Polymorphisms of tumor necrosis factor alpha in Middle Eastern population with colorectal cancer

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Abstract Tumor necrosis factor-alpha (TNF-α) contributes in inflammation and has been implicated in the development of colorectal cancer (CRC). Single nucleotide polymorphisms (SNPs) in TNF-α promoter could affect the risk of CRC by regulating TNF-α production. This is the first study to investigate TNF-α SNPs in a Middle Eastern population. In this study, we examined three SNPs in TNF-α for association with CRC. One hundred CRC patients and 100 controls were genotyped for TNF-α -308, -238, and -857 using TaqMan allelic discrimination assay. The TNF-α -238 (G/A) genotype was significantly associated with high risk of CRC ($p=0.003552$). The distribution of three genotypes of -238 G/A was significantly different between the controls and CRC patients even after Bonferroni’s correction. The AA genotype of -238 G/A SNP was observed at considerably higher proportion (13 %) in CRCs compared to controls (1 %). Additionally, similar to genotypes, the allelic frequencies of -238 G/A were significantly different between the CRC cases and controls (odds ratios (OR)=7.647, $\chi^2=18.50$, $p=0.00002$). The genotype frequencies of -308 and -857 were not notably different between the cases and controls. TNF-α -238A may be useful as a screening marker to identify individuals prior to their acquiring CRC in the Saudi population although, further validations in larger cohorts are needed.

Keywords Tumor necrosis factor · Polymorphisms · Colorectal cancer

Introduction

Colorectal cancer (CRC) is one of the most common cancers worldwide. It is the third most common cancer in males and the second in females. Incidence rates of CRC are rapidly increasing in several areas in the world, probably related to a combination of genetic factors as well as lifestyle changes including diet, obesity, and smoking [1–3]. In the Kingdom of Saudi Arabia, it ranks first in incidence among Saudi males and third among Saudi females according to the Saudi Cancer Registry data [4]. Reports from Saudi Arabia suggest that CRC is a more aggressive disease in that population [5, 6].

Inflammation is one of the key factors involved in carcinogenesis, and people with inflammatory bowel disease are at higher risk of CRC [7]. Therefore, polymorphisms of inflammation-related genes have been regarded as potential sources of cancer risk biomarkers [8]. CRC arises from the accumulation of mutations in oncogenes and tumor suppressor genes during tumorigenesis [9]. Biological and epidemiological studies indicated a clear association between chronic inflammation and colorectal cancer [10, 11]. In the recent years, cytokines have received much attention due to their possible role in tumorigenesis [12, 13]. Cytokines are important inflammatory mediators that may act in a regulatory network to directly or indirectly activate downstream signaling pathways key to the development of malignancies [11, 14]. Human predisposition to cancer could be influenced by single nucleotide
polymorphisms (SNPs) located in genes encoding cytokines and their receptors, especially in promoter regions [15].

Tumor necrosis factor-alpha (TNF-α) is an important pro-inflammatory cytokine involved in cell growth, differentiation, and apoptosis [16, 17]. It has also been reported to play a critical role in the carcinogenesis [18]. The TNF-α gene is located on chromosome 6p21.3. Cytokine production is controlled at the transcriptional level, and many promoter polymorphisms have been described [19]. Previous studies indicated that TNF-α-related cellular functions were influenced by polymorphism in the promoter region of the TNF-α gene [20–22]. Recently, TNF-α was suggested to be one of the immunomodulatory genes in the progression of sporadic CRC [23].

The aim of our study was to examine the allelic frequencies of three promoter SNPs (-857 C/T, -308 G/A and -238 G/A) in Saudi patients with CRC versus healthy controls, in order to identify any association of these SNPs with susceptibility to CRC. These SNPs were selected as they were the common TNF-α SNP sites that were examined in other populations besides being included in the SNP500Cancer project that aims to identify and characterize genetic variants in genes important in cancer.

Material and methods

Study population

A total of 200 blood samples were obtained from King Khalid University Hospital. These encompassed 100 patients with sporadic colorectal cancer and 100 healthy controls with no history of cancer. Colorectal cancer patients included patients of all ages (median age=57 years) and all stages of the disease. Patient blood samples were collected preoperatively. None of the patients underwent preoperative irradiation or chemotherapy. Diagnosis of CRC were established by standard diagnostic procedures and confirmed histopathologically. Patients and controls were from Saudi Arabian ethnicity. All controls were gender- and age-matched and recruited from physical examinations after diagnostic exclusion of cancer. The study complied with the requirements, and has been approved by the Ethics Committee of the King Saud University. Patient consent was obtained for this study.

DNA extraction

Approximately 3 ml of blood samples were collected in vacutainers containing ethylenediaminetetraacetic acid (EDTA) from all subjects enrolled in the study. Genomic DNA was extracted from peripheral blood using the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA) and stored at −80 °C until the time of study. After extraction and purification, the DNA was quantitated spectrophotometrically on NanoDrop 8000 (Thermo Scientific, USA), and its purity examined using standard A260/A280 and A260/A230 ratios.

SNP selection and genotyping

A total of three important TNF-α SNPs were selected from the SNP500 Cancer project and previous literature [24–26]. SNPs were genotyped using TaqMan allelic discrimination assay as previously described [27]. TaqMan TNF-α SNP Genotyping Assays having catalogue number 4351379 and assay numbers C_11918223_10 (rs1799724), C_7514879_10 (rs1800629), and C_2215707_10 (rs361525) were acquired from Applied Biosystems. These assays were supplied at 40X concentration. For each PCR, a 5-ng DNA sample was used with 12.5 μL of 2X Universal Master Mix and 1X assay mix in a total of 25 μL reaction volume (Applied Biosystems, Foster City, CA, USA). PCR conditions are as follows: pre-read stage 60 °C for 30 s, hold stage 95 °C for 10 min, PCR stage 95 °C for 15 s and 60 °C for 1 min for 40 cycles, and post-read stage 60 °C for 30 s. All genotypes were determined by endpoint reading on ViiA™ 7 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). For quality control, 5 % of the samples were randomly selected and subjected to repeat analysis as measure for verification of genotyping procedures. The results were reproducible without any discrepancies.

Statistical analysis

Comparisons of genotype and allelic frequencies in the CRC group and healthy individuals were calculated according to Pearson’s goodness-of-fit chi-square. Deviation from Hardy-Weinberg equilibrium, χ² values, odds ratios (OR), 95 % confidence intervals (CI), and p values were calculated using the web-based programs (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl, http://www.socsicistatistics.com). A p value of <0.05 was considered as significant. Additionally, for multiple comparisons of the three SNPs in the TNF-α gene that were examined, Bonferroni’s correction was applied with an α=0.0167 considered as significant.

Results

Genotype and allele association with CRC risk

To determine the risk of predisposition to colorectal cancer with the genetic variants in the TNF-α gene, three different loci comprising SNPs rs1800629, rs361525, and rs1799724 were examined in Saudi Arabian patients. All the three SNPs are within 2 kb upstream of the 5′ region of the gene and hence likely to be in the promoter. The study population comprised 100 colorectal cancer cases and the same number of age- and gender-matched normal healthy individuals. The
clinico-pathological characteristics of CRC patients are presented in Table 1. The distribution of genotype and allele frequencies of the analyzed SNPs along with odds ratio and significance are shown in Table 2. The homozygous ancestral allele was used as a reference to determine the risk of acquiring CRCs associated with the other two genotypes. Of the three SNPs in the TNF-α gene that were analyzed, statistically significant association with CRC risk was observed only for rs361525. The distribution of the three genotypes of rs361525 was significantly different between the CRC cases and controls (OR=7.647, χ²=18.50, p=0.00002). The AA genotype as well as A allele of rs361525 was highly significant even after Bonferroni’s correction for multiple testing.

