MDR1 polymorphisms are not related to Xeliri and Xelox chemoresistance in colorectal cancer

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Abstract

Background Multidrug resistance member 1 (MDR1) is located on chromosome 7 and encodes P-glycoprotein (Pgp), which is universally accepted as a drug resistance biomarker. MDR1 polymorphisms may change either the protein expression or function, suggesting its possible association with cancers, including colorectal cancer (CRC). Thus, this study aimed to determine the effects of MDR1 polymorphisms on the drug response of Saudi CRC patients. **Methods** DNA samples were obtained from 62 CRC patients and 100 healthy controls. The genotypes and allele frequencies of the MDR1 polymorphisms G2677T and T1236C were determined by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP). **Results** No significant difference was observed in the genotype distribution and allele frequency of T1236C between the CRC the patients and the controls. However, G2677T was found to play a highly significant protective role against the progression of CRC. Moreover, the results showed that none of the genotypes in SNPs T1236C and G2677T affected chemoresistance to Xeliri and Xelox. **Conclusions** T1236C in the MDR1 gene is not related to CRC risk, and G2677T protects against the development of CRC. Both MDR1 polymorphisms are not associated with the risk of chemoresistance.

Background

Colorectal cancer (CRC) is considered the third most common cancer and the fourth most common cause of cancer death worldwide [1,2]. According to the latest annual cancer incidence report in 2015 from the Saudi Cancer Registry, CRC is the first and the third common cancer in men and women, respectively [3]. Although CRC treatment has evolved in recent years, it is ineffective in some patients for many reasons, including change in the absorption, metabolism or drug uptake of the target cells [4]. Another major reason contributing to the failure of the currently used treatment regimen in CRC patients is the development of drug resistance to multiple anticancer agents. Cancer multidrug resistance (MDR) occurs when cancer cells are treated with primary chemotherapy or during recurrence after primary chemotherapy. There are many mechanisms for drug resistance, such as decreasing the uptake of drug, activating the signaling of growth and DNA repair pathways, inducing the anti-apoptotic molecules to inhibit the apoptosis signaling pathways, and increasing the efflux of drug through cellular transporters [5].

ATP-binding cassette (ABC) transporter genes play an important role in cancer MDR. The family of human ABC transporters consists of 49 members [5], which are divided into seven subfamilies from A to G [4]. The ABCB subfamily is a subclass of ABC transporters containing 11 members, and among them is ABCB1/MDR1 which has physiological functional sites such as the blood–brain barrier and liver [6]. MDR1 was the first human ABC transporter that was cloned and characterized through its ability to confer MDR phenotype to cancer cells that had developed resistance to chemotherapy drugs [6].

The *MDR1* gene is located on chromosome 7 and encodes P-glycoprotein (Pgp), which is universally accepted as a drug resistance biomarker [5,7]. The *MDR1* gene is greatly expressed in many sites, such as the proximal and distal human intestines, and it causes the excretion of several carcinogens from the gut into the intestinal lumen. The excretion increases in drug-resistant tumors that pump out various anticancer drugs [5]. Pgp has an important role in the detoxification system of normal tissues by transporting substrates and protecting the tissues from physiologically active substances, cytotoxic agents, and xenobiotics [8]. The expression of the *MDR1* gene and the activity of its product may differ between individuals as a result of genetic polymorphisms. Thus, this condition could change its ability to react with several toxins, carcinogens, and drugs, suggesting its possible association with cancers including CRC [9,10].

