commonly occurring cancer in Saudi Arabia; the highest rates of the incidence of CRC have been identified in Riyadh.[3]

Although the exact cause of CRC is currently unknown, some factors are associated with an increased risk of developing the disease: advanced age, lifestyle, diet, alcohol intake, lack of physical activity, smoking, family history of CRC and colon polyps, presence of colon polyps, exposure to radiation, and inherited genetic disorders.[7-9] These risk factors may play a role at the genetic level. Therefore, it is necessary to identify risk factors affecting the progression of CRC that may have an effective role in its diagnosis and treatment.[9]

It is necessary to improve the diagnosis and treatment of CRC by identifying new biomarkers related to its clinicopathological characteristics.[10] Several genetic variants involved in CRC pathogenesis have been identified through the development of sophisticated molecular techniques for genome analysis.[11]

Currently, more than 300 cytokines have been identified and CRC has been linked to both systemic and local changes in the cytokine profiles.[12] Genetic variations, such as single nucleotide polymorphisms (SNPs), sometimes lead to changes in the transcription, production, and functional activity of cytokines.[13]

Interleukin (IL) -8, -10, -22, and -27, and transforming growth factor beta 1 (TGFβ1), have been recognized as critical factors involved in CRC development.[12] Additionally, relationships between polymorphisms and cytokine levels, including those of TGFβ1 and IL-10, -22, and -27, have been reported in CRC patients.[14-17] However, the effects of these cytokine gene polymorphisms on CRC patients in Saudi Arabia are understudied. The present study aims to explore how CRC patients in Saudi Arabia are affected by the presence of several specific cytokine polymorphisms: IL-8 (*rs4073* T/A), IL-10 (*rs1800896* A/G), IL-22 (*rs1179251* C/G and *rs2227485* C/T), IL-27 (*rs17855750* T/G), and TGFß1 (*rs1800469* C/T).

Methods. This study was carried out in King Khaled University Hospital, Riyadh, Saudi Arabia. The case-control study was carried out between July 2019 and January 2020. We used the PubMed Modular for Biotechnology Information (NCBI) website to search for relevant publications. This study included 70 Saudi patients with CRC from the out-patient department in King Khaled University Hospital. All patients in the study were previously diagnosed with CRC by colonoscopy.[18]

Inclusion criteria of case group are patients who need partial colectomy of both genders and ≥18 years old. Exclusion criteria of case group are ≤18 years old, All patients had no previous diagnosis of inflammatory bowel disease. The control group consisted of 70 healthy Saudi subjects from the outpatient department at the King Khaled University Hospital, Riyadh, Saudi Arabia who is undergoing colonoscopy as preventive measure and who had a normal result. Participants were selected consecutively. The study performed was according to the principles of Helsinki Declaration. Ethical approval for the study was obtained from the Ethical Committee of King Saud University (Ref.No.19/0830/IRB). All patients and controls provided written informed consent and agreed to provide blood samples for this case-control study.

Deoxyribonucleic acid (DNA) preparation. Blood samples (10 ml) was obtained by venepuncture from both groups between July 2019 and November 2019, in tubes containing anti-coagulant EDTA. From peripheral blood genomic DNA was extracted by using a Puregene Purification Kit (Qiagen; Hilden, Germany), according to the manufacturer's protocol. Using a NanoDrop

Disclosur. This study was funded by the Deanship of Scientific Research at King Saud University, Riyadh, Kingdom of Saudi Arabia (Grant No. RG-1441-356).

ND-2000c Spectrophotometer (Thermo Scientific, Wilmington, DE, USA) for assay quantification of the extracted DNA.

