

RESEARCH ARTICLE

Low Incidence of the DPD IVS14+1G>A Polymorphism in Jordanian Breast and Colorectal Cancer patients

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Abstract

Background: Dihydropyrimidine dehydrogenase (DPD) is a crucial enzyme in the catabolism of 5-fluorouracil (5-FU), a drug that is frequently used in cancer therapy. Patients with deficient DPD activity are at risk of developing severe 5-FU-associated toxicity. One possible cause of deficiency is genetic polymorphisms in the DPD gene, such as IVS14+1G>A. **Aim:** The present study was conducted to screen for the IVS14+1G>A polymorphism in cancer patients receiving 5-FU and a control group. **Methods:** A total of 40 cancer patients (30 colorectal cancer (CRC) and 10 breast cancer patients) were enrolled in this study. One hundred healthy controls were also tested using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). DNA sequence analysis was carried out to confirm the presence of the IVS14+1G>A polymorphism. **Results:** Only one CRC patient showed heterozygous IVS14+1G>A polymorphism in the DPD gene. **Conclusion:** The results of this study demonstrated a very low frequency of the IVS14+1G>A polymorphism among Jordanian patients with colorectal and breast cancer.

Keywords: Colorectal cancer- breast cancer- DPYD gene- Capecitabine- 5-FU

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Introduction

Cancer is a major health problem facing most of the world regions, and Jordan is no exception. Colorectal cancer (CRC) is the most common cancer among males and breast cancer is the most common cancer among females in Jordan during 2001-2009 periods (1). 5-Fluorouracil (5-FU) is a commonly used drug to treat cancer; including colorectal, breast, gastrointestinal tract, and ovarian carcinomas (Goetz et al., 2004; van Kuilenburg et al., 2004). An oral form of 5-FU is called capecitabine, a novel oral fluoropyrimidine that mimics continuous infusion of 5-FU and generates 5-FU preferentially at the tumor site through exploitation of high intratumoral thymidine phosphorylase (TP) concentrations (Miwa et al., 1998; Blum Joanne, 2001; van Cutsem et al., 2004; Bradford et al., 2001; Chintala et al., 2011). Capecitabine has become an important treatment option for patients with metastatic breast cancer that has progressed following taxane therapy, and has shown promise in anthracycline- pretreated patients, as a first-line monotherapy (Blum Joanne, 2001). The use of capecitabine has spread to a number of off-label indications, including the treatment of advanced or metastatic colorectal cancer and the neoadjuvant treatment of rectal cancer (Bradford et al., 2001).

Dihydropyrimidine dehydrogenase (DPD) is the rate-controlling enzyme of endogenous pyrimidine and

fluoropyrimidine catabolism responsible for the elimination of approximately 80% of 5-FU (Heggie et al., 1987). More than 30 single-nucleotide polymorphisms and deletion mutations of dihydropyrimidine dehydrogenase (DPYD) gene have been identified, to date. The most prevailing is G to A substitution in the splicing-recognition sequence of intron 14, known as IVS14+1G>A polymorphism. This polymorphism leads to the absence of exon 14 immediately upstream of the mutated splice donor site in the process of DPD pre-mRNA splicing. As a result, the mature DPD mRNA lacks a 165 nucleotide segment encoding the amino acids 581-635, resulting in a partial deficiency of DPD enzyme activity (Goetz et al., 2004; Meinsma et al., 1995). The IVS14+1G>A polymorphism is thought to result in an increased half-life of 5-FU, leading to an increase risk of 5-FU toxicity (Cai et al., 2014; Deenen et al., 2011). The potential toxic effects of 5-FU is schedule dependent; some toxicities are associated with bolus doses schedule or with continuous infusions schedule and other toxicities such as diarrhea and dermatitis are associated with both bolus and continuous infusion schedules (Macdonald, 1999). Clinical data demonstrates that blood concentration of 5-FU can vary by over 10-fold despite equal dose administration depending on body surface area. Clinical data also demonstrates that pharmacokinetically guided dose adjustment of blood levels of 5-FU can result in lowered toxicity, improved

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overall response, and increased survival (Capitain et al., 2008; Gamelin et al., 2008).

The goal of this study was to screen colorectal and breast cancer patients for IVS14+1G>A polymorphism indicating partial or complete DPD deficiency and to compare the prevalence of IVS14+1G>A polymorphism among this group of patients with normal control population.

Materials and Methods

Sample Collection

The study included 30 patients with CRC and 10 patients with breast cancer (20 males and 20 females: average age: 60.65±11.7); they were all Jordanian patients referred to Jordan university hospital (JUH) / Hematology and Oncology clinic during the period from August 2015 to January 2016. All patients were on capecitabine chemotherapy treatment. We have also tested 100 gender matched normal controls (50 males and 50 females); they were all unrelated Jordanians with no history of any malignant diseases. After having the consent form signed from each participant including patients and controls, 4.5ml of peripheral blood was collected in EDTA tubes under aseptic conditions. Samples were stored at 4 oC until DNA extraction was performed at the Faculty of medicine / Haemostasis and Thrombosis laboratory.

DNA Extraction

DNA was extracted using Wizard® Genomic DNA purification kit (Promega, Madison, WI, USA) according to the manufacture instructions. Briefly, the whole blood sample was incubated with cell lysis solution. After obtaining the nucleus pellet, a nucleus lysis solution was added to the pellet and mixed. Then, a protein precipitation solution was added to the nucleus lysed samples and mixed vigorously. After that, the DNA was precipitated by isopropanol and washed with 70% ethanol and lastly dissolved in nuclease free water. All DNA samples were stored at -20°C until use.

PCR-RFLP

The genomic DNA region flanking the IVS14+1G>A polymorphism site was amplified using the following primers (forward) 5'-ATCAGGA CATTGTGACATATGTTTC-3' and (reverse) 5'-CTTGTTTTAGATGTTAAATCACACATA-3' in a final concentration of was 5 Pmol, as van Kuilenburg, (2001) previously described.

The PCR reaction was performed under the following conditions: initial denaturation at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 1 minute, with final extension at 72°C for 5 minutes. The PCR product (198bp) was digested for overnight at 37°C using the NdeI restriction enzyme (New England Biolab, Inc.). The digested PCR products were then separated on 3% agarose gel yielded fragments of 154 bp, 27 bp and 17 bp for the mutant allele and 181 bp and 17 bp fragments for the wild type allele (Figure 1).

DNA sequencing

The PCR products were sent to GENEWIZ company (US, GENEWIZ) for sequencing using the forward primer. Sequence analysis was performed using Applied Biosystems Model (ABI3730x1) automated DNA sequencer using the dye terminator method. Then sequence alignment was performed for all patients and compared with the same number of normal controls using the software BioEdit Sequence Alignment Editor Version 7.2.

Statistical analysis

Genotype frequencies of the IVS14+1G>A polymorphism were tested for Hardy-Weinberg equilibrium by using the Chi-square test. Genotype and allele frequencies of this polymorphism were compared with Fisher's exact test by using the SPSS statistics program. The level $p < 0.05$ was considered as the cut-off value for significance.

Ethics

The institutional review board at JUH approved the study (IRB number 46/2015), and written informed consent was obtained from each participant in according with the Declaration of Helsinki (WMA, 2013).

Results

Genotyping and allelic frequency of IVS14+1G>A

One patient with CRC showed heterozygous genotype for IVS14+1G>A polymorphism of the DPYD gene in the Jordanian group of patients with colorectal and breast cancer (out of the total of 280 alleles) both in the patients and control group. The wild type genotype (GG) and allele (G) frequencies were 97.5% and 98.75% respectively. No individuals homozygous for the IVS14+1G>A polymorphism were found among the patients or the normal control individuals. The IVS14+1G>A frequency was 1.25 in our group of patients and control (Table 1).

Sequence analysis

Analysis of exon 14 of the DPYD gene for all patients

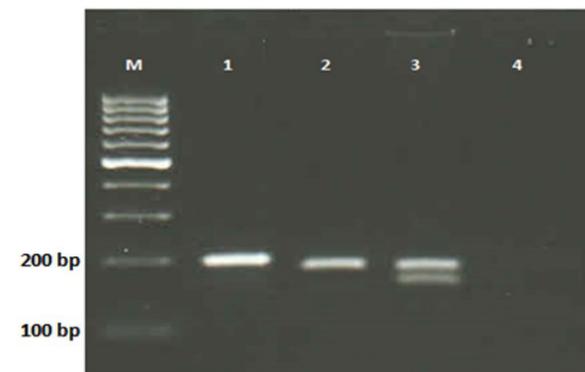
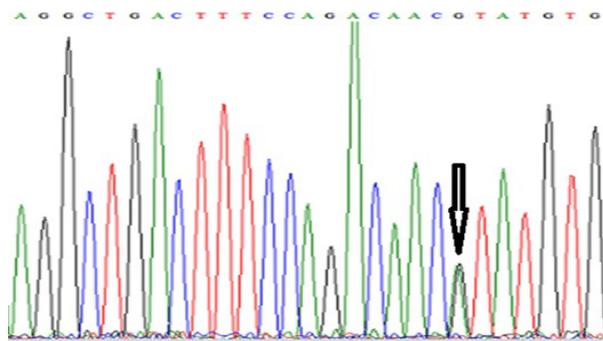