The distribution of genotype frequencies of rs1800629 was not statistically significant between the CRC cases and controls (χ²=2.79, df=2, p=0.247381). Although the AA genotype of rs1800629 was found in CRC cases at a lower frequency (0.10) compared to controls (0.18) and conferred about twofold decreased risk against CRCs, this association was not statistically significant (OR=0.489, χ²=2.79, p=0.09464) (Table 2). In case of rs1799724 polymorphism, the distribution of genotype and allele frequencies between the CRCs and healthy normal subjects were exactly similar (Table 2). Only the CC and CT genotypes were observed while the TT homozygotes were absent in both the cases and control populations.

**Association of TNF-α SNPs with CRC risk based on age at disease diagnosis and gender**

To investigate whether TNF-α SNPs rs1800629, rs361525, and rs1799724 are associated with the age at CRC diagnosis, patients were stratified based on the median age at the time of disease diagnosis as ≤57 (n=50) and >57 (n=50) years and the genotype and allele frequencies were compared to the age-matched controls. The distribution of genotype and allele frequencies along with the statistical analysis of the three TNF-α SNPs in CRC cases and normal control population in the two age groups are shown in Table 3. Similar to the findings in the overall study population, individuals with the AA homozygosity compared to the GG homozygosity in rs361525 were at significantly higher risk of developing CRCs. Additionally, examination of the allelic frequencies of rs361525 suggested that the minor allele A confers significantly higher risk of developing CRCs. However, the genotype as well as allele associations were not affected by the age at disease diagnosis as they were observed in both age groups (Table 3). The genotypes of rs1800629 and rs1799724 did not show any predisposition to CRCs in younger as well as older age group patients. Nonetheless, it was noted that the minor allele A of rs1800629 exerts about twofold protective effect in patients >57 years of age (OR=0.508, χ²=4.34, p=0.03726).

The prevalence of genotype and allele frequencies of the three TNF-α SNPs that were examined in CRC cases and normal control population according to the gender are presented in Table 4. In both the male as well as female populations, individuals with the AA genotype of rs361525 has significantly higher risk of developing CRCs compared with those having GG homozygosity (male—OR=10.333, χ²=6.95, p=

**Table 1** Clinico-pathological characteristics of colorectal cancer patients

| Variable                              | Number of patients | Percent |
|---------------------------------------|--------------------|---------|
| Median age (57 years)                 |                    |         |
| ≤57                                   | 50                 | 50      |
| >57                                   | 50                 | 50      |
| Gender                                |                    |         |
| Male                                  | 64                 | 64      |
| Female                                | 36                 | 36      |
| Primary tumor                         |                    |         |
| Colon                                 | 85                 | 85      |
| Rectum                                | 15                 | 15      |
| Tumor staging (UICC 2010)             |                    |         |
| pT1                                   | 4                  | 4       |
| pT2                                   | 13                 | 13      |
| pT3                                   | 73                 | 73      |
| pT4                                   | 10                 | 10      |
| Lymph node status (UICC 2010)         |                    |         |
| pN0                                   | 68                 | 68      |
| pN1                                   | 20                 | 20      |
| pN2                                   | 12                 | 12      |
| Clinical staging (UICC 2010) and metastasis |            |         |
| Stage I                               | 11                 | 11      |
| Stage II                              | 58                 | 58      |
| Stage III                             | 17                 | 17      |
| Metastasis                            | 14                 | 14      |
| Histological grading (UICC 2010)      |                    |         |
| G1                                    | 1                  | 1       |
| G2                                    | 97                 | 97      |
| G3                                    | 2                  | 2       |
Furthermore, based on the allelic model, the minor A allele of rs361525 exhibited significant association with CRCs albeit not showing gender specificity (Table 4). The TNF-α polymorphisms rs1800629 and rs1799724 did not show any association with the risk of developing CRCs in males as well as in females as was observed in the overall study population.