Genotyping. Genotyping assays were performed using Assays-on-Demand TaqMan®SNP genotyping assays, according to the manufacturer's instructions (Thermo Fisher Scientific, Applied Biosystems, USA). Interlukin-8 (*rs4073* T/A, assay ID: C_11748116_10), IL-10 (*rs1800896* A/G, assay ID: C_1747360_10), IL-22 (*rs1179251* C/G, assay ID: C_3125110_10) and IL-22 (*rs2227485* C/T, assay ID: C_15955719_20), IL-27 (*rs17855750* T/G, assay ID: C_25926696_10), and TGFß1 (*rs1800469* C/T, assay ID: C_8708473_10). The TaqMan genotyping assay mix contained 2 primers for the sequence of interest and 2 TaqMan® Minor Groove Binder (MGB) probes for alleles detecting. The TaqMan® MGB Probes involved of target-specific oligonucleotides with VIC® dye (linked to the 5′ end of the Allele 1 probe) and 6FAM™ dye (linked to the 5′ end of the allele 2 probe). The presence of 2 probe pairs in each reaction allowed genotyping of the 2 possible variant alleles at the SNP site in a DNA target sequence. The presence or absence of an SNP determined by genotyping assay based on probed-associated dye fluorescence change. Assays were run at a final volume of 10 µl involving 5.5 µl of TaqMan® Genotyping MasterMix, 0.26 µl TaqMan®SNP Genotyping Assay mix containing primers and probes (Thermo Fisher Scientific, Applied Biosystems, USA), and 50 ng/µl DNA template. The thermocycling protocol contained an initial AmpliTaq Gold, UP enzyme activation step for 10 min at 95°C and 40 cycles for 15 sec at 95°C, and 40 cycles for 60 sec at 60°C. Reactions were achieved using ViiA™7 real-time PCR. Analyses of amplification products were achieved using ViiA™7, v.1.1. (Applied Biosystems, USA). All experimental conditions are available upon request. Each assay was replicate 2 times.

Statistical analysis. The data was entered in an Excel program and was carried out using IBM SPSS Statistics for Windows, version 22 (IBM Corp, Armonk, NY, USA). Mean and standard (SD) deviation were calculated by using the students' t-test for the comparison of results for the 2 groups. Genotypes were calculated manually and the normal and patient groups were compared using the online statistical platform (MEDCALC®, Belgium). Odds ratio (OR), 95% confidence interval (CI), Chi-square (X^2), and the p-value were calculated for small expected values using the 2-tailed Fisher's exact test in the same statistical software. Difference were considered statistically significant for $p<0.05$. Observed and expected all SNP were tested for deviation from Hardy-Weinberg equilibrium using the statical website.

Results. Genotyping was carried out by an allele discrimination assay using the TaqMan (ViiA™ 7 Real-Time PCR) method. The genotype and allele frequencies in the patient and control groups were calculated separately, and the strength of association was assessed by estimating the OR with 95% Cl.

All SNPs obeyed the Hardy-Weinberg Equilibrium ($p>0.05$) except for the TGF-ß1 (*rs1800469* C/T) SNP ($p=0.01$). This may be due to the limited population of subjects with the study (Riyadh city only).

Demographic and clinical characteristics of patients and control participating in this study are reported in Table 1. The mean age of the CRC patient was 55.90±1.56 years and that of the control was 55.20±1.42 years. The patient and the control groups were matched for gender and BMI ($p>0.05$).

Interleukin-8 gene (rs4073 T/A) polymorphism. The *rs4073* T/A polymorphism in the promoter region of the IL-8 gene was assayed in samples from patients with CRC and controls. Table 2 presents the base pairs in the wild type homozygous (TT), heterozygous (TA), and homozygous for the variant (AA). Overall, distributions of the various alleles of IL-8 (*rs4073*) T/A did not differ significantly between patients with CRC and controls. The majority of patients and controls were with heterozygous variant genotype (TA). The *p*-value was >0.05 for all genotypes and alleles.

Interleukin-10 gene (rs1800896 A/G) polymorphism. For *rs1800896* A/G polymorphism in the promoter region of the IL-10 gene in. (Table 3) presents the genotype and allele frequency of the A and

Table 1 - Demographic data of colorectal cancer (CRC) patients and control subjects included in this study.

Parameter	Patient mean±SEM	Control Mean±SEM	*P*-value
Number of subjects	70	70	
Age (yrs)	55.90±1.56	55.20±1.42	0.372
Body mass index (kg/m²)	30.85±0.87	30.70±0.85	0.452
Gender (%)			
Male	45 (64.29)	43 (61.43)	0.726
Fmale	25 (35.71)	27 (38.57)	
Grading and staging of disease (%)			
Stage II	13 (18.57)		
Stage III	40 (57.14)		
Stage IV	17 (24.28)		

G alleles in the patients and controls. We observed that the heterozygous AG was significantly decreased among patients with CRC (p=0.027) and this was confirmed by an OR=0.44. When considering the recessive model (AG+GG versus AA), we found significant association between AA genotype and risk of CRC (p=0.025, OR=0.45).