When the polymorphisms of genes have the ability to affect the clinical response to chemotherapy, they may affect the absorption, distribution, metabolism, and excretion of drugs [11]. The *MDR1* gene has many mutations, and 40 of its SNPs have been described for exon and intron regions and for promoters. Two of the most popularly studied *MDR1* gene polymorphisms, (C3435T) and (G2677T), have been found to decrease the expression of Pgp in the intestine [11]. Regarding CRC, many studies found that several *MDR1* variants could increase the risk of CRC, including the G2677T and T1236C polymorphisms [8,9,12,13,14,15]. The G2677T SNP of the *MDR1* gene is located in exon 21, and it leads to the change from alanine to serine or threonine, which affects the function of Pgp as a pump. SNP T1236C is one of the most common polymorphisms of the *MDR1* gene and is located in exon 12, where a silent mutation occurs similar to SNP C3435T [8]. Recently, several studies found that the *MDR1* gene polymorphisms contributed to the risk of CRC in several ethnic groups either by changing the structure and function of the pump or by affecting the response of cancer cells to the currently used drugs [11]. CRC chemotherapeutic drugs have evolved in recent years, and they show promising survival rates for CRC patients. Xeliri and Xelox are widely recommended for CRC patients. Xeliri™ is composed of two chemotherapeutic drugs (Xeloda and irinotecan) and is commonly used to treat different cancers, including metastatic CRC. It is usually given to patients in cycles, each one lasting for 2–3 weeks depending on the extent of the disease. Patients usually take Xeloda orally as tablets and irinotecan intravenously [16]. Conversely, Xelox™ is a chemotherapeutic agent comprising Xeloda and Oxaliplatin. It is widely used for bowel cancers including CRC. As in the Xeliri treatment regimen, CRC patients usually take Xeloda orally as tablets and Oxaliplatin intravenously [17]. Both drugs kill cancer cells by exerting their toxic effects that cause DNA damage through different mechanisms. Thus, this study aimed to determine the genotype distribution and allele frequency of two major *MDR1* polymorphisms, namely, T1236C and G2677T, in Saudi CRC patients. The genetic results were correlated with the drug response to the major chemotherapeutic drugs (Xeliri and Xelox) used to treat metastatic CRC patients.

Methods

Subjects and samples

A total of 162 subjects (30–80 years old) volunteered to participate in the study, and they were divided into two groups: 62 CRC patients and 100 healthy controls. The samples of the CRC patients were collected from King Abdulaziz University Hospital. The patients were divided according to TNM staging system into stage I (n=7), stage II (n=6), stage III (n=17), and stage IV (n=32). Stage I patients did not receive any chemotherapy treatment, and other patients with other stages (II, III, and IV) received chemotherapy either as Xeliri treatment, which lasted for 2–3 weeks (cycles) (irinotecan intravenously and xeloda tablets orally twice a day) or Xelox treatment, which lasted for 3 weeks (oxaliplatin intravenously and xeloda tablets orally twice a day). Stages III and IV patients with metastatic tumor received a combination of Xeliri, Xelox, Xeloda, and Bevacizumab. The levels of carcinoembryonic antigen (CEA), a CRC tumor marker, were taken after the chemotherapy cycles were completed to assess the response of cancer cells to the treatment with Xeliri or Xelox. Conversely, the healthy control samples were collected from the blood bank unit of King Fahad General Hospital in Jeddah, Kingdom of Saudi Arabia. The purpose of the research was explained to the participants. To participate in the study, their written consent and answers to the questionnaire were obtained. The study was approved by the General Directorate of Health Affairs in Jeddah, Kingdom of Saudi Arabia (approval number: A00221).

From each subject, a 2 ml whole blood sample was collected and used in the DNA extraction using the QIAamp DNA mini kit (Qiagen, Germany) following the manufacturer's instructions. The range of DNA concentration was 3–12 µg with purity of (1.7–1.9), which was determined by calculating the ratio of absorbance at 260/280 nm.

Polymerase chain reaction-restriction fragment length polymorphism

A 25 µl polymerase chain reaction (PCR) containing 100 ng/µl DNA was mixed with 12.5 µl HotStart-IT® Fidelity™ PCR Master Mix (2X) (Affymetrix, USA), 9.5 µl RNase free water, and 1 µl of (100 nmol) of each forward and reverse primer. The primers and PCR thermocycling reactions were previously published [14,18] and are listed in **Table 1**.

Statistical analysis

All statistical comparisons were made on the GraphPad Prism version 5.0 software. The one-way analysis of variance (ANOVA) test was used to compare the parametrical variables between more than two groups, and the Mann–Whitney and unpaired t tests were used to compare the parametric values between two groups only. The chi-square test and the two-tailed *P* values of Fisher's exact probability test were applied to determine the genotype distribution and allele frequency of the two SNPs under the concept of the Hardy–Weinberg equilibrium. *P* values less than 0.05 were considered statistically significant.

