Potential of Dihydropyrimidine Dehydrogenase Genotypes in Personalizing 5-Fluorouracil Therapy Among Colorectal Cancer Patients

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Background: Dihydropyrimidine dehydrogenase (DPD) is a pyrimidine catabolic enzyme involved in the initial and rate-limiting step of the catabolic pathway of toxic metabolites of 5-fluorouracil (5-FU). Several studies have reported that deficiency of DPD and polymorphisms of its gene are related to 5-FU toxicities and death. Association between serum concentration of 5-FU and its related toxicity has also been previously demonstrated. Hence, this study aims to understand the role of *DPYD* variants in serum level of 5-FU and the risk of developing toxicity to prevent adverse reactions and maximize therapy outcome for personalized medicine.

Methods: A total of 26 patients comprising 3 different ethnic groups (Malay, Chinese, and Indian) diagnosed with colorectal cancer and treated with 5-FU chemotherapy regimen from local hospital were recruited. Polymerase chain reaction and denaturing high-performance liquid chromatography methods were developed to screen polymorphisms of *DPYD* gene. High-performance liquid chromatography–based quantification assay was developed to measure the serum concentration of 5-FU among these patients.

Results: Patients with *DPYD* genotypes of deficient enzyme activity had higher median serum levels of 5-FU compared with normal DPD group (median, 11.51 mcg/mL; 95% confidence interval, 10.18–16.11 versus median, 0.83 mcg/mL; 95% confidence interval, 0.55–5.90, Mann–Whitney *U* test; $P = 0.010$). Patients with neutropenia (n = 11) had significantly higher serum concentrations of 5-FU as compared with those with normal white blood cell count (n = 15) (Mann–Whitney *U* test, $P = 0.031$). Combined regression analysis showed that the predictive power of *DPYD*5* (rs1801159) and 1896 T>C (rs17376848) for serum concentrations of 5-FU in the studied group was 36.6% ($P = 0.04$). Similarly, *DPYD*5* and 1896 T>C accounted for 29.9% of the occurrences of neutropenia (analysis of variance, $P = 0.017$).

Conclusions: This study revealed that *DPYD*5* (rs1801159) and 1896 T>C (rs17376848) are potentially useful predictive markers of patients' responses to 5-FU chemotherapy. Pharmacogenotyping is therefore recommended to guide dosing of 5-FU and prevent neutropenia.

Key Words: 5-fluorouracil, dihydropyrimidine dehydrogenase, pharmacogenotypes, adverse effect, neutropenia

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INTRODUCTION

Association between serum concentration of 5-fluorouracil (5-FU) and its related toxicity was demonstrated by Findlay et al.[1] Subsequently, several more studies have reported that deficiency of dihydropyrimidine dehydrogenase (DPD) is related to 5-FU–related toxicities such as diarrhea, stomatitis, mucositis, and neurotoxicity, and in some cases, death due to 5-FU.[2–4] DPD is a pyrimidine catabolic enzyme. It is the initial and rate-limiting factor in the catabolism pathway of toxic metabolites of 5-FU. It is polymorphic, and many *DPYD* variants were reported to be associated with 5-FU–induced toxicity.[5–7] For example, Kleibl et al[8] reported that patients with IVS14+1G>A or *DPYD*6* (V732I) have higher risk of mucositis and leukopenia after 5-FU chemotherapy.

However, there is wide interethnic and intergeographical difference in the allele types and frequency of *DPYD*. It has been reported that 3% of the Caucasian population carry *DPYD*2A* in exon 14 of *DPYD*, whereas none of the Japanese population was found having this variant.[9] Moreover, *DPYD*5* (rs1801159) in exon 13 of *DPYD* gene was reported to be 11.5% in Egyptian, 14% in Caucasians, and 12% in Tunisians.[10] Higher frequencies of *DPYD*5* (rs1801159) were observed in Japanese (35%), Taiwanese (21%), and African Americans (22.7%). Hence, understanding the role of *DPYD* polymorphism in serum levels of 5-FU and associated toxicity is crucial to prevent adverse reactions and maximize therapy outcome. The information is essential in our country with

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population consisting of multiethnic groups, which has posed great challenge in the clinical management of patients.

Function tests that aim to determine DPD enzyme activity and predict severe 5-FU–related toxicity have been sought after. This includes the use of 2-[^{13}C]-uracil breath test, uracil/dihydrouracil, and [^{14}C]-thymine to dihydrothymine plasma ratio. However, these tests may not be suitable for clinical routine as radiolabeled chemicals are used.[7,11,12] The most convenient and accurate platform useful clinically for the prediction of DPD enzyme activity and 5-FU efficacy is genetic analysis. We therefore developed easy to use polymerase chain reaction (PCR) and denaturing high-performance liquid chromatography (dHPLC) methods for rapid screening of *DPYD* variants. Serum level of 5-FU was quantified using HPLC method, and *DPYD* genotypes correlated with the serum level of 5-FU and clinical outcomes.

MATERIALS AND METHODS

Patients

The protocol of this project was approved by the Human Research Ethics Committee of the Universiti Teknologi MARA and the Research Ethics Committee of the Malaysian Ministry of Health. Also, it was performed in accordance with the Declaration of Helsinki and guidelines for good clinical practice. Explanation on the study protocol was given to all patients, and written informed consent was obtained before sampling was done.

A total of 26 patients consisting of 16 Malays, 9 Chinese, and 1 Indian diagnosed with colorectal cancer from a local hospital were recruited. All patients involved were prescribed with 5-FU chemotherapy regimen for the first time. 5-FU was given based on the body surface area (BSA). Patients included in this study had adequate hematological and cardiac status because physician prescribed 5-FU only to patients with adequate hematological status to avoid serious hematological side effects. They did not have chronic liver disease or any liver dysfunction that may affect the metabolism of 5-FU. Patients taking drugs that may increase adverse reactions of 5-FU (ie, allopurinol, cimetidine, digoxin, metronidazole, and vaccines) were excluded.

Details of demographic data, medical history, adverse drug reaction, serum creatinine levels, carcinoembryonic antigen measurements, and staging of cancer were recorded (Table 1). Assessment of 5-FU toxicity was based on the National Cancer Institute's Common Terminology Criteria for Adverse Events.[13] Staging was done according to the American Joint Committee on Cancer TNM (Tumor–Node–Metastasis) classification system.[13] The Common Toxicity Criteria of the National Cancer Institute was used for classification of neutropenia. Absolute neutrophil count value less than 1.5×10^9/L was considered as neutropenia.

Collections of Blood Samples

After obtaining consent from patients, 10 mL of blood was collected after 30 minutes after 5-FU infusion. Five milliliters of the blood was transferred into a sodium citrate containing tube, whereas another 5 mL into a plain glass tubes for DNA extraction and 5-FU level analysis, respectively. Whole blood in the second tube was left to coagulate for 30 minutes, and serum was obtained by centrifugation at 3000*g* for 15 minutes. All samples were kept at −80°C until analysis.

TABLE 1. Demographic Characteristic of Patients

Patients Demographic Data (N = 26)	
Age (yr)	
Median ± SD	56.5 ± 9.35
Range	33–90
Gender, n (%)	
Male	16 (61.5)
Female	10 (38.5)
Race, n (%)	
Malay	16 (61.5)
Chinese	9 (34.6)
Indian	1 (3.8)
Serum creatinine (mmol/L)	
Mean ± SD	85.2 ± 13.5
Range	58–101
BSA (m^2)	
Mean ± SD	1.59 ± 0.18
Range	1.26–2.1
Weight (kg)	
Mean ± SD	57.7 ± 11.7
Range	39–90
Body mass index	
Mean ± SD	22.8
Range	15.9–31.1

Quantitation of Serum Levels of 5-FU Using HPLC

Quantitation of 5-FU were performed on Agilent 1100 HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with quaternary pump, autosampler, thermostat column compartment, vacuum degasser, and diode-array detector (Model 1100; Agilent). Data were analyzed using ChemStation acquisition and data analysis software (Agilent). Quantitation of serum level using HPLC method was optimized according to several criteria, including separation, specificity, recovery, linearity, precision, and accuracy. Food and Drug Administration (http://www.fda.gov/downloads/Drugs/…/Guidances/ucm070107.pdf)[14] guideline on the analysis of biological samples were followed.

Extraction of Serum Samples

Three hundred microliters of sodium sulfate and 30 μL of sodium acetate were added into each sample of 500-μL serum in a glass tube and vortexed for 10 seconds. Subsequently, 6 mL of ethyl acetate was added. The samples were gently mixed by a slow rotation for 20 minutes using rotary mixer (Stuart SB3; Bibbly Scientific, Staffordshire, United Kingdom). Then, the samples were centrifuged at 3000*g* for 10 minutes. The organic layer was transferred into a clean reaction vial and evaporated to dryness under nitrogen stream at room temperature. Finally, the samples were reconstituted in 100 μL of mobile phase. The samples were vortexed briefly

for 10 seconds before transferred into a glass insert of autosampler vials. An aliquot of 60 μL was injected into the HPLC.

Each sample was analyzed with UV detection at 264 nm on C18 column with an inner diameter of 4.6 × 250 mm and particle size 5 μm (D18 Atlantis, Waters, United Kingdom). Mobile phase used consisted of 95% of 0.01 mol/L phosphate buffer (pH 3) and 5% of acetonitrile (vol/vol). The mobile phase was run in isocratic mode, at flow rate of 0.8 mL/min, and the total run was 25 minutes for 1 sample.

Preparation of Calibrators and Quality Control

Calibration curves with 6 selected concentrations including blank were prepared (1, 2.5, 5, 10, 50, and 80 mcg/mL). Chlorouracil with a concentration of 10 mcg/mL was added as an internal standard. Quality control (QC) solutions were prepared from different stock solutions at final concentrations of 0.5, 25, and 100 mcg/mL. QC solutions were stored at −20°C for no more than 3 months. The mean percentages of 5-FU recovery were 66.9, 65.7, and 66.5 for the concentrations of 0.5, 25, and 100 mcg/mL, respectively. The interday coefficient of variation was 11.7%, 9.2%, and 10.2% for the 3 different concentrations of QC samples, whereas the recovery for the internal control was 78.6%.

Genotyping of *DPYD* Using dHPLC

Complete human DNA sequences were obtained from the Web site of the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov) for *DPYD* (chromosome 1; accession number: NC_000001.9). Sequences of interest encoding *DPYD* gene including exon 4, exon 13, and exon 14 were amplified using PCR System 9700 (PerkinElmer, Perkin Elmer Inc, MA, USA). PCR amplification was carried out in a mixture of reagents containing 1 unit of DNA GoPhorIT*Taq*, 0.2 μmol/L of primers (Fig. 1), 0.2 mmol/L of dNTP, 1× detergent-free buffer, and 50–100 ng of DNA template. "Touch-down" PCR condition was optimized to maximize specific product amplification and minimize the nonspecific products resulting from mispriming to the target template. Touch-down PCR program with initial denaturation at 95°C for 2 minutes, 12 cycles at 95°C for 1 minute, 60°C to 48°C (1°C decrements per cycle) for 1 minute, and 72°C for 45 seconds, followed by 32 cycles at 95°C for 1 minute, 50°C for 1 minute, and 72°C for 45 seconds was performed. The successfully produced PCR amplicons were subjected to dHPLC analysis.

Before dHPLC, all PCR products were first evaluated by visualizing the PCR amplicons using 1.5% agarose gel electrophoresis with ethidium bromide staining. Standard 100-base pair (bp) ladder was used as an indicator for the band sizes. Three exons including exon 4 (239 bp), exon 13 (296 bp), and exon 14 (285) were amplified for subsequent dHPLC analysis.

The optimum partial denaturing temperatures to differentiate the peak profiles of heterozygosity or homozygosity of polymorphic sites were determined. The optimum temperature was 53°C for exon 4, although 2 optimum temperatures

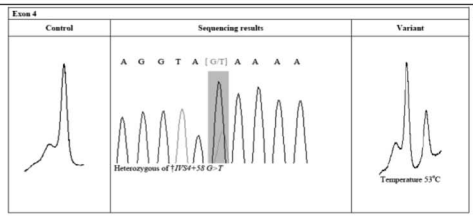
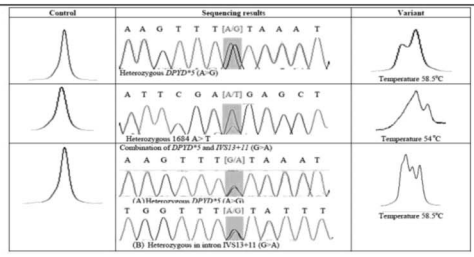
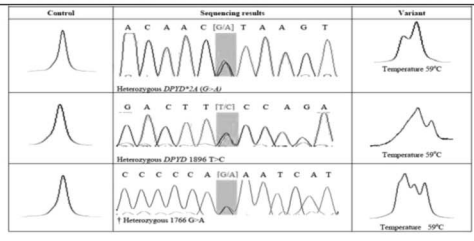
Exon	Primers sequence (5' – 3')	T_m (ºC)	Result of sequencing
Exon 4	FW- ACACGGACTCTGAATGAGTATAAGG	58.1	
	RV- GATCTTCGTTCTCTATTATCTGTGG	58.1	
Exon 13	FW-GATGTAATATGAAACCAAGTATTGG	54.5	
	RV- GAAGTCATATAGAAATGTCTATCAT	52.8	
Exon 14	FW-GGAAATGAGCAGATAATAAAGATTATAGC	58.3	
	RV- CATCTAAAACAAGAGAATTGGCATAAGTTGG	61.7	

FIGURE 1. List of primers, dHPLC peak profiles, and results of sequencing for different *DPYD* genotypes.

were used to screen for single-nucleotide polymorphism at exon 13 (54°C and 58.5°C) and exon 14 (55°C and 59°C). All DNA templates were screened using these selected optimum temperatures and compared with peak profiles of known DNA sequence, which act as a control to assure reproducibility in each run. The mobile phase consisted of 100 mmol/L triethylammonium acetate, 0.1 mmol/L EDTA (Helix BufferPaks A), and 100 mmol/L triethylammonium acetate, 0.1 mmol/L EDTA with 25% (vol/vol) acetonitrile (Helix BufferPaks B). Random samples were chosen and sent for direct sequencing to validate the dHPLC results (Fig. 1).

Statistical Analysis

Statistical test was performed using PASW 18.0 software for Windows (SPSS, Inc, Chicago, IL, USA). Test for normality was performed using Shapiro–Wilk normality test. For nonparametric test, 2 type of statistical tests were used: (1) Mann–Whitney *U* test for categorical data with 2 groups; (2) Kruskal–Wallis test for categorical data with more than 2 groups. Confidence intervals (CI) were presented at 95%. *P* value <0.05 was considered statistically significant.

Correlation between the presence of a genetic variant and toxicity was evaluated by relative risk (RR). RR was calculated using MedCalc software available online at http://www.medcalc.org. In this study, multiple linear regression analysis was performed to determine the influence of each predictor variables on dependent variables. Predictor factors with *P* value <0.05 was used for the analysis.

RESULTS

Association Between Concentrations of 5-FU and Clinical Outcomes

Patients who experienced neutropenia had significantly higher serum concentrations of 5-FU as compared with those without neutropenia (Mann–Whitney *U* test, $P = 0.031$). Levels of 5-FU were found to be higher in patients with weight loss; the difference was not statistically significant (Mann–Whitney *U* test, $P = 0.217$). 5-FU concentrations were elevated in patients with fever but not statistically significant (Mann–Whitney *U* test, $P = 0.355$). Prolonged and high concentrations of 5-FU can decrease patient's white blood cell count, and this can increase the risk of infection, which may be presented as fever. No difference in the incidence of diarrhea, fever, cough, vomiting, nausea, and gastrointestinal toxicity was observed among patients with different concentrations of 5-FU (data not shown).

Influence of *DPYD* Genotypes on the Concentrations of 5-FU (Toxicities of 5-FU)

We predicted the degree of DPD activity or deficiency based on the presence of *DPYD* variants (Fig. 2). Concentrations of 5-FU among patients were different according to *DPYD*5* (rs1801159) or 1896 T>C (rs17376848).

Patients with *DPYD* allele of deficient enzyme activity had higher median serum levels of 5-FU compared with the normal DPD group (Mann–Whitney test, $P = 0.010$) (Fig. 2). Regression analysis was performed to determine the influence of *DPYD*5* and 1896 T>C on 5-FU levels; *DPYD*5*

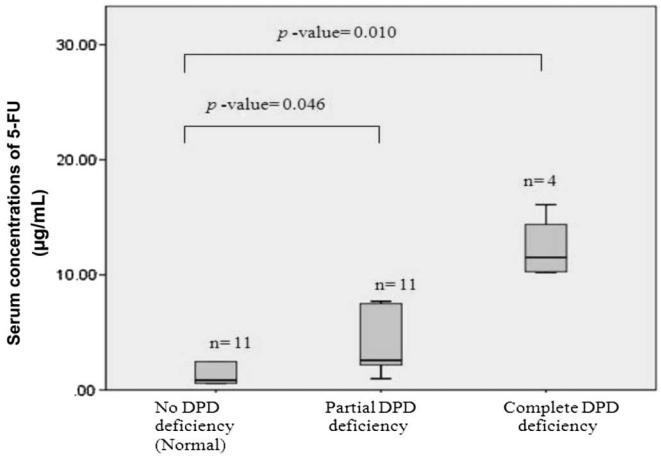


FIGURE 2. Association of genetic polymorphism of *DPYD* and serum concentrations of 5-FU. The middle line in the box plot corresponds to the median values. This figure shows that patients with complete DPD deficiency have higher median serum concentrations of 5-FU compared with normal DPD group (median, 11.51 mcg/mL; 95% CI, 10.18–16.11 versus median, 0.83 mcg/mL; 95% CI, 0.55–5.90). Patients were classified to 3 categories, where (1) no DPD deficiency are patients with homozygous wild-type genotype for *DPYD*5* and 1896 T>C (median serum concentrations of 5-FU, 0.83 mcg/mL; 95% CI, 0.55–5.90); (2) partial DPD deficiency are those who are heterozygous in either *DPYD*5* or 1896 T>C (median serum concentrations of 5-FU, 2.57 mcg/mL; 95% CI, 0.97–27.592); and (3) complete DPD deficiency are those with homozygous mutant in either *DPYD*5* or 1896 T>C (median serum concentrations of 5-FU; 11.51 mcg/mL; 95% CI, 10.18–16.11).

(rs1801159) and 1896 T>C (rs17376848) were accountable for 5-FU levels when tested as individual variable at 3.4% and 27.6%, respectively. *DPYD*5* (rs1801159) and 1896 T>C (rs17376848) were accountable for 36.6% of interindividual variation of 5-FU serum levels achieved among patients. Significant correlation ($P < 0.05$) was observed in patients who were carrier of 1896 T>C (rs17376848) and neutropenia (RR, 2.3; 95% CI, 1.01–5.09) (Table 2). The regression equation for the prediction of 5-FU levels and risk of neutropenia obtained were as follows: 5-FU levels = −0.8628 + 3.605 (*DPYD*5*/rs1801159) + 6.844 (1896 T>C/rs17376848) ($P = 0.0041$) and neutropenia = −0.612 + 0.349 (*DPYD*5*/rs1801159) + 0.411 (1896 T>C/rs17376848) ($P = 0.017$).

*DPYD*5* (rs1801159) was the most common variant detected in the colorectal cancer patients (19%). The RR of experiencing toxicity in patients with *DPYD*5* (rs1801159) was 7.7 for hematoma, 1.60 for upper respiratory tract infection, 3.2 for diarrhea, 1.9 for neutropenia, 1.3 for leukopenia, and 1.4 for anemia (Table 2). In this study, 242 patients, 23.1% (n = 6) and 26.9% (n = 7) who experienced neutropenia and anemia, respectively, were the patients with *DPYD*5* (rs1801159). Significant correlation ($P < 0.05$) was observed in patients with 1896 T>C (rs17376848) genotype and the occurrences of neutropenia (RR, 2.3; 95% CI, 1.01–5.09) (Table 2). Knowing that *DPYD* 1896 T>C (rs17376848) predisposes patients to other adverse effects, therefore, it should be accounted seriously in clinical

TABLE 2. Relative Risk of Adverse Effects and *DPYD* Genotypes

	*DPYD*5*					
	Wild-Type Genotype, n (%)		Variant Genotype, n (%)			
	No toxicity	Toxicity	No toxicity	Toxicity	RR	95% CI
Neutropenia	11 (42.31)	5 (19.23)	4 (15.38)	6 (19.23)	1.9	0.79–4.66
Hematoma	16 (61.54)	0 (0)	8 (30.77)	2 (7.69)	7.7	0.40–146.1
Diarrhea	15 (57.69)	1 (3.85)	8 (30.77)	2 (7.69)	3.2	033–30.87
Anemia	8 (30.77)	8 (30.77)	3 (11.54)	7 (26.92)	1.4	0.74–2.65
Upper respiratory tract infection	14 (53.85)	2 (7.69)	8 (30.77)	2 (7.69)	1.3	0.22–7.74
Cough	13 (50.00)	3 (11.54)	8 (30.77)	2 (7.69)	1.1	0.21–5.31
Leukopenia	11 (42.31)	5 (19.23)	6 (19.23)	4 (15.38)	1.1	0.39–2.87
Vomit and nausea	11 (42.31)	5 (19.23)	7 (26.92)	3 (11.54)	1.0	0.29–3.17
Hand tremor	15 (57.69)	0	10 (38.46)	0	0.4	0.02–9.55
Fever	12 (46.15)	4 (15.38)	9 (34.62)	1 (3.85)	0.4	0.05–3.10
Numb fingers	14 (53.85)	2 (7.69)	9 (34.62)	1 (3.85)	0.8	0.08–7.72
Loss of weight	11 (42.31)	5 (19.23)	9 (34.62)	1 (3.85)	0.3	0.04–2.36
Loss of appetite	9 (34.62)	7 (26.92)	7 (26.92)	3 (11.54)	0.7	0.23–2.10
Hematuria	15 (57.69)	1 (3.85)	10 (38.46)	0 (0)	0.2	0.01–4.41
	1896T>C					
	Wild-Type Genotype, n (%)		Variant Genotype, n (%)			
	No toxicity	Toxicity	No toxicity	Toxicity	RR	95% CI
Neutropenia	13 (53.85)	6 (19.23)	2 (7.69)	5 (19.23)	2.3*	1.01–5.09
Hematoma	17(65.39)	2 (7.69)	7 (26.92)	0	0.5	0.03–9.30
Diarrhea	16 (61.54)	3 (11.54)	7 (26.92)	0	0.3	0.02–5.52
Anemia	9 (34.62)	10 (38.46)	2 (7.69)	5 (19.23)	1.4	0.72–2.56
Upper respiratory tract infection	15 (57.69)	4 (15.38)	7 (26.92)	0	0.3	0.02–4.71
Cough	15 (57.69)	4 (15.38)	6 (23.08)	1 (3.85)	0.7	0.09–5.08
Leukopenia	14 (53.85)	5 (19.23)	7 (26.92)	0	2.0	0.80–4.98
Vomit and nausea	14 (53.85)	5 (19.23)	4 (15.38)	3 (11.54)	1.6	0.52–5.09
Hand tremor	19 (76.92)	0	6 (19.23)	1 (3.85)	6.9	0.32–149
Fever	16 (61.54)	3 (11.54)	5 (19.23)	2 (7.69)	1.8	0.38–8.66
Numb fingers	17 (69.23)	2 (7.69)	6 (19.23)	1 (3.85)	1.4	0.14–0.73
Loss of weight	15 (57.69)	4 (15.38)	5 (19.23)	2 (7.69)	1.4	0.32–5.84
Loss of appetite	12 (46.15)	7 (26.92)	4 (15.38)	3 (11.54)	1.2	0.41–3.29
Hematuria	18 (69.23)	1 (3.85)	7 (26.92)	—	0.8	0.03–18.4

*Variant of *DPYD 1896T>C* is a significant predictor of RR for neutropenia (analysis of variance, $P = 0.017$).

management. The small sample size of study failed to show significant correlation of this variant and other adverse effects, but it should not be ruled out as clinically important. The *DPYD*2A* (IVS14+1G>A) (rs3918290) allele was not detected in this group of patients.

Combined regression analysis was performed to determine the influence of *DPYD*5* (rs1801159) and 1896 T>C (rs17376848) on the prediction of neutropenia and 5-FU level. Both *DPYD*5* (rs1801159) and 1896 T>C (rs17376848) were significant variables (analysis of variance, $P = 0.017$) and accounted for 29.9% of the occurrences of neutropenia and 36.6% of interindividual variation of 5-FU serum levels.

DISCUSSION

Current approach in cancer treatment is often a "trial and error" that is inefficient and often causes severe toxicity. Application of molecular analysis to reduce the trial-and-error prescription and safer dosing is desirable in reducing the occurrence of adverse drug reactions.[15] BSA has been used as the standard method for dosing of 5-FU in clinical settings. However, this method lacks accuracy and reliability because BSA does not consider other factors that could affect 5-FU metabolism such as genotype, age, gender, and drug–drug interaction.[16] Therefore, we investigated the value of genotyping in monitoring the outcome of patients. DPD enzyme plays an important role in catabolism of the metabolites of 5-FU, and more than 80% of 5-FU is cleared from the body via catabolism pathways.[17] 5-FU–induced toxicity has been reported in DPD-deficient patients with higher levels of 5-FU.[18,19]

Previous study had identified the splice mutation IVS14+1G>A (*DPYD*2A*) (rs3918290) as the most common variant associated with 5-FU toxicity. According to Morel et al,[20] 50%–60% patients who carry genetic variations of *DPYD* developed severe 5-FU toxicity. A study by Kristensen

et al[21] indicated that prescreening of *DPYD* variant before 5-FU administration could prevent 20% of 5-FU–related toxicities. In this study, *DPYD*5* (rs1801159) and 1896 T>C (rs17376848) were found to be responsible for 36.6% of interindividual variation of 5-FU levels, whereby median levels of 5-FU were found significantly higher in patients bearing these variants (Mann–Whitney test, $P = 0.010$). Therefore, genotyping of *DPYD*5* (rs1801159) and 1896 T>C (rs17376848) serves as a good tool to monitor and predict the efficacy of 5-FU because phenotyping using drug level is tedious and laborious. Measuring drug level is tedious and less practical compared with genotyping for the following reasons: (1) sampling needs to be done at a specified optimal time point during the elimination phase; (2) patient's compliance in fasting before sampling or before next dose for trough level determination may not be reliable; and (3) samples need to be sent to laboratory within an hour to reduced degradation of drug in serum. Conversely, DNA is relatively stable, and genotype needs to be determined only once.

An increased level of 5-FU was significantly associated with neutropenia events (Mann–Whitney test, $P = 0.031$). The present study demonstrated that patients who developed neutropenia had up to 9 times higher 5-FU levels as compared with those who did not encounter it. This implicates that *DPYD*5* (rs1801159) and 1896 T>C (rs17376848) could potentially be used as predictive markers for neutropenia. This result is in accordance with another study where patients with reduced DPD activity had higher risk of neutropenia when compared with those with normal DPD activity.[22] In addition, van Kuilenburg et al[7] had demonstrated that impairment of DPD activity was associated with high level of 5-FU and lead to severe neutropenia (grade III/IV). High concentration of 5-FU is also linked to various adverse effects, including diarrhea, nausea, vomiting, mucositis, and even death.[23] Besides 5-FU levels, age and stage of cancer were additional risk factors of toxicity determined in this study. We observed that patients aged 70 years with advanced stage of cancer (metastasis) were more likely to develop 5-FU–related toxicity, such as loss of weight, neutropenia, and fever.

CONCLUSIONS

*DPYD*5* (rs1801159) and 1896 T>C (rs17376848) are useful predictors of patient's response to 5-FU chemotherapy. The phenotype–genotype correlations demonstrated in this study prove the usefulness of genotyping as a tool to monitor 5-FU efficacy instead of using conventional drug level measurements. Genotyping of *DPYD* variants is therefore recommended to prevent severe toxicity, particularly neutropenia, due to 5-FU. An alternative drug or a reduced dose of 5-FU should be recommended for a poor metabolizer or an intermediate metabolizer to avoid severe adverse effects due to 5-FU administration.

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