Interleukin-22 gene (rs1179251 C/G and rs2227485 C/T) polymorphisms. Two IL-22 polymorphisms were studied in Saudi patients with CRC and compared to the controls in **Table 4**. Genotypes (*rs1179251* C/G) of in the wild type homozygous (CC), heterozygous (CG), and homozygous for the variant GG. The results indicate the associated risk of IL-22 (*rs1179251* C/G) genotypes with CRC susceptibility in this population. Allele frequency showed significant decreased of the G allele to CC with p=0.022, and this was also confirmed by an OR of 0.47. Having one or more copy of allele (G) conferred an decrease in CRC risk (p=0.062, OR=0.13). The homozygous for the variant GG was decreased among patients with CRC (OR=0.06) but did not reach significance.

The IL-22 (*rs2227485*: C/T) polymorphism in the promoter region was assessed. The base sequence identified in this region in the wild type homozygous (CC), heterozygous (CT), and homozygous for the variant (TT). We did not found significant association between *rs2227485* C/T SNPs of IL-22 and the risk of developing CRC in the overall investigated Saudi population. In addition, the genotype and allelic frequencies were similar for the entire investigated population.

Table 2 - Interleukin-8 (*rs4073* T/A) genotype in patients with colorectal cancer (CRC) compared to the controls.

Genotype	CRC n (%)	Control n (%)	Control vs Patients			
			OR	CI	X^2	*P*-value
rs4073						
TT	15 (21.4)	14 (20.0)		Reference=1		
TA	30 (42.9)	35 (50.0)	0.80	0.33-1.92	0.25	0.396
AA	25 (35.7)	21 (30.0)	1.09	0.43-2.81	0.05	0.824
TA + AA	55	56	0.91	0.40-2.07	0.04	0.834
Allele						
T	0.43	0.45		Reference=1		
A	0.57	0.55	1.09	0.68-1.75	0.13	0.717

Abbreviations: no-number of individuals, OR: odds ratio, CI: confidence Interval; X^2: Chi Square, Freq: allele frequencies, p<0.05 was considered statistically significant, vr: versus

Table 3 - Interleukin-10 (*rs1800896* A/G) genotype in patients with colorectal cancer (CRC) compared to controls.

Genotype	CRC n (%)	Control n (%)	Control vs. Patients			
			OR	CI	X^2	*P*-value
rs1800896						
AA	35 (50.0)	22 (31.4)		Reference=1		
AG	29 (41.4)	43 (61.4)	0.44	0.23-0.87	5.68	0.027*
GG	6 (8.6)	5 (7.2)	0.75	0.20-0.91	0.18	0.670
AG + GG vs AA	35	48	0.45	0.23-0.91	5.00	0.025*
Allele						
A	0.71	0.62		Reference=1		
G	0.29	0.38	0.68	0.41-1.12	2.31	0.160

Abbreviations: no-number of individuals, OR: odds ratio, CI: confidence interval; X^2: Chi square, Freq: allele frequencies, p<0.05 was considered statistically significant, *: significant

Interleukin-27 gene (rs17855750T/G) polymorphism. The frequencies of the IL-27 (*rs17855750* T/G) promoter SNP were analyzed. These results are summarized in Table 5. The majority of patients and controls were homozygous for the variant type allele (GG). However, a slightly association between IL-27 (rs17855750 T/G) genotypes and risk of developing CRC was observed as a decrease in the GG genotype was observed in the CRC group (85.7% vs. 95.7%, OR=0.09). A significant association between the G allele protective effect against CRC is observed (OR=0.20, *p*=0.005).

Transforming growth factor-ß1 gene (rs1800469 C/T) polymorphism. The TGFß1 (rs1800469 C/T) polymorphism in the promoter region was assayed. Table 6 shows the genotype and allele frequency of the C and T alleles in patients and controls. It presents the SNP profile in the wild type homozygous (CC), heterozygous (CT), and mutated homozygous (TT) types. Clear tendency for a decrease in the CT genotype was observed in the CRC group (45.7% vs. 54.3%, OR=0.15, *p*=0.009). The TT genotype was significantly decreased in the patients (OR= 0.16, *p*=0.014). When considering the recessive model (CT + TT versus CC), high significant association was observed between CC genotype and occurrence of CRC (*p*=0.009). T allele was slightly lower in the CRC patients compared to the control group but failed to reach significance (*p*=0.161, OR=0.68).