Results

Demographic distribution of the participants

A total of 62 CRC patients (males *n*=48, representing 77.42% and females *n*=14, representing 22.58%) participated in the study. The (*n*=100) control subjects were divided into males (*n*=70, representing 70%) and females (*n*=30, representing 30%). The Mann–Whitney test comparison (**Table 2**) showed a significant difference in weight, which affected the body mass index between the two groups because of the loss of appetite of patients as a consequence of chemotherapy.

Relationship between the genetic variations of the *MDR1* gene and drug response of CRC patients

To correlate the genetic variants with the drug response, the genotype distribution and allele frequency of the two *MDR1* SNPs were determined by the chi-square test and then correlated with the CEA level, which is a major CRC tumor marker.

For SNP T1236C, the genotypic frequencies of the patients were 0% (*n*=0) normal (TT), 66.13% (*n*=41) heterozygous (TC), and 33.87% (*n*=21) homozygous (CC). The frequencies of the T and C alleles were 33.07% and 66.93%, respectively. The genotype distribution for the CRC patients was beyond the Hardy–Weinberg equilibrium ($\chi^2 = 15.12$, degree of freedom (DF)=1, *P* < 0.05). In the controls, the results showed 0% (*n*=0) normal (TT), 46% (*n*=46) heterozygous (TC), and 54% (*n*=54) homozygous (CC). The frequencies of the T and C alleles were 23% and 77%, respectively. The genotype distribution for the controls was beyond the Hardy–Weinberg equilibrium ($\chi^2 = 8.92$, DF=1, *P* < 0.05). Comparing the genotype frequency was difficult because no subjects carried the normal genotype (TT) either in the patients or in the controls. Therefore, concluding the relation of this SNP with the risk of developing CRC in our study subjects was difficult (**Table 3**).

For SNP G2677T, the genotypic frequencies of the patients were 69% (n=43) normal (GG), 6% (n=4) heterozygous (GT), and 24% (n=15) homozygous (TT). The frequencies of the G and T alleles were 72% and 28%, respectively. The genotype distribution for the CRC patients was beyond the Hardy–Weinberg equilibrium ($\chi^2=42.39$, DF=1, $P < 0.05$). For the controls, the results showed 1% (n=1) normal (GG), 24% (n=24) heterozygous (GT), and 75% (n=75) homozygous (TT). The frequencies of the G and T alleles were 13% and 87%, respectively. The genotype distribution for the controls was within the Hardy–Weinberg equilibrium ($\chi^2=0.59$, DF=1, $P > 0.05$). The results showed that SNP G2677T has a highly significant protection against the development of CRC, particularly when comparing the heterozygous (GT) and homozygous (TT) frequencies of the subjects carrying normal (GG) genotype between the CRC patients and the control group (**Table 3**).

Among the 62 patients in this study, only 32 were drug resistant as shown by their CEA level and smoking status. A comparison between the drug-sensitive and the drug-resistant patients in each genotype was made using the unpaired t test, and a final comparison was performed for the six genotype groups using the one-way ANOVA test. **Table 4** shows the mean of the CEA level $\pm$ SEM for each genotype and its contributions to drug resistance for SNPs T1236C and G2677T. As shown in the results, none of the genotypes in the two SNPs of *MDR1* gene increased the risk of developing chemoresistance to both Xeliri and Xelox.

Discussion

In this study, SNP T1236C was investigated and correlated with the risk of developing CRC and drug resistance. Although the chi-square test was performed to calculate the genotype distribution, allele frequency, odds ratio, and *P* values, comparing the genotypes frequency was not possible because there were no subjects carrying the normal genotype (TT) either in the patient group or in the controls. Thus, concluding the relation of this SNP with the risk of developing CRC was difficult in our study subjects. Nevertheless, a few studies have been conducted to assess the association between T1236C polymorphism with CRC risk and showed contradictory results [8,19,20].

Regarding SNP G2677T, we found that it played a highly significant protective role against the development of CRC particularly when comparing the heterozygous (GT) and homozygous (TT) genotypes with subjects carrying the normal (GG) genotype. Interestingly, the comparisons in the genotype distributions showed a non-significant difference in risk ratio in the males between the patient group and the controls. However, we found a significant difference in the genotype distribution of heterozygous (GT) and a highly significant difference in the homozygous (TT) and combined GT and TT genotypes distribution among females between the patient group and the controls. This result showed that the heterozygous (GT) and homozygous (TT) subjects were protected against the development of CRC. Several studies have been conducted to assess the association between the G2677T polymorphism and CRC risk, including a study conducted on an Italian population. This study found no significant effect of the G2677T polymorphism on the increased incidence of CRC or its prognosis [21]. Another study conducted on Bulgarian patients suggested that the G2677T polymorphism is not a risk factor for CRC [12]. A study found that variant G2677T in *MDR1* was not related to the risk of CRC [11]. A meta-analysis study showed no association between the *MDR1* G2677T polymorphism and risk of CRC [10]. Conversely, many studies found that the G2677T polymorphism of *MDR1* was associated with increased CRC risk [8,11,15,22].

