Review Article

A Detailed Review on Dihydropyrimidine Dehydrogenase Enzyme Deficiency-Autosomal Recessive Condition

P. Sharmila Nirojini, A. Asma Fathumuthu*

Department of Pharmacy Practice, Swamy Vivekanandha College of Pharmacy, Tiruchengode, Namakkal, Tamil Nadu, INDIA.

ABSTRACT

Dihydropyrimidine dehydrogenase deficiency (DPD) is an autosomal recessive condition. The DPD enzyme is in charge of the 5-fluorouracil drug's metabolism. Patients having DPD deficiency, when get administered with 5-fluorouracil drug, leads to drug toxicity due to increased concentration of drug in the body. Determining the DPYD (gene encoding DPD) genotyping and DPYD phenotyping will provide the best strategy to know the DPD deficiency in patients. Tegafur and Capecitabine are available prodrugs for the 5-fluorouracil medication. Genomic techniques appear to be a superior indirect evaluation for screening DPD deficiency in the clinical environment. Other than this there are several diagnostic tests such as Uracil oral loading dose, Uracil Breath test, Rapid UPLC-UV (Ultra performance liquid chromatography-Ultravoilet) method, and DPD activity in mononuclear cells from the peripheral circulation. An antidote Uridine Triacetate is available for treating the toxicity produced by 5-FU (5-Fluorouracil) in DPD deficient patient. Hence, this review explains briefly about the DPD enzyme, its deficiency, its diagnosis, and how to manage the DPD deficient patient undergoing chemotherapy.

Keywords: DPD, 5-Fluorouracil, Capecitabine, Tegafur, Uracil Breath test, Uridine triacetate.

INTRODUCTION

Dihydropyrimidine dehydrogenase (DPD) is an enzyme present in human cells for the degradation of pyrimidine, thymine, and uracil.[1] It is a rate-limiting enzyme and also a very important agents for the metabolism of cancer drugs like 5-fluorouracil. Lack of the DPD enzyme activity leads to an increase in the half-life of many cytotoxic drugs those results in increased drug content in the body which leads to many serious toxic effects on humans.[2]

Today cancer is one of the alarming diseases of the 21st century. Many cancers are curable only when they are detected at a prior stage of the disease. Cancer is said to occur when the size of the cells is compromised by 1 million cells which are referred to as tumors or lumps or nodule.[3] For solid cancer, one of the most widely used anticancer drugs is 5-fluorouracil. Fluorouracil drug is a pyrimidine analog which works as an antimetabolite inhibiting the RNA (Ribonucleic acid) and DNA (Deoxyribonucleic acid) synthesis that leads to the destruction of malignant cells and the DPD enzyme does this work via the pyrimidine degradation route.[4] One of the world's most prevalent causes of death is cancer. In 2018, there were 18.1 million new cancer diagnoses worldwide, with 9.5 million cancer-related deaths. By 2040, the number of new cancer diagnoses will

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Address for correspondence:
Ms. A Asma Fathumuthu
M.Pharm,
Department of Pharmacy Practice, Swamy Vivekanandha College of Pharmacy, Namakkal-637205, Tamil Nadu, INDIA.
Email id: hfasma_18@hotmail.com

www.ijopp.org

have risen to 29.5 million per year, with 16.4 million deaths due to cancer as per WHO estimation. Cancer rates are often higher in countries with the highest life expectancy, educational attainment, and living standards. However, for other cancers, such as cervical cancer, the opposite is true, and the incidence rate is greater in nations with the lowest population on this criteria.[5] Figure 1 indicates the scenario of cancer in the year 2020.[6]

Dihydro Pyrimidine Dehydrogenase Enzyme

Dihydropyrimidine dehydrogenase is an enzyme produced in the liver. This enzyme is responsible for the metabolism of endogenous pyrimidine and fluoropyrimidine catabolism.[7] Figure 2 indicates the metabolism of thymine and uracil by DPD.[55] It is encoded with the DPYD gene. The DPYD gene is present in the 1p21.3 chromosome.[8] In the PBMCs (Peripheral blood mononuclear cells), DPD is employed as a representation of the body's overall DPD activity. On the 1p22 chromosome, the DPYD gene was revealed to have 4400 nucleotides and 23 coding exons spanning 950 Kbs. The transcription and translation processes affect the activity of this enzyme, and the transcription factors include SP1, SP3, and microRNA-27a, microRNA-27b.[4] The dihydropyrimidine dehydrogenase enzyme is critical for the catabolism of the anticancer medication fluoropyrimidine. DPD has been identified to predict anti-tumor response and a high level of DPD will lead to an increase in the metabolism of the 5-fluorouracil drug that leads to a decrease in the cytotoxic effect.[9]

Other than the metabolism of endogenous pyrimidine and fluoