The aim of this study was to investigate the possible association of three single nucleotide polymorphisms (SNPs), C194T, G399A, and G280A, in the X-ray repair cross-complementing group 1 (XRCC1) gene with the risk of developing colon cancer in Saudi patients. Samples of 65 colon cancer patients, aged 45 to 80 years old, and 65 age-matched controls were genotyped. The results demonstrated that carriers of the variant A-allele of 280 had a higher risk for colon cancer than carriers of the normal (GG) genotype (OR=2.27; 95% CI 0.89-5.83; P=0.08), and carriers of the variant A-allele of 399 were found to have a lower risk for colon cancer than carriers of the normal (GG) genotype (OR=0.73; 95% CI 0.43-1.23; P=0.23). Additionally, the genotype combination showed that the individuals carrying XRCC1 280GA and 399GG genotypes had an elevated risk for colon cancer (OR=3.73; 95% CI 1.14-12.27; P=0.03), whereas subjects carrying the XRCC1 280GA and 399GA genotypes had a decrease risk for colon cancer (OR=0.44; 95% CI 0.04-4.63; P=0.63). These results suggest that the A-allele of 280 might be a risk factor and the A-allele of 399 a protective factor for colon cancer risk.

Key words: XRCC1 gene, SNPs, colon cancer, Saudi.

INTRODUCTION

Deoxyribonucleic acid (DNA) repair genes play a critical role in protecting the genome of the cell from cancer-causing agents (Kovacs and Almendral, 1987; Knight et al., 1993; Scott et al., 1994; Helzlsouer et al., 1995; Scott et al., 1996; Wei et al., 1996; Spitz et al., 1997). In humans, more than 150 genes are involved in DNA repair, which are crucial for maintaining genomic integrity (Wood et al., 2001, 2005). Genetic variants associated with repair of DNA substantially increase the risk of cancer in carriers because of biochemical alterations caused by polymorphisms (Goode et al., 2002; Spitz et al., 2003; Weiss et al., 2005). Several polymorphisms in DNA repair genes representing different repair pathways have been reported. Base excision repair (BER) is the predominant DNA damage repair pathway for the processing of small base lesions, derived from oxidation and alteration damage. X-ray repair cross-complementing group 1 (XRCC1) is one of the most important proteins in BER and is closely associated with BER pathway coordination by interacting with most components of the BER short-patch pathway.

Human XRCC1 was cloned in 1990 and it is the first mammalian gene to be isolated (Thompson, 1990). The size of the gene was identified to be 31.9 kb (Trask et al., 1993). The gene is mapped to chromosome 19q 13.2-13.3 and consists of 17 exons. It encodes a 2.2 kb transcript, which corresponds to a putative protein of 633 amino acids (Trask et al., 1993). The XRCC1 gene exhibits more than 300 single nucleotide polymorphisms (SNPs), among these, approximately 35 variants are located in the exons or the promoter region of the gene. The most common SNPs that result in amino acid substitutions are in exon 6 (Arg194Trp), exon 9 (Arg280His), and exon 10 (Arg399Gln). These non-conservative amino acid alteration may influence DNA repair capability by altering the protein-protein interaction between XRCC1 and other BER proteins. A growing number of reports
on XRCC1 SNPs have been shown to be associated with measurable reduced DNA repair capacity and increased risk of several types of cancers including colon cancer (Krupa and Blasiak, 2004, Duarte et al., 2005, Moreno et al., 2006, Yin et al., 2008, Stern et al., 2009, Yin et al., 2009, Kim et al., 2010, Jelonek et al., 2010).

Colon cancer is a multifactoral disease and a leading cause of cancer death around the world. In the Kingdom of Saudi Arabia (KSA), colon cancer ranks first in frequency among males and third among females after breast and thyroid cancer (Saudi cancer registry, 2005; Bodmer and Bonilla, 2008). Epidemiological studies have provided clues to the etiology of colon cancer and revealed an environmental role as well as genetic factors that place certain individuals at a higher risk. Genes such as DNA repair are involved in the genetic susceptibility to colon cancer (Vettriselvi et al., 2007). We conducted this case-control study to detect the distribution of XRCC1 gene polymorphisms (C194T, G399A, and G280A) and to assess the potential role of the three SNPs on the risk of colon cancer; to compare the allele frequency of the XRCC1 gene variants in the Saudi population to the frequencies of the XRCC1 gene polymorphisms in different countries.

