EFFECT OF DIHYDROPYRIMIDINE DEHYDROGENASE GENE POLYMORPHISM ON TOLERABILITY OF ORAL CAPECITABINE THERAPY IN SOLID TUMORS.

Usha Adiga*

Abstract

Background:5-fluorouracil(5-FU) has been extensively used as monotherapy or in combination therapy for a variety of solid cancers. Capecitabine, a novel oral fluoropyrimidine derivative may be converted to 5-FU selectively in tumors. Although treatment with 5-FU is generally well tolerated, few people often experience fluoropyrimidine-related toxicity. This can be explained by clinical factors. However, much variability in toxicity remains unexplained. Dihydropyrimidine dehydrogenase **(**DPYD) is a rate limiting metabolizing enzyme for fluorouracil and its prodrug capecitabine. Reports show that G>A single nucleotide polymorphism at the 5'-splice sequence of exon 14 (DPYD*2A) of DPYD gene to be highly polymorphic, which leads to the formation of a truncated non-functional protein. It is considered to be predictive marker as about 50% DPYD*2A allele carriers actually develop severe toxicities with 5-FU. However, the polymorphism pattern of DPYD*9 and DPYD*2 and its association with tolerability are limited in Indian setting.

In silico analysis of the SNPs using bioinformatics tools, SIFT, Polyphen 2 and I Mutant 3 showed that all the four SNPs were deleterious. Hence selection of these SNPs is justifiable.

Objectives:

a) To determine the pattern of DPYD*9 and DPYD*2 gene polymorphisms in cancer patients with solid tumors (colorectal cancer, TNBC, Ca stomach)
b) To estimate the plasma concentration of 5FU at the end of first and 4th cycle.
c) To evaluate the association of myelosuppression, GIT and skin toxicity due to capecitabine therapy in patients of DPYD*9 and DPYD*2 gene polymorphism in our population.

Methodology: Eligible cancer patients who receive oral capecitabine will be recruited. Blood sample will be collected for Genotyping of DPYD*9 and DPYD*2 polymorphisms by PCR-RFLP/gene sequencing. Myelosuppression, Febrile Neutropenia and other toxicities will be monitored.

Conclusion: A reduced functional DPD enzyme is often caused by genetic polymorphisms in DPYD. The heterozygous carriers of such DPYD polymorphisms have a partial DPD deficiency. When such patients are treated with a full dose of fluoropyrimidines, they are generally exposed to toxic levels of 5-FU and its metabolites.

Keywords: DPDY*2, DPYD*9, capecitabine, colorectal cancer.

*Professor, Dept of Biochemistry, KS Hegde Medical Academy, Nitte Deemed to be University, Mangalore, India.

***Corresponding Author:** Dr Usha Adiga,
Professor, Dept of Biochemistry, KS Hegde Medical Academy, Mangalore, Email: ushachidu@yahoo.com

DOI: 10.48047/ecb/2023.12.si10.00201

Background:
5-fluorouracil (5-FU) has been extensively used for almost five decades either as monotherapy or in combination therapy for a variety of solid cancers [1]. Capecitabine, a novel oral fuoropyrimidine derivative may be converted to 5-FU selectively in tumors through a cascade of different enzymes. Several metabolic pathways are involved by which 5-FU and the prodrug capecitabine are converted to active nucleotide analogues[2].The main mechanism of action is by inhibition of thymedylate synthase, which plays an important role in the folate homocysteine cycle and pyrimidine synthesis pathways. Also, the active metabolite (5-FU) can be incorporated directly into the RNA and DNA ultimately leading to cell death [3].

The most common dose-limiting capecitabine toxicities are hand-foot syndrome (HFS), diarrhea febrile neutropenia and thrombocytopenia which may demand either dose reduction or discontinuation of therapy. This can be explained by clinical factors, such as age, gender, local clinical practice and, possibly, diet. However, much variability in toxicity remains unexplained [4].

Dihydropyrimidine dehydrogenase (DPYD): DPYD is a rate limiting metabolizing enzyme for -flurouracil and its prodrug capecitabine. Overexpression of DPYD enzyme is related to drug resistance [5]. A meta-analysis reported that DPYD gene to be highly polymorphic in different ethnic population. G>A single nucleotide polymorphism at the 5'-splice sequence of exon 14 (DPYD*2A) is the most commonly reported variation of DPYD gene which leads to the formation of a truncated non-functional protein [6]. The major limitation of this polymorphism of being used as a predictive marker for toxicity is its lower minor allele frequency in different ethnic groups and only about 50% DPYD*2A allele carriers actually develop severe toxicities with 5-FU[7]. Despite the documented data from different ethnic groups, the polymorphism pattern of DPYD*9 and DPYD*2 and its association with tolerability are limited in Indian setting.

In six RCTs, patients receiving capecitabine as neoadjuvant or adjuvant treatment for breast cancer showed grade 3–4 leucopenia [8-13]. 25.73% patients in the capecitabine group developed grade 3–4 leucopenia after neoadjuvant or adjuvant treatment and 39.11% in capecitabine-free group.

In six RCTs, the rates of grade 3 HFS were reported [8, 10-14], overall, 14.87% patients in the capecitabine group developed grade 3 HFS after neoadjuvant or adjuvant treatment and 1.70% experienced in the capecitabine-free group.

Rosmarin in his study concluded that DPYD polymorphisms was consistently associated with capecitabine toxicity, rs12132152 and rs12022243 rs12132152 were particularly strongly associated with HFS [15].

The clinical pharmacogenetic implementation consortium guidelines stated the DPYD*2A polymorphism patients should receive 30–50% dose reduction in heterozygous (GA) patients and switching to an alternate drug in homozygous (AA) DPYD*2A mutants [16].

Lokanayagam et al concluded four DPYD sequence variants (c.1905 + 1G>A [rs3918290], c.2846A4T [rs67376798] c.1601G4A [rs1801158] and c.1679T>G [rs55886062] were found in 6% of the cohort and were significantly associated with grade 3–4 toxicity[17].

Varma et al studied the influence of DPYD*9A polymorphism on plasma concentration of 5-FU concluded that DPYD *9 polymorphism had a significant influence on the plasma levels of 5-FU after capecitabine administration. [18]

Ramalaksmi S et al conducted genetic study in south Indian origin suffering from colorectal cancer on CAPOX therapy. She reported exon 14 skipping IV +1g>A (DPYD*9A) which is related to DPYD deficiency occurred in only 1of 16 (5.5%) patients and this was associated commonly with more toxic side effects and nonresponsive to treatment. [19]

In silico analysis of genes using bioinformatics tools avoids screening of a huge number of individuals to detect an association between the gene and the disease at a reasonable level of statistical significance. In other words, these tools help in the pre-selection of SNPs.

Before taking up wet lab-based approaches, if disease-associated SNPs can be identified from neutral SNPs, it would be of great use. In silico analyses are useful when the disease associations could not be established by subsequent independent studies. Hence, independent evidence of functionality of SNPs obtained by using prediction tools could also serve as additional

resources to discriminate true associations from false positives.

Table 1 shows the in-silico analysis of the concerned SNPs using SIFT, Polyphen and I Mutant bioinformatics tools. In missense mutations, 4 SNPs were selected, D949V, S534N, I560S and I560S. All the 4 SNPs were found to be Damaging with SIFT Score of 0.00. These SNPs were analysed by the PolyPhen tool with score of 0.996,0.993, 1.00 and 1.00 respectively all of them being probably damaging. I-mutant 3.0 was used to predict the effects of single point mutation on the protein stability. DDG values of binary classification showed values of <0 implying a decreased stability of the protein as a result of the gene polymorphism. Hence it is justifiable to find its association with the effect of drug and therapeutic outcome.

Table 1:IN SILICO analysis of the SNPs of DPYD using bioinformatics tools

Common Allele ID	Codons	Substitution	dbSNP	SNP type	Prediction	SIFT Score	Polyphen Score	Sensitivity	Specificity	Prediction	SVM 2 Prediction effect	DDG value Prediction by I Mutant
1,97547947,1,T/AT>A	GAT-GtT	D949V	rs67376798	Non synonymous	Damaging	0.00	0.996	0.55	0.98	Probably damaging	-0.12 Kcal/mol	Decrease
1,97981421,1,C/T	AGT-AaT	S534N	rs1801158:T	Non synonymous	Damaging	0.00	0.993	0.7	0.97	Probably damaging	-0.54 Kcal/mol	Decrease
1,97981343,1,A/C	ATT-AgT	I560S	rs55886062:C	Non synonymous	Damaging	0.00	1.00	0.00	1.00	Probably damaging	-1.80 Kcal/mol	Decrease
1,97981343,1,A/T	ATT-AaT	I560S	rs55886062:T	Non synonymous	Damaging	0.00	1.00	0.00	1.00	Probably damaging	-1.80 Kcal/mol	Decrease

Hypothesis:

Myelosuppression, GIT and skin toxicity are more pronounced in our patient population with DPYD *9 and DPYD*2 gene polymorphism in patients receiving capecitabine therapy.

Specific Objectives:

1. To determine the pattern of DPYD*9 and DPYD*2 gene polymorphism in patients of solid cancers (Colorectal carcinoma, TNBC and Ca Stomach)
2. To estimate the plasma concentration of 5FU at the end of first and 4th cycle
3. To evaluate the association of myelosuppression, GIT and skin toxicity due to capecitabine therapy in patients of DPYD*9 and DPYD*2 gene polymorphism in our population.

Methodology:

Study Design: Prospective Observational Study

Study Setting: Medical Oncology Dept of JKSHCH, Mangalore & KMC Attavar
Central Research Laboratory, KSHEMA

Study Period: 2 years from IEC approval

Inclusion Criteria:

1. Patients of more than 18 years of age diagnosed with any solid cancer eligible to receive oral capecitabine chemotherapy for 8 cycles.
2. Patients willing to participate in the study.

Exclusion criteria:

1. Known contraindications for Fluorouracil therapy – coronary insufficiency, pregnancy, lactation.

Sample size:

Formula used :$4pq/d^2$
p- Prevalence of ADR of 30% in DPYD *9 polymorphism and 8 % of precision rate
4x0.30x0.0.07/ 0.08x0.08= 132
With 15% attrition=150

Methodology:

Clinical Work: Patients suffering from any type of solid cancer eligible to receive oral capecitabine in a dose of 625- 850 mg/m2 twice daily for 2 weeks followed by 1 week rest for 8 cycles (fulfilling inclusion and exclusion criteria) will be enrolled after obtaining the written informed consent.

Sample collection: 4ml of venous blood sample for genotyping of DPYD*9 AND DPYD*2 was drawn prior to initiation of capecitabine therapy. The baseline parameters such as CBC, (Hb%, TC, DC, ESR, Platelet count, LFT were performed and followed with a complete blood count done every 3 weeks. The patient will be clinically evaluated for signs of gastrointestinal, hemopoietic, skin toxicity in addition to routine oncology evaluation for the respective cancer for which patient is receiving the therapy.

Parameters observed: Myelosuppression: Absolute neutrophil count (ANC) , platelet count,

Episodes of febrile neutropenia, cumulative dose of capecitabine and duration of treatment interruptions were recorded for 24 weeks. Dose adjustment, if any, was done as per routine to maintain an ANC between 750–1500/cumm will be noted.

Definitions: Myelosuppression ANC<750 cumm and or thrombocytopenia<75000cumm
Febrile Neutropenia (Temperature higher than 38 °C with ANC).

Thrombocytopenia, Hand foot syndrome and GIT toxicity will be categorized as per NCI- CTC revised criteria.

Patients will be evaluated for tolerating the dose initiated, Percentage of patients completing the planned cycle of capecitabine therapy at 24 weeks. Patients will also be monitored for symptoms of GIT (stomatitis, vomiting, Diarrhea, hand-foot syndrome.

Laboratory work:
Blood sample collection for genetic analysis: 3 ml of venous blood samples will be collected in EDTA (2%) vial. EDTA blood will be utilized for DNA extraction and genotyping. DNA will be extracted from leukocytes by using DNA extraction mini kit.

Quality analysis of the extracted DNA: The quality of the DNA will be checked by electrophoresis on 0.8% Agarose gel, containing ethidium bromide (0.5 µg/ml) in TAE buffer.

Quantification of genomic DNA: The quantification and purity of DNA will be checked by the spectrophotometer (ratio of OD260 / OD280). DNA concentration was calculated using the following formula:
Concentration (µg/ml) of DNA in original solution= Absorbance x 100 x 50 µg/ml.

Amplification and Genotyping of the gene polymorphism: Genotyping of all genes will be confirmed by PCR-RFLP.

Genotyping of DPYD*9 and DPYD*2 polymorphisms:
Genotyping will be performed using PCR RFLP for DPYD*9 (DPD (IVSI14+1G>A) and genotyping was performed using PCR and gel electrophoresis. 5'-ATCAGGACATTGTGACATATGTTTC-3' will be the forward primer and 5'-CTTGTTT TAGATGTTAAATCACACATA-3' will be the reverse primer used for DPYD*9 gene with an annealing temperature of 58 degree C and Ndel will be used as restriction enzyme. All the reactions were set in a final volume of 25 µl consisting 10 µl of DNA (100 ng), 1 µl forward primer, 1 µl reverse primer and 13 µl of 2X PCR master mix. Except the annealing temperatures the PCR cycling conditions were Initial denaturation at 95° C for 5 min, Denaturation at 95°C for 1 min, Extension at 72°C for 40 sec, Final extension at 72°C for 7 min which will be common for all primers. All the amplifications will be completed within 35 PCR cycles. The amplified PCR products will be resolved on 2% agarose gels and visualized under UV light.

Statistical Analysis: Statistical analysis will be performed using IBM SPSS software version 22.0. Allelic and genotypic frequencies, disequilibrium coefficients and the associated standard error for co-dominant traits will be calculated. Hardy Weinberg equilibrium (HWE) will be calculated for each polymorphism studied. Association between the genetic variants and toxicity will be assessed by Chi-square test. Relative risk for toxicity will be determined by Fischer's test. P value < 0.05 was considered as statistically significant.

Significance of the proposed study
The study will help the clinician in deciding whether to start or not to initiate the capecitabine therapy with particular genetic pattern and also to adjust the dose of capecitabine according to the tolerability to the drug.

Conclusion:
The association of DPYD*9 and DPYD*2 gene polymorphisms have been less explored among solid cancer patients. There are only a few studies which focused on influence of DPYD*9 and DPYD*2 gene polymorphism on the plasma concentration of 5FU and on the toxic effects of the drug among cancer patients in terms of myelosuppression, GIT and skin toxicity due to capecitabine therapy.

The study will help the clinician in deciding whether to start or not to initiate the capecitabine therapy with particular genetic pattern and also to adjust the dose of capecitabine according to the tolerability to the drug. Hence it is essential to treat patients through personalized approach.
The study is on the large inter-individual variability, that may reflect functional

consequences of genetic polymorphisms in genes encoding drug-metabolizing enzymes. The concept of "individualized medicine" is evolving and there has been a paradigm shift from the concept of "one drug fits all" to "right drug for the right patient at the right dose and time." Hence it is very important to investigate the possible roles of genetic polymorphisms in the metabolism of 5FU. There are only a few studies on the effect of DPYD gene polymorphism on 5FU metabolism, especially in Indian settings to the best of our knowledge.

References:

1. Ezzeldin H, Diasio R. Dihydropyrimidine dehydrogenase deficiency, a pharmacogenetic syndrome associated with potentially life-threatening toxicity following 5-fluorouracil administration. Clin Colorectal Cancer. 2004; 4(3): 181–18
2. Thorn CF, Marsh S, Carrillo MW, McLeod HL, Klein TE, Altman RB. Pharm GKB summary: fuoropyrimidine pathways. Pharmacogenet Genomics. 2011; 21(4):237–42.
3. CM, Lindley C. Capecitabine: a review. Clin Therapeut 2005; 27:23–44.
4. Cassidy J, Twelves C, Van Cutsem E, Hoff P, Bajetta E, Boyer M et al. First-line oral capecitabine therapy in metastatic colorectal cancer: a favorable safety profile compared with intravenous 5-fluorouracil/leucovorin. Ann Oncol 2002; 13(4):566–75
5. Del RM, Loupakis F, Barbara C, Lombardo L, Latiano T, Zafarana E et al. A clinical Pharmacogenetic characterization of DPD polymorphism for pre-treatment screening of patients candidates to fluoropyridine therapy. EPMA J.2014; 5(1) A24.
6. Li Q, Liu Y,Zhang HM, Huang YP,Wang TY, Li DS et al. Infuence of DPYD Genetic polymorphism on 5-Fluorouracil Toxicities in Patients with Colorectal Cancer: A meta-Analysis. Gastroenterol Res Pract. 2014; 2014: 827989. doi: 10.1155/2014/827989.
7. Borro M, Botticelli A, Mazzuca F, Onesti EC, Gentile G, Romiti Adriana et al. Pre-treatment assay of 5 Fluorouracil degradation rate(5-FUDR) to improve prediction of 5-fluorouracil toxicity in gastro-esophageal cancer. Oncotarget. 2017; 8:14050-57.
8. Joensuu H, Kellokumpu-Lehtinen PL, Huovinen R, Jukkola-Vuorinen A, Tanner M, Kokko R, et al. Adjuvant capecitabine in combination with docetaxel, epirubicin, and cyclophosphamide for early breast cancer: the randomized clinical FinXX trial. JAMA Oncol. 2017; 3(6):793–800.
9. von Minckwitz G, Möbus V, Schneeweiss A, Huober J, Thomssen C, Untch M, et al. German adjuvant intergroup node-positive study: a phase III trial to compare oral ibandronate versus observation in patients with high-risk early breast cancer. J Clin Oncol. 2013; 31(28):3531–9.
10. O'Shaughnessy J, Koeppen H, Xiao Y, Lackner MR, Paul D, Stokoe C, et al. Patients with slowly proliferative early breast cancer have low five-year recurrence rates in a phase III adjuvant trial of capecitabine. Clin Cancer Res. 2015; 21(19):4305–11.
11. 11.Masuda N, Lee SJ, Ohtani S,Im YH, Lee ES, Yokoto I et al. Adjuvant capecitabine for breast cancer after preoperative chemotherapy. N Engl J Med. 2017; 376(22):2147–59.
12. Lluch A, Barrios CH, Torrecillas L, Ruiz-Borrego M, Bines J, Segalla J, et al. Phase III trial of adjuvant Capecitabine after standard neo−/adjuvant chemotherapy in patients with early triple-negative breast Cancer (GEICAM/2003-11_CIBOMA/2004-01). J Clin Oncol. 2020; 38:203–13.
13. Li J, Yu K, Pang D, Wang C, Jiang J, Yang S, et al. Adjuvant Capecitabine with Docetaxel and cyclophosphamide plus Epirubicin for triple-negative breast Cancer (CBCSG010): an open-label, randomized, multicenter. Phase III Trial JCO. 2020; 38:1774–84.
14. Martín M, Ruiz Simón A, Ruiz Borrego M, Ribelles N, Rodríguez-Lescure Á, Muñoz-Mateu M, et al. Epirubicin plus cyclophosphamide followed by docetaxel versus epirubicin plus docetaxel followed by capecitabine as adjuvant therapy for node-positive early breast cancer: results from the GEICAM/2003-10 study. J Clin Oncol. 2015; 33:378
15. Rosmarin D, Palles C, Pagnamenta A, Kaur K, Pita G, Martin M, at al. A candidate gene study of capecitabine-related toxicity in colorectal cancer identifies new toxicity variants at DPYD and a putative role for ENOSF1 rather than TYMS. Gut 2015; 64:111–120. doi:10.1136/gutjnl-2013-306571.
16. Amstutz U, Henricks LM, Offer SM, Barbarino J, Schellens JHM, Swen JJ, et al. Clinical pharmacogenetics implementation consortium (CPIC) guideline for dihydropyrimidine dehydrogenase genotype and fluoropyrimidine dosing. Clin Pharmacol Ther 2018; 103:210–16.

17. Loganayagam A , Hernandez MA , Corrigan A , Fairbanks L , Lewis CM , P Harper P et al. Pharmacogenetic variants in the DPYD, TYMS, CDA and MTHFR genes are clinically significant predictors of fluoropyrimidine toxicity. British Journal of Cancer.2013; 108: 2505–2515. doi: 10.1038/bjc.2013.262
18. Varma A, Jayanthi M*, Dubashi B, Shewade DG, Sundaram R. Genetic influence of DPYD*9A polymorphism on plasma levels of 5-fluorouracil and subsequent toxicity after oral administration of capecitabine in colorectal cancer patients of South Indian origin. Drug Metabol Pers Ther 2020; 35(4): 20200133
19. Ramalakshmi S, Kavimani S, Srinevas S, V. Vettriselvi V and Bhaskar LVKS. A pharmacogenetic study of capecitabine-oxaliplatin therapy in colorectal cancer patients among south Indian population. European Journal of Pharmaceutical and Medical Research. 2016; 3(9): 520-528.