Figure 1. The NdeI Restriction Profiles of DPYD Gene IVS14+1G>A Polymorphism on 3% Agarose Gel Electrophoresis (1X TBE). Lane M shows DNA ladder (100bp); Lane 1 shows undigested PCR product (198 bp); Lane 2 shows wild type genotype (181 bp); Lane 3 shows Heterozygous genotype (181 bp and 154 bp); Lane 4 shows negative control.

Table 1. The Genotype and Allele Frequencies of the IVS14+1G>A Polymorphism in Jordanian Population

Genotype/ Allele	CRC and Breast cancer patients (n=40)	Healthy controls (n=100)	X ²	p value
<i>DPYD</i>				
IVS14+1G>A				
GG	39 (97.5%)	100 (100%)	0.032	0.86
GA	1 (2.5 %)	0	2.5	0.11
AA	0	0	-	-
Alleles				
G	79(98.75%)	200 (100%)	0.008	0.93
A	1 (1.25%)	0 (0%)	1.25	0.26

Figure 2. Sequence Analysis of Exon 14 of *DPYD* Gene. One Patient was shown to be heterozygous for the splice-site IVS14+1G>A polymorphism.

and the controls confirmed the presence of IVS14+1G>A polymorphism in one patient heterozygous for the splice-site IVS14+1G>A polymorphism (Figure 2).

Discussion

This case-control study analyzed the IVS14+1G>A Polymorphism in Jordanian cancer patients with breast and colorectal cancer. To the best of our knowledge, this is the first study that reports the prevalence of this polymorphism in Jordanian cancer patients. Furthermore, IVS14+G>A polymorphism screening with the PCR-RFLP technique is simple and inexpensive and sequencing the exon 14 allows the detection of novel polymorphisms in addition to known polymorphisms.

Several reports have been published regarding the prevalence of IVS14+1G>A polymorphism in different populations. The allele frequency of this polymorphism has been found to be 0.6%, 1.1%, 0.91%, 0.94 and 0.7 in Turkish (Uzunkoy et al., 2007), French (Magne et al., 2005), Dutch (van Kuilenburg et al., 2001), German (Raida et al., 2001) and Portuguese populations (Salgueiro et al., 2004), respectively. No IVS14+1G>A polymorphism was found among the 121 Korean (Cho et al., 2007), 300 Taiwanese (Hsiao et al., 2004), 190 African Caucasian-African (Ahluwalia et al., 2003) and 105 African-Americans populations (van Kuilenburg et al., 2004). The allele frequency of the IVS14+1G>A polymorphism found in this study was higher than other reported studies (Table 2) (A allele p-value=0.26) (Table

Table 2. Prevalence of IVS14+1G>A Polymorphism in Different Populations

Population	Number of allele analyzed	Population studied	Allele frequency (%)	Reference
Jordanian	280	Patients and controls	1.25	This study
Turkish	436	Patients and controls	0.6	Uzunkoy et al., 2007
French	186	Cancer patients	1.1	Magne et al., 2005
Dutch	2714	Healthy individuals	0.91	van Kuilenburg et al., 2001
German	1702	Healthy individuals	0.94	Raida et al., 2001
Portuguese	146	Cancer patients	0.7	Salgueiro et al., 2004
Korean	242	Healthy individuals	0	Cho et al., 2007
Taiwanese	600	Healthy individuals	0	Hsiao et al., 2004
African Caucasian-African	380	Healthy individuals	0	Ahluwalia et al., 2003
African-Americans	210	Healthy individuals	0	van Kuilenburg et al., 2004

1). This may be due to the small sample size; therefore increasing the sample size will confirm or refute our finding.

It has subsequently been demonstrated that a number of these individuals were genotypically heterozygous for IVS14+1G>A polymorphism (Raida et al., 2001; van Kuilenburg et al., 2001; Uzunkoy et al., 2007; Magne et al., 2005; Salgueiro et al., 2004), the heterozygote genotype results in partial deficiency of DPD. Therefore this plays an important role in the etiology of 5-FU associated toxicity (Cho et al., 2007).

Although the IVS14+1G>A polymorphism plays an important role in 5-FU toxicity, its rarity in this study group of patients indicates that other polymorphisms in the *DPYD* gene may also play an important role in 5-FU toxicity in Jordan.

Conflict of interest

None.

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