Discussion. This study examined the relationship between some candidate polymorphism and CRC in a Saudi population following a case control study based on a group of CRC patients and confirmed unrelated healthy group at a 1:1 ratio. Both groups were age and BMI matched.

A polymorphism in the 251 position in the promoter region of the IL-8 gene (rs4073 T/A) has been identified. Several studies have identified the functional role of the *rs4073* T/A polymorphism.[19,20]

In this study, the IL-8 (*rs4073* T/A) polymorphism in the promoter region in Saudi patients with CRC and controls was assessed using the TaqMan sequencing method. The presence of TT, TA, and AA genotypes were confirmed. The results indicated no noticeable change in allele frequency in the CRC and control

Table 4 - Interleukin-22 (*rs1179251* C/G) and (*rs2227485* C/T) genotype in patients with colorectal cancer (CRC) compared to the controls.

Genotype	CRC n (%)	Control n (%)	Control vs. Patients			
			OR	CI	X^2	*P*-value
rs1179251						
CC	6 (8.6)	0 (0.0)		Reference=1		
CG	23 (32.9)	19 (27.1)	0.09	0.01-1.75	0.54	0.132†
GG	41 (58.5)	51 (72.9)	0.06	0.00-1.13		0.870†
CG + GG vs CC	64	70	0.13	0.02	1.09	0.062†
Allele						
C	0.25	0.14	Reference=1			
G	0.75	0.86	0.47	0.25-0.87	5.87	0.022*
rs2227485						
CC	14 (20)	18 (25.7)		Reference=1		
CT	29 (41.4)	25 (35.7)	1.49	0.62-3.59	0.80	0.372
TT	27 (38.6)	27 (38.6)	1.28	0.53-3.09	0.31	0.574
Total	70	70				
Allele						
C	0.41	0.44		Reference=1		
T	0.59	0.56	1.10	0.68-1.77	0.17	0.708

Abbreviations: no-number of individuals, OR: odds ratio, CI: confidence interval, χ^2: Chi square, Freq: allele frequencies, $p<0.05$ was considered statistically significant, *: sinificant, †: Haldane's correction was applied to overcome the zero case

Table 5 - Interleukin-27 (*rs17855750* T/G) genotype in patients with colorectal cancer (CRC) compared to the controls.

Genotype	CRC n (%)	Control n (%)	Control vs. Patients			
			OR	CI	X^2	*P*-value
rs17855750						
TT	4 (5.7)	0 (0.0)		Reference=1		
TG	6 (8.6)	3 (4.3)	0.21	0.01-5.05		0.591
GG	60 (85.7)	67 (95.7)	0.10	0.01-1.88		0.160
TG + GG vs TT	66	70	0.10	0.05-1.98		0.187†
Allele						
T	0.10	0.02		Reference=1		
G	0.90	0.98	0.20	0.06-0.70	7.58	0.005§

Abbreviations: no-number of individuals, OR: odds ratio, CI: confidence interval; X^2: Chi square, Freq: allele frequencies, $p<0.05$ was considered statistically significant, vs: versus, †: Haldane's correction was applied to overcome the zero case, §: high significant

Table 6 - Transforming growth factor-ß1 (*rs1800469* C/T) genotype in patients with colorectal cancer (CRC) compared to the controls.

Genotype	CRC n (%)	Control n (%)	Control vs. Patients			
			OR	CI	X^2	*P*-value
rs1800469						
CC	11 (15.7)	2 (2.8)		Reference=1		
CT	32 (45.7)	38 (54.3)	0.15	0.03-0.74	6.65	0.009§
TT	27 (38.6)	30 (42.9)	0.16	0.03-0.81	5.92	0.014*
CT + TT vs CC	59	68	0.16	0.03-0.74	6.87	0.009§
Allele						
C	0.39	0.30		Reference=1		
T	0.61	0.70	0.68	0.42-1.12	2.28	0.161

Abbreviations: no-number of individuals, OR: odds ratio, CI: confidence interval; X^2: Chi square, Freq: allele frequencies, $p<0.05$ was considered statistically significant, vs: versus, *: significant, §: high significance

groups. However, there were no significant differences in allele frequency between the CRC and control groups.