Response to chemotherapy was also examined in our study. The results showed that none of the two studied SNPs (T1236C and G2677T) in the *MDR1* gene correlated with increasing or decreasing the risk or chemoresistance to the currently used drugs, such as Xeliri and Xelox. Consistent with our research, a Korean study found a non-significant association between SNPs C3435T, G2677T, and T1236C in *MDR1* and drug resistance among Korean epileptic

patients [23]. Moreover, a study conducted on Romanian children found that the T1236C and G2677T polymorphisms of the *MDR1* gene were not associated with drug resistance in epilepsy but that the genotypes 1236TT, 1236TC, and 2677TT were related to drug-responsive idiopathic epilepsy [24]. Another study concluded that the T1236C polymorphism of the *MDR1* gene greatly influenced the drug response “low response to therapy” in breast cancer patients from the Arab population of Saudi Arabia [25]. In another study, the T1236C polymorphism was associated with drug resistance in female epileptic patients in the Iranian population, while the T129C polymorphism was not associated with drug resistance in epilepsy in the Iranian population [14]. A study suggested that the *MDR1* SNP T1236C could contribute to the response to chemotherapy for cancers, especially osteosarcoma and breast cancer, of Asians [26].

Conclusions

The results showed no association between SNP T1236C in the *MDR1* drug-resistant gene and the risk of CRC development. However, the SNP G2677T played a highly significant protective role against the development of CRC in our population. Moreover, a non-significant association was found between the two SNPs and the risk of chemoresistance. Therefore, these two SNPs cannot be used as a molecular marker for drug response in CRC patients. Further examinations using a larger number of samples and tissues are necessary to confirm our findings with more reliable conclusions.

Abbreviations

ABC: ATP-binding cassette; CEA: carcinoembryonic antigen; CRC: colorectal cancer; DF: degree of freedom; MDR: multidrug resistance; MDR-1: Multidrug resistance member 1; PCR-RFLP: polymerase chain reaction–restriction fragment length polymorphism; Pgp: P-glycoprotein; CEA: Carcinoembryonic antigen, SNP: Single nucleotide polymorphism.

Declarations

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Author's contributions

A.B.A. designed the project, A.M.A performed experimental work, S.N.A collected blood samples, H.A.A revised and edited the manuscript, H.M.T. and A.M.A. provided the samples and diagnosed the patients, S.S.A provide the ethical approval and wrote the questionnaire and the consent, U.M.O. helped in statistical analysis, and A.A.Z provided the samples and diagnosed the patients.

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Availability of data materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study was approved by the General Directorate of Health Affairs in Jeddah, Kingdom of Saudi Arabia (approval number: A00221).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interest.

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Tables

Table 1 Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) conditions for *MDR1* polymorphisms

<i>MDR1</i>	PCR-RFLP conditions
SNPs	
ID	
T1236C	<p>Primers: forward (5'-TTTTTCTCACGGTCCTGGTAG-3') and reverse (5'-CATCCCCTCTGTGGGGTCATA-3').</p> <p>(exon 12) PCR condition: Initial denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min and an extension at 72°C for 2 min, as well as a final extension at 72°C for 5 min.</p> <p>PCR Product size: 147 bp</p> <p>RFLP conditions: 5µl PCR product, 16.5µl nuclease-free water, 2.5µl cutsmart buffer and 1µl of <i>HaeIII</i> (NEB, UK) were added. Then tubes were incubated at 37°C for 1 hour with an inactivation step at 80°C for 20 minutes.</p> <p>RFLP sizes: Wild=68,79 bp; heterozygous=33, 35, 68,79 bp; and homozygous=33,35,79 bp</p>
G2677T	<p>Primers: forward (5'-TACCCATCATTGCAATAGCAG-3') and reverse (5'-TTTAGTTTGACTCACCTTTCTAG-3').</p> <p>(exon 21) PCR condition: Initial denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and an extension at 72°C for 2 min, as well as a final extension at 72°C for 5 min.</p> <p>PCR Product size: 107 bp</p> <p>RFLP conditions: 5µl PCR product, 16.5µl nuclease-free water, 2.5µl cutsmart buffer and 1µl of <i>XbaI</i> (NEB, UK) were added. Then tubes were incubated at 37°C for 1 hour with an inactivation step at 65°C for 20 minutes.</p> <p>RFLP sizes: Wild=107 bp; heterozygous=24,83,107 bp; homozygous=24,83 bp</p>