MATERIALS AND METHODS
Subjects
This case-control study was conducted with 65 colon cancer patients and 65 controls. All patients were recruited between September 2008 and February 2010 from King Abdulaziz Hospital and Oncology Center in Jeddah, Saudi Arabia. All cases (32 males and 33 females) had positive colonoscopic results for malignancy, histologically confirmed as colon cancer. The healthy subjects (30 males and 35 females) were unrelated individuals and were selected from the family clinic of King Abdulaziz Hospital. They were judged to be in good health according to their medical history, routine laboratory tests, and colonoscopy preventive examination. None were taking any medication. There were no significant differences between colon cancer patients and controls with respect to age and to the number of females and males in each group. Controls were more likely than cases to be smokers. This study was approved by the ethical committee of the faculty of medicine at King Abdul-Aziz University. All participants gave informed written consent.

DNA extraction and amplification
Genomic DNA was extracted from 1.5 ml peripheral blood samples using the QiAamp Blood kit (QIAGEN). Polymorphisms of XRCC1 were detected using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) assays. Three sets of primers (5.0 nmol) from TIB (TIB-MOLBIOL Inc., Germany) were used. PCR technique was used to amplify the regions (exon 6, exon 9, and exon 10) that contain C194T, G280A, and G399A, respectively. The following primers were included in the reactions: 5’-GCCGGTCCACCGTA-3’ and 5’-CCCAGACCTTCACCTACT-3’ for SNP194 (C194T), 5’-CTGTCACATTGCTCCACCAG-3’ and 5’-GGAAGGTGCTTGGGGAAT-3’ for SNP280 (G280A), and 5’-TGCTTTCTCCTGTGCCA-3’ and 5’-TCCAGCCTTTTCTGATA-3’ for SNP390 (G399A). All of the PCR reactions were carried out by a MastercyclerGradient Thermocycler (Eppendorf, Hamburg, Germany). Each 25 µl of the PCR reactions contained 2 µl genomic DNA (0.2 µg), 12.5 µl HotStarTaq Master Mix, 10.1 µl RNase-free water, and 0.2 µl of each primer (0.1 µM) were used. After the first denaturing for 5 min at 96°C, PCR was carried out for 40 cycles with denaturing at 96°C for 35 s, annealing at (55°C for G280A and G399A, and 60°C for C194T) for 35 s and extension at 72°C for 45 s, with a final extension for 4 min. The amplified PCR products were visualized on 2.6% agarose gels. DNA molecular weight markers (GelPilot 50 bp ladder (100), QIAGEN and pGEM, Promega) were used to estimate the size of DNA fragments.

Genotyping
To distinguish the polymorphic alleles, XRCC1 C194T PCR products (466 bp) were digested with MspI, and the result showed 100% wild type (GG) genotype in both patients and controls. Heterozygous (GA) and homozygous (AA) genotypes were not detected in all the patients and the controls. XRCC1 G280A PCR (188 bp) products were digested with RsaI enzyme, the normal genotype (GG) produced two bands of sizes 126 and 62 bp, the heterozygous variant genotype (GA) resulted in three fragments of sizes 188, 126, and 62 bp, and homozygous genotype (AA) produced one fragment of size 188 bp (Figure 1). XRCC1 G399A PCR products (615 bp) were digested with MspI enzyme. The digestion resulted in two fragments of 374 and 241bp for the wild type (GG); three fragments of 615, 374 and 241 bp for the variant heterozygous (GA), and one fragment of 615bp for the homozygotes (AA) (Figure 2).

Statistical analysis
All statistical analyses were performed with the SPSS (v.16) for Windows software. Continuous variables were expressed as mean and standard deviation (SD). Genotype distributions, allele frequencies, odds ratio, and risk ratio between the study groups were computed by 2×2 contingency table analysis. The Hardy-Weinberg equilibrium was tested by a goodness-of-fit χ² test to compare the observed genotype frequencies to the expected genotype frequencies among the patients and the controls. Statistical significance was defined as the probability of P value less than 0.05 (two-sided).

RESULTS
In this study, we analyzed the distribution of The XRCC1 C194T, G280A, and G399A polymorphisms in a sample of 65 colon cancer patients and 65 healthy controls in the Saudi population and evaluated their association with colon cancer development. The distribution of the genotype and the allele frequencies among cases and controls as well as the genotype specific risk for the G280A and G399A polymorphisms are shown in Tables 1 and 2. The genotype distributions of the G280A SNP did not deviate from the Hardy-Weinberg equilibrium among the controls and the patients (P=0.28 and P=0.60, respectively). The genotypic frequencies of the patients were 75.8% (n=47) normal (GG) and 24.2% (n=18) heterozygous (GA). In the controls, the results showed 87.7% (n=57) wild type (GG) and 12.3% (n=8) heterozygous genotype (GA). The
Figure 1: Photograph of a 2.6% agarose gel showing the result of Rsal digest of the PCR product that contains SNP 280. Lane 1: GelPilot DNA marker. Lane 2: Homozygous (AA) genotype that produced on band of size 188 bp. Lanes 3: Homozygous normal (GG) genotype that produced two bands of sizes 126 and 62 bp. Lane 4: Heterozygous (GA) genotype that produced three bands of sizes 188, 126, and 62 bp.

Figure 2: Photograph of a 2.6% agarose gel showing the result of MspI digest of the PCR product that contains SNP 399. Lane 1: GelPilot DNA marker. Lanes 2: Homozygous normal (GG) genotype that produced two bands of sizes 374 and 241 bp. Lane 3: Homozygous (AA) genotype that produced 615 bp. Lane 4: Heterozygous (GA) genotype that produced three bands of sizes 615, 374, and 241 bp. Lane 5: is an uncut PCR product of size 615 bp. Lane 6: pGEM DNA marker.
homozygous genotype (AA) was not detected in both the patients and the controls. For G399A polymorphism, the genotype distributions among cases and controls were also consistent with those expected from the Hardy-Weinberg equilibrium ($P=0.66$ and $P=0.31$, respectively). The genotypic frequencies of the patients were 52.3% (n=34) wild type (GG), 38.5% (n=25) heterozygous genotype (GA), and 9.2% (n=6) homozygous genotype (AA). In the controls, 44.6% (n=29) wild type (GG), 40.0% (n=26) heterozygous genotype (GA), and 15.4% (n=10) homozygous genotype (AA).

With respect to the risk of colon cancer development, G280A and G399A were analyzed in patients and controls. In both variant forms, the wild type genotype and the wild type allele were taken as the reference. In the case of G280A polymorphism, the analysis showed that carriers of the variant A-allele of 280 were a 2.27-fold higher risk of colon cancer than the wild type carriers of the G-allele (OR=2.27; 95% CI 0.89-5.83; $P=0.08$). In the case of G399A polymorphism, our results demonstrated that carriers of the variant A-allele of 399 were found to have lower risk for colon cancer than wild type carriers of the G-allele (OR=0.73; 95% CI 0.43-1.23; $P=0.38$). When the associations of these polymorphisms with colon cancer were taken into account by gender, no significant increase or decrease in risk among both males and females were shown.

The combined effects of the $XRCC1$ G280A genotypes with the G399A genotypes were analyzed. Using the combined low-risk genotypes (280GG and 399GG) as the reference group, the combination of the 280GA and 399GG genotypes showed a significantly positive association with colon cancer (OR=3.73; 95% CI 1.14-12.27; $P=0.03$), whereas the combination of the 280GA and 399GA genotypes showed a decrease risk for colon cancer (OR=0.44; 95% CI 0.04-4.63; $P=0.63$) (Table 3).