**Discussion**

Inflammation plays an important role in the pathogenesis of CRC. TNF-α is a critical component in the inflammatory pathway whose level is known to be upregulated in CRC including in Saudi patients [28, 29]. Hence, in this study, we investigated the link between genetic variants in the TNF-α gene promoter and susceptibility to CRC in a Saudi population. Of the three SNPs in the TNF-α gene that were analyzed, a significant association with CRC was observed only for TNF-α -238. The other two SNPs, -308 and -857, were not significantly associated. It was found that in our population except for the SNP -857 (rs1799724), the other two SNPs, -308 (rs1800629) and -238 (rs361525), did not follow the Hardy-Weinberg equilibrium (Table 5). A high rate of consanguinity in Saudi Arabia could be a probable cause for this lack of Hardy-Weinberg equilibrium [30, 31]. A significantly low frequency of the homozygous “A” allele of TNF-α SNP -238 in the normal control subjects (0.01), compared to the CRC cases (0.13), implicates this genotype as a risk factor in the Saudi population. The odds of acquiring CRC in individuals with AA homozygosity of SNP -238 were 14-fold higher compared to GG homozygotes. The high level of association between the AA genotype of TNF-α -238 and CRC existed even after Bonferroni’s correction for multiple comparisons, suggesting this association was not by chance. We hypothesize that the -238A allele of the TNF-α gene might be inducing higher affinity for transcription factor binding, leading to increased transcription in the tumors. Carriers of the GG genotype of TNF-α -238 were characterized by low production of TNF-α by the peripheral blood mononuclear cells and in the sputum of chronic obstructive pulmonary disease patients [32, 33]. Additionally, Maxwell and colleagues demonstrated that the GA genotype at TNF-α -238 was associated with a poorer response to infliximab, an anti-TNF agent in the treatment of rheumatoid arthritis [34].

Recently, Yu et al and Ming et al conducted a comprehensive meta-analysis about TNF-α -238 polymorphism and gastric cancer susceptibility and reported significant associations in Asian populations [35, 36]. However, another meta-analysis showed no association with gastric cancer in a different patient cohort [37, 38]. A number of studies that investigated the association between TNF-α -238 promoter polymorphism and risk of CRC have yielded contradictory results.

| SNP ID   | Genotype | CRC n (frequency) | Controls n (frequency) | OR (95 % CI) | χ² value | p value* |
|----------|----------|------------------|------------------------|--------------|----------|---------|
| rs1800629 | GG       | 67 (0.67)        | 59 (0.59)              | Ref          |          |         |
|          | GA       | 23 (0.23)        | 23 (0.23)              | 0.881 (0.448–1.731) | 0.14     | 0.71215 |
|          | AA       | 10 (0.10)        | 18 (0.18)              | 0.489 (0.209–1.143) | 2.79     | 0.09464 |
| Allele   | G        | 157 (0.785)      | 141 (0.705)            | Ref          |          |         |
|          | A        | 43 (0.215)       | 59 (0.295)             | 0.655 (0.416–1.031) | 3.37     | 0.06644 |
| rs361525  | GG       | 86 (0.86)        | 97 (0.97)              | Ref          |          |         |
|          | GA       | 1 (0.01)         | 2 (0.02)               | 0.564 (0.050–6.329) | 0.22     | 0.63808 |
|          | AA       | 13 (0.13)        | 1 (0.01)               | 14.663 (1.879–114.420) | 10.94    | 0.00094 |
| Allele   | G        | 173 (0.865)      | 196 (0.98)             | Ref          |          |         |
|          | A        | 27 (0.135)       | 4 (0.02)               | 7.647 (2.624–22.290) | 18.50    | 0.00002 |
| rs1799724 | CC       | 85 (0.85)        | 85 (0.85)              | Ref          |          |         |
|          | CT       | 15 (0.15)        | 15 (0.15)              | 1.000 (0.460–2.173) | 0.00     | 1.00000 |
|          | TT       | 0 (0.00)         | 0 (0.00)               | na           | na       | na      |
| Allele   | C        | 185 (0.925)      | 185 (0.925)            | Ref          |          |         |
|          | T        | 15 (0.075)       | 15 (0.075)             | 1.000 (0.475–2.105) | 0.00     | 1.00000 |

CRC colorectal cancer, OR odds ratio, 95 % CI 95 % confidence interval

*p<0.05 was considered significant and are depicted in bold.
A recent meta-analysis on TNF-α-238 polymorphism did not find a significant association with CRC risk [25]. This difference in the predisposition of the disease related to the SNP could be due to ethnic diversity between populations. Larger studies in the Saudi population as well as other cohorts are required to confirm the association of -238 with the risk of CRC observed in our study.