Meta-analysis of IL-8 (*rs4073* T/A) polymorphism association with risk of prostate cancer are in agreement with the results of the present study. Their study did not identify any significant association between the IL-8 (rs4073) TA and AA genotypes.[21]

In contrast with our study, Gonzalez-Hormazabal et al[20] reported that the polymorphism in the IL-8 gene was associated significantly with gastric cancer risk. The (rows) AA genotype was significantly associated with an increased risk of CRC when compared with the *rs4073* TT genotype. Additionally, for allele frequencies, compared with the wild type allele T of IL-8 -251, the variant allele A showed significant increases for CRC susceptibility risk. Similarly, Liu et al[19] investigated the association of the IL-8 -251 T/A and +781 C/T polymorphisms and glioma risk. They found patients with glioma had a significantly higher frequency of IL-8 -251 AA genotype. We suggests that further analyses of the IL-8 genotypes should be performed.

In our study, we also examined the association between the IL-10-1082 A/G (*rs1800896*) polymorphism and CRC. The results showed that the AA genotype vs AG+GG of the investigated IL-10 polymorphism increased the risk of CRC in the studied population. Also, a significant association was observed wherein those with heterozygous variant genotype

(AG) had a lower risk of developing CRC (OR=0.44, p=0.027). Additionally, we found that G allele was associated with a decreased risk of CRC, but this result was not statistically significant (OR=0.68, p=0.160). The higher producing G allele could be a protective factor in CRC susceptibility. These findings are in agreement with those of Gulubova et al,[17] who studied a Caucasian population. A previous study involving a Kashmiri population aimed to evaluate the association of IL-10 -592C/A and IL-10 -1082A/G promoter SNPs with CRC risk. This study found that the association between the IL-10 -592C/A promoter SNP and a decreased risk of CRC is significant, but found no significant association between IL-10-1082A/G SNP and the risk of CRC.[22] Basavaraju et al,[23] did not identify any statistically significant associations for the IL10-1082 A/G and IL10-592 A/C SNP risk of CRC in a population of Northeast Scotland.

The strong association that we observed in patients with CRC suggests that the IL-10 (*rs1800896* A/G) polymorphism indicates G allele possible protective role against CRC.

Polymorphisms in the IL-22 gene have been suggested as risk factors for cancers.[24] In the present study, we investigated 2 SNPs for the IL-22 (*rs1179251* C/G) and *(rs2227485*: C/T) polymorphism in Saudi patients with CRC and controls. The results showed that the polymorphism in IL-22 (rs1179251 C/G) Variant G allele was significantly associated with decreased risk of CRC (p=0.022) that confirmed by OR=0.47. Similarly, for variant GG homozygote, such an association was observed but not significance (OR=0.06).

Increasing evidence indicates that the IL-22 *rs1179251* C/G polymorphism is associated with many other cancers. It has been demonstrated that the *rs1179251* C/G polymorphism of IL-22 is associated with gastric cancer and might influence its progression.[25] A meta-analysis of 3 case control studies involving 799 cancer cases and 1129 controls investigated the *rs1179251* polymorphism. The results showed that the *rs1179251* C/G polymorphism was correlated with an increased risk of developing cancer.[24]

However, we did not identify any association between IL-22 (*rs2227485* C/T) and the risk of CRC. Comparison of the results for patients and controls showed no significant differences (p=0.708) in frequencies of C and T alleles of IL-22 *rs2227485* C/T.

In the present study, the results were in accordance with those found in the meta-analysis investigating the association between IL-22 (*rs2227485* C/T) polymorphism and cancer risk. Those results indicated that IL-22 (*rs2227485* C/T) polymorphisms were not associated with a risk of cancer.[24] We noted that data from previous studies in different ethnic populations are inconsistent with our results. Lin et al[16] found that CRC cases had a significantly frequency of the IL-22 (*rs2227485*) TT genotype and T allele compared to the controls when stratifying by the differentiation of CRC in a Chinese population. This suggests that the distribution of the IL-22 gene frequencies vary between different ethnic groups. Therefore, ethnic differences may be a plausible explanation for the absence of an association between *rs2227485* C/T polymorphisms with CRC risk compared to previous reports. Our results indicated that the IL-22 (*rs1179251* C/G) polymorphism was considered as a protective for CRC susceptibility in a Saudi population, while the (*rs2227485* C/T) polymorphism was not associated with CRC risk.