Table 2 Demographic analysis of all study participants

Physical parameters	CEC patients (n=62)	Controls (n=100)	Mann-Whitney <i>P</i> value
Age (years)	55.89±1.60	53±1.19	0.1367
Weight (kg)	73.37±2.01	84.41±1.78	< 0.0001
Height (cm)	165.5±1.19	165.5±0.96	0.5414
Body Mass Index (kg/m ²)	26.80±0.72	30.91±0.60	0.0001
Waist (cm)	100.1±2.54	102.8±2.22	0.5671
Hip (cm)	109.8±2.43	108.1±1.84	0.9546
Waist-to-hip ratio	0.92±0.02	0.96±0.01	0.3566

Table 3 Genotype distribution and allele frequency analysis of *MDR1* SNPs

Genotypes and alleles	Patients (n=62)	Controls (n=100)	Fisher's exact <i>P</i> value	Odds ratio (95% CI)
T1236C				
Wild (TT)	0% (n=0)	0% (n=0)		1.00 (Reference)
Heterozygous (TC)	66.13% (n=41)	46% (n=46)	1	Not Applicable
Homozygous (CC)	33.87% (n=21)	54% (n=54)	1	Not Applicable
Combined (TC+CC)	100% (n=62)	100% (n=100)	1	Not Applicable
Dominant (T)	33.07%	23%		1.00 (Reference)
Recessive (C)	66.93%	77%	0.16	0.61 (0.33- 1.13)
G2677T				
Wild (GG)	69% (n=43)	1% (n=1)		1.00 (Reference)
Heterozygous (GT)	6% (n=4)	24% (n=24)	<0.0001	0.004 (0.0004-0.04)
Homozygous (TT)	24% (n=15)	75% (n=75)	<0.0001	0.005 (0.0006-0.04)
GT+TT	30% (n=19)	99% (n=99)	<0.0001	0.005 (0.0006-0.03)
Dominant (G)	72%	13%		1.00 (Reference)
Recessive (T)	28%	87%	1.68	0.05 (0.02-0.10)

Table 4 Correlation of CEA level and genotypes of SNP T1236C in *MDR1* gene with drug response

Genotypes of SNP T1236C in <i>MDR1</i> gene	CEA* level (ng/ml) (Mean±SEM)	Unpaired t test <i>P</i> value	One-way ANOVA <i>P</i> value
Normal (TT) drug resistant (n=0)	0	Not Applicable	0.2722
Normal (TT) drug sensitive (n=0)	0		
Heterozygous (TC) drug resistant (n=21)	576.8± 385.9	0.15	
Heterozygous (TC) drug sensitive (n=20)	2.011± 0.145		
Homozygous (CC) drug resistant (n=15)	51.73± 29.65	0.30	
Homozygous (CC) drug sensitive (n=6)	1.265± 0.162		
Genotypes of SNP G2677T in <i>MDR1</i> gene	CEA* level (ng/ml) (Mean±SEM)	Unpaired t test <i>P</i> value	One-way ANOVA <i>P</i> value
Normal (GG) drug resistant (n=25)	498.5± 325.3	0.20	Not applicable
Normal (GG) drug sensitive (n=18)	1.909± 0.148		
Heterozygous (GT) drug resistant (n=3)	25.64± 8.215	Not applicable	
Heterozygous (GT) drug sensitive (n=1)	2.450±0.0		
Homozygous (TT) drug resistant (n=8)	43.85± 30.58	0.22	
Homozygous (TT) drug sensitive (n=7)	1.571± 0.297		

*CEA: Carcinoembryonic antigen