We found no significant association between TNF-α-308 polymorphism and CRC patients in the overall analysis. Our results are in agreement with other investigations that did not find a correlation between TNF-α-308 and sporadic colon

Table 3  Distribution of TNF-α SNPs genotype and allele frequencies in colorectal cancer cases and control population based on age

| SNP ID    | Genotype | CRC       | Controls  | OR (95 % CI) | $\chi^2$ value | p value* |
|-----------|----------|-----------|-----------|--------------|----------------|----------|
|           |          | n (frequency) | n (frequency) |              |                |          |
| ≤57       |          |            |           |              |                |          |
| rs1800629 | GG       | 31 (0.62)  | 30 (0.60) | Ref          |                |          |
|           | GA       | 15 (0.30)  | 14 (0.28) | 1.037 (0.428–2.511) | 0.01 | 0.93606 |
|           | AA       | 04 (0.08)  | 06 (0.12) | 0.645 (0.165–2.516) | 0.40 | 0.52586 |
| Allele    | G        | 77 (0.77)  | 74 (0.74) | Ref          |                |          |
|           | A        | 23 (0.23)  | 26 (0.26) | 0.850 (0.446–1.621) | 0.24 | 0.62185 |
| rs361525  | GG       | 43 (0.86)  | 47 (0.94) | Ref          |                |          |
|           | GA       | 00 (0.00)  | 02 (0.04) | 0.218 (0.010–4.677) | 1.79 | 0.18043 |
|           | AA       | 07 (0.14)  | 01 (0.02) | 7.651 (0.904–64.753) | 4.64 | 0.03126 |
| Allele    | G        | 86 (0.86)  | 96 (0.96) | Ref          |                |          |
|           | A        | 14 (0.14)  | 04 (0.04) | 3.907 (1.239–12.323) | 6.11 | 0.01348 |
| rs1799724 | CC       | 43 (0.86)  | 43 (0.86) | Ref          |                |          |
|           | CT       | 07 (0.14)  | 07 (0.14) | 1.000 (0.323–3.095) | 0.00 | 1.00000 |
|           | TT       | 00 (0.00)  | 00 (0.00) | 1.000 (0.019–51.542) | na  | 1.00000 |
| Allele    | C        | 93 (0.93)  | 93 (0.93) | Ref          |                |          |
|           | T        | 07 (0.07)  | 07 (0.07) | 1.000 (0.337–2.963) | 0.00 | 1.00000 |
| >57       |          |            |           |              |                |          |
| rs1800629 | GG       | 36 (0.72)  | 29 (0.58) | Ref          |                |          |
|           | GA       | 08 (0.16)  | 09 (0.18) | 0.716 (0.245–2.089) | 0.38 | 0.53994 |
|           | AA       | 06 (0.12)  | 12 (0.24) | 0.403 (0.135–1.204) | 2.74 | 0.09773 |
| Allele    | G        | 80 (0.80)  | 67 (0.67) | Ref          |                |          |
|           | A        | 20 (0.20)  | 33 (0.33) | 0.508 (0.267–0.966) | 4.34 | 0.03726 |
| rs361525  | GG       | 43 (0.86)  | 50 (1.00) | Ref          |                |          |
|           | GA       | 01 (0.02)  | 00 (0.00) | 3.483 (0.138–87.708) | 1.15 | 0.28385 |
|           | AA       | 06 (0.12)  | 00 (0.00) | 15.092 (0.826–275.623) | 6.52 | 0.01068 |
| Allele    | G        | 87 (0.87)  | 100 (1.00) | Ref          |                |          |
|           | A        | 13 (0.13)  | 00 (0.00) | 31.011 (1.817–529.290) | 13.90 | 0.00019 |
| rs1799724 | CC       | 42 (0.84)  | 42 (0.84) | Ref          |                |          |
|           | CT       | 08 (0.16)  | 08 (0.16) | 1.000 (0.343–2.913) | 0.00 | 1.00000 |
|           | TT       | 00 (0.00)  | 00 (0.00) | 1.000 (0.019–51.569) | na  | 1.00000 |
| Allele    | C        | 92 (0.92)  | 92 (0.92) | Ref          |                |          |
|           | T        | 08 (0.08)  | 08 (0.08) | 1.000 (0.360–2.778) | 0.00 | 1.00000 |