Interleukin-27 shown notable antitumor effects in different models of cancer. Thus, IL-27 has been suggested as a new potential agent in cancer immunotherapy studies.[26] Therefore, we suggest that SNPs of IL-27 may impact IL-27 anti-cancer activity, which might increase the risk of developing CRC.

We explored the *rs17855750* T/G polymorphism located 2905 bp upstream of the transcription start site of the IL-27 gene, and CRC risk. The results suggest that the IL-27 *rs17855750* T/G polymorphism is associated with CRC risk.

However, the IL-27 *rs17855750* T/G polymorphism has been identified, and a relationship between this gene polymorphism and a risk of developing endometrial cancer,[27] thyroid carcinomas,[28] cervical cancer,[29] or acute lymphoblastic leukemia[30] has been reported. Additionally, a meta-analysis aiming to determine the effects of IL-27 polymorphisms (*rs153109*, *rs17855750*, and *rs181206*) on cancer predisposition showed that the IL-27 *rs17855750* T/G polymorphism is significantly associated with increased susceptibility to cancer.[31]

We found that non significant differences in the GG genotype (OR=0.10, p=0.160) and significant differences in G allele (p=0.005, OR=0.20) frequencies were observed between CRC patients and controls, indicating that the G alleles may act as protection against CRC.

In contrast with our study, a meta-analysis reported that the polymorphism in the IL-27 gene was associated significantly with CRC risk in Chinese population.[32] The difference with our results may be due to ethnic variability in the allelic distribution of Il-27 polymorphism, which suggests that further analyses of the IL-27 genotypes should be performed.

Transforming growth factor-β1 It is known to affect many marks of cancer that contain angiogenesis, tissue invasion, immune suppression, metastasis and immune suppression.[33] We found that the carriers of the CT genotype had a lower risk of developing CRC (OR=0.15, *p*=0.009), also the carriers of the TT genotype (OR=0.16, *p*=0.014). Our analyses showed a strong association with having one or more copy of the T allele and decrease in predisposition to CRC. However, we also found that significantly high frequency of the TGF-β1 (*rs1800469*) CC genotype vs TT+TC with CRC risk (OR=0.16, *p*=0.009).

These results are consistent with those of a case-control study that found a significantly increased risk of advanced CRC cancer for the TGF-β1 (*rs1800469*) CC genotype while T allele was protective against development and progression of CRC in a Bulgarian population.[34]

Transforming growth factor-β have important role in promoting progression of tumor.[35] TGF-β1 gene mRNA expression in CRC tissue relates with tumor progression and metastasis.[34] We speculate that the TGF-β1 (rs1800469) CC genotype carriers could a high level of TGF-β expression, which increased CRC susceptibility compared to its counterpart.

Study limitations. The limitation of this study were the relatively small sampled size and conduct only in one city.

In conclusion, statistically significant reduction of CRC risk was found for carriers of the IL-10 (rs1800896 A/G) AG genotype, IL-22 (rs1179251 C/G) G allele, IL-27 (rs17855750T/G) G allele and TGFß1 (rs1800469 C/T) CT and TT genotype. Also, we found significant associations between the cytokine polymorphisms of IL-10 (rs1800896 A/G) AA genotype and TGFß1 (rs1800469 C/T) CC genotype and increase CRC risk. We did not identify significant associations between the cytokine polymorphisms of IL-8 (rs4073 T/A) and IL-22 (rs2227485 C/T) and CRC risk. However, additional studies are needed to determine the associations of IL-8, -10, -22, and -27 and TGF-ß1 polymorphisms with CRC risk under several genetic association models in a Saudi population. Kindly

Acknowledgment. *We would like extend our appreciation to the Deanship of Scientific Research at King Saud University, Riyadh, Kingdom of Saudi Arabia for funding this work through research group no. RG-1441-356. We would also like to thank Editage (www.editage.com) for English language editing.*

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