In comparing the allele frequency of the $XRCC1$ gene variants, the frequency of the 280-A allele for cases and controls were 0.12 and 0.06, respectively, no significant differences in frequencies of alleles were observed between patients and controls ($P=0.10$) as well as between male and female subjects in patients and controls ($P=0.89$ and 1.0, respectively). The frequency of the 399-A allele for cases and controls were 0.29 and 0.35, respectively. No significant differences in frequencies of alleles were observed between patients and controls ($P=0.23$) as well as between males and females in patients and controls ($P=0.92$ and 0.41, respectively).
In the present study, a case-control analysis was performed to examine the association between three common polymorphisms of XRCC1 gene (G280A, G399A, and C194T) and colon cancer risk in Saudi patients. In addition, we were interested to compare the allele frequency of the XRCC1 gene variants in the Saudi population to the frequencies of the XRCC1 gene polymorphisms in different countries. The study was conducted in Jeddah, a city in the Western province of Saudi Arabia. To the best of our knowledge, this is the first report on genotype distribution and allele frequency of the XRCC1 in the Saudi population.

In this study, we examined the hypothesis that the XRCC1 gene polymorphisms may be one of the genetic factors that affect colon cancer susceptibility. Our results showed an increase risk for individuals who featured the polymorphism in the XRCC1 280 heterozygous (GA). Individuals with the 280-GA genotype were found to have a two times (OR=2.27; 95% CI 0.89-5.83) greater risk of colon cancer than those with 280-G homozygous normal genotype. No risk could be assigned to subjects with the 280-AA homozygous due to its rarity in the selected samples. In comparing our outcome to previous work, our result is in agreement with the few reports that have been conducted on the effect of the SNP 280 polymorphism. In a Japanese study, the result suggested that the XRCC1 280-GA variant protein has an unsuccessful ability to localize a damaged site in the DNA, consequently reduced the repair efficiency, accumulated unrepaird damages, and increased the risk of carcinogenesis (Takanami et al., 2005). The analysis by Hong et al. (2005) showed association between 280-GA genotype and increased colorectal cancer risk. Skjelbred et al. (2006) also found the 280-GA variant to be associated with increased adenomas risk (OR=2.30; 95% CI 1.19-4.46). Our results are in agreement with those reports that have been conducted on the effect of the SNP 280 polymorphism. It is difficult to explain the mechanism for this association, but it shows that this polymorphism may have a functional significance with regard to the XRCC1 SNP 399 variant allele. The data of the present study showed a decreased association with the risk of colon cancer (OR=0.73; 95% CI 0.43-1.23). This result suggests that the SNP 399 variant allele has either a protective role or no biological impact on colon cancer susceptibility. Our results support reports showed an association between SNP 399 variant allele and decreased risk of colorectal cancer (Stern et al., 2007; Slawinski et al., 2008; Canbay et al., 2011). However, an almost equally impressive number of studies have reported an association between SNP 399 variant allele and increased risk of colorectal cancer (Hong et al., 2005; Moreno et al., 2006). Discrepancies across these studies may be explained by false positive or false negative results, or the different populations studied.

Combined effect of polymorphism of the XRCC1 G280A and G399A with regard to colon cancer risk was observed in the present study. The results of the genotype combinations showed that individuals heterozygous (GA) for 280-GA and normal (GG) for 399-GA were characterized with a high risk for colon cancer (OR=3.73; 95% CI 1.14-12.27). Also a reduction in cancer risk was observed for patients heterozygous for 280-GA and 399-GA genotype (OR=0.44; 95% CI 0.04-4.63). This result indicates and confirms the protection role of the SNP 399 variant genotype (GA). In addition, the genotype distribution and allele frequency for the SNPs 280 and 399 polymorphic forms for males and females in control and patient groups did not differ significantly. This result supports previous work of Olshan et al. (2002), and Park et al. (2002) that showed the genotype distribution of the XRCC1 gene does not vary between sexes.