CRC colorectal cancer, OR odds ratio, 95 % CI 95 % confidence interval, na not analyzable
*p<0.05 was considered significant and are depicted in bold
cancer in a Spanish population [42], a Hungarian population [43], a Korean population [39], and a Croatian population [23]. In addition, TNF-α -308 polymorphism was not associated with colon cancer in any European population in a meta-analysis conducted by Fan et al, 2011 [44]. Two previous meta-analyses also reported lack of association of TNF-α -308 polymorphism with CRC risk [45, 46]. However, a meta-analysis by Min et al reported TNF-α -308 to be moderately associated with an increased risk of CRC in some Western populations [25].

Our investigation of the risk of CRC associated with -308 polymorphism found an inverse association with the AA genotype in patients >57 years of age as well as in the unstratified subjects. An approximately twofold lower risk of CRC

| SNP ID     | Genotype | CRC n (frequency) | Controls n (frequency) | OR (95 % CI) | χ² value | p value* |
|------------|----------|-------------------|------------------------|--------------|----------|---------|
| rs1800629  | GG       | 43 (0.67)         | 36 (0.56)              | Ref          |          |         |
|            | GA       | 12 (0.19)         | 14 (0.22)              | 0.718 (0.295–1.746) | 0.54     | 0.46359 |
|            | AA       | 09 (0.14)         | 14 (0.22)              | 0.538 (0.209–1.388) | 1.67     | 0.19644 |
| Allele     | G        | 98 (0.766)        | 86 (0.672)             | Ref          |          |         |
|            | A        | 30 (0.234)        | 42 (0.328)             | 0.627 (0.361–1.087) | 2.78     | 0.09529 |
| rs361525   | GG       | 54 (0.844)        | 62 (0.968)             | Ref          |          |         |
|            | GA       | 01 (0.016)        | 01 (0.016)             | 1.148 (0.070–18.800) | 0.01     | 0.92279 |
|            | AA       | 09 (0.140)        | 01 (0.016)             | 10.333 (1.268–84.211) | 6.95     | 0.00837 |
| Allele     | G        | 109 (0.852)       | 125 (0.977)            | Ref          |          |         |
|            | A        | 19 (0.148)        | 03 (0.023)             | 7.263 (2.092–25.210) | 12.73    | 0.00036 |
| rs1799724  | CC       | 54 (0.84)         | 52 (0.81)              | Ref          |          |         |
|            | CT       | 10 (0.16)         | 12 (0.19)              | 0.802 (0.319–2.017) | 0.22     | 0.63938 |
|            | TT       | 00 (0.00)         | 00 (0.00)              | 0.963 (0.019–49.443) | na       | 1.00000 |
| Allele     | C        | 118 (0.922)       | 116 (0.906)            | Ref          |          |         |
|            | T        | 10 (0.078)        | 12 (0.094)             | 0.819 (0.341–1.970) | 0.20     | 0.65560 |
| rs1800629  | GG       | 24 (0.67)         | 23 (0.64)              | Ref          |          |         |
|            | GA       | 11 (0.30)         | 09 (0.25)              | 1.171 (0.410–3.348) | 0.09     | 0.76787 |
|            | AA       | 01 (0.03)         | 04 (0.11)              | 0.240 (0.025–2.307) | 1.75     | 0.18626 |
| Allele     | G        | 59 (0.819)        | 55 (0.764)             | Ref          |          |         |
|            | A        | 13 (0.181)        | 17 (0.236)             | 0.713 (0.317–1.603) | 0.67     | 0.41177 |
| rs361525   | GG       | 32 (0.89)         | 35 (0.97)              | Ref          |          |         |
|            | GA       | 00 (0.00)         | 01 (0.03)              | 0.364 (0.014–9.258) | 0.90     | 0.34220 |
|            | AA       | 04 (0.11)         | 00 (0.00)              | 9.831 (0.509–189.758) | 4.12     | 0.04235 |
| Allele     | G        | 64 (0.889)        | 71 (0.986)             | Ref          |          |         |
|            | A        | 08 (0.111)        | 01 (0.014)             | 8.875 (1.080–72.920) | 5.81     | 0.03345 |
| rs1799724  | CC       | 31 (0.86)         | 33 (0.92)              | Ref          |          |         |
|            | CT       | 05 (0.14)         | 03 (0.08)              | 1.774 (0.391–8.055) | 0.56     | 0.45325 |
|            | TT       | 00 (0.00)         | 00 (0.00)              | 1.063 (0.020–55.233) | na       | 1.00000 |
| Allele     | C        | 67 (0.93)         | 69                    | Ref          |          |         |
|            | T        | 05 (0.07)         | 03                    | 1.716 (0.395–7.467) | 0.53     | 0.71900 |