An interesting finding in the current study was the absence of 194 SNPs. Although the 194-T allele showed a high frequency in Asian populations (Hu et al., 2005) and has been associated with several cancer-related biomarkers, our data did not show heterozygous (CT) or homoyzgous (TT) genotypes for the C194T SNP. It seems that there is a need for expanding genotype studies with respect to SNP 194 on larger population numbers to clarify if the result is due to its rarity in the Saudi population.

In comparing the allele frequency for SNP 280 to previous published data for different ethnic groups, this

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**Table 3. Genotype combination of SNPs G280A and G399A in XRCC1 gene.**

<table>
<thead>
<tr>
<th>XRCC1 polymorphisms</th>
<th>Frequencies (%)</th>
<th>P value</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotypes G280A-G399A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal/Normal GG/GG</td>
<td>0.29 (n=18)</td>
<td>0.37 (n=24)</td>
<td>0.57&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Normal/Hetero GG/GA</td>
<td>0.37 (n=23)</td>
<td>0.37 (n=24)</td>
<td>0.89 (0.27-2.95)</td>
</tr>
<tr>
<td>Normal/Homo GG/AA</td>
<td>0.10 (n=6)</td>
<td>0.14 (n=9)</td>
<td>0.63&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hetero/Normal GA/GG</td>
<td>0.23 (n=14)</td>
<td>0.08 (n=5)</td>
<td>0.44 (0.04-4.63)</td>
</tr>
<tr>
<td>Hetero/Hetero GA/GA</td>
<td>0.02 (n=1)</td>
<td>0.05 (n=3)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Two sided χ² test; <sup>b</sup> two sided fisher exact test; OR, odds ratio; CI, confidence interval.
study indicates that the frequency of the 280-A allele (0.06) for the control group is higher than those reported by Skjelbred et al. (2006) for Norwegians (0.02), African Americans (0.03), and white Americans (0.04). It was also lower than the published work by Hong et al (2005) for Koreans (0.2). The frequency of the 399-A allele have also lower than the published work by Hong et al. (2005) for Americans (0.03), and white Americans (0.04). It was also lower than the frequency in the USA (Duell et al., 2001), France (Moullan et al., 2003) Turkey (Deligezer and Dalay, 2004), Norway (Skjelbred et al., 2006), and Poland populations (Sliwinski et al., 2008). On the other hand, it was slightly higher than the frequency in Portugal (Varzim et al., 2003), Finland (Forsti et al., 2004) Iran (Mohamadynejad and Saadat, 2007), and Italy populations (Importa et al., 2008). However, the frequency for 399-A allele in African Americans (Lunn et al., 1999; Duell et al., 2001), Egyptians (Abdel-Rahman et al., 2000; Cardecott, 2003), Chinese (Shu et al., 2003), Japanese (Matsuo et al., 2004), Taiwanese (Yeh et al., 2005), Indians (Chacko et al., 2005), and Singaporeans (Stern et al., 2007) was much lower than the frequency reported in this study for the Saudi population. These differences suggest a possible ethnic variability in the allelic distribution of XRCC1 and indicate the need for more studies of polymorphism frequencies in different populations on a larger group.

These findings should be considered in light of the small number of subjects that limit the statistical power. Despite this limitation, the design of this study is relatively strong because the controls were recruited from the same cohort as the colon cancer patients. Also, the cases and controls have been matched by age and sex. Moreover, the genotype distributions for the patients and the controls were in agreement with Hardy-Weinberg equilibrium (HWE) suggesting no selection bias. We evaluated the influence of polymorphisms in the XRCC1 gene, not only based on SNP, but also on combined alleles. The allele combination approach yielded a more informative relationship between the XRCC1 and colon cancer.

In summary, our results suggest that the 280-GA polymorphism of XRCC1 DNA repair gene may contribute to genetic susceptibility to colon cancer and 399-GA may have a protective role to decrease colon cancer risk. These findings suggest that genetic polymorphisms in XRCC1 gene (G280A and G399A) may play an important role in the development of colon cancer in the Saudi population.

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