CRC colorectal cancer, OR odds ratio and 95 % CI 95 % confidence interval, na not analyzable
*p<0.05 was considered significant and are depicted in bold
for individuals with the AA genotype was noted compared to the GG genotype. However, this association was not statistically significant. Additionally, in the allelic model, we found that the “A” allele of TNF-α -308 presents a significant protective effect against CRC in individuals >57 years of age. The mechanism behind this protection cannot be explained and needs further investigation as the “A” allele of TNF-α -308 is associated with higher expression of the cytokine [47]. The three TNF-α promoter polymorphisms examined in this study were also included in an investigation to find an association of these SNPs with ulcerative colitis-associated CRC by Garrity-Park et al [26]. The authors found a strong association only with TNF-α -308 polymorphism and ulcerative colitis-associated CRC both at the genotype and allele levels when compared with ulcerative colitis that did not progress to CRC as controls. The probable cause for the discrepancy between the results by Garrity-Park et al and our study could be due to the differences in the patient study population as they examined a homogenous patient group with ulcerative colitis-associated CRCs while our cases mainly included sporadic CRCs without a history of ulcerative colitis. Further, we examined the DNA from blood samples and hence the genotypes represented in our study subjects were germline while the likelihood of somatic mutations in the DNA extracted from formalin-fixed paraffin-embedded biopsies by Garrity-Park and colleagues cannot be ruled out [26].

Increased local expression of pro-inflammatory cytokines such as TNF-α is often detected at sites of inflammation [48]. Studies exploring the genetic association between TNF-α promoter SNPs and altered cytokines expression have been demonstrated in vivo and in vitro including a link between -308 SNP and increased TNF-α expression [49–52]. Higuchi et al reported that the T allele of TNF-α -857 C/T and the A allele of TNF-α -308 G/A resulted in higher transcriptional activity and an increase in TNF-α cytokine production [53]. Thus, it is suggested that the “A” allele of TNF-α -238 SNP, highly represented in the CRC cases in our population, may lead to higher expression of TNF-α in the tumors.

The genotype frequencies -857 were the same in our CRC patients compared to control. In our study, the distribution of the three genotypes in the Saudi population (CC/CT/TT—cases 85/15/0, controls 85/15/0) were different compared to a Croatian population (CC/CT/TT—cases 130/64/6, controls 126/67/7) [23]. Moreover, significant correlation between the TNF-α mRNA expression level in CRC tumor tissue, and prevalence of TNF-α -857 CT and TT genotypes were reported and may be involved in the progression of colon cancer [23]. The differences in the distribution of genotype frequencies of TNF-α -857 could be due to the ethnic diversity between the two populations.

In conclusion, our study assessed CRC predisposition with genetic variants in the TNF-α gene in the Saudi population. While the TNF-α -238 SNP was significantly associated with CRC risk, SNPs -308 and -857 did not correlate with susceptibility to CRC in our population. Because the population size in our study was relatively small, these findings need to be confirmed in larger cohorts. The TNF-α -238 may be a potential marker for CRC screening in the Saudi population.

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Compliance with ethical standards

Conflicts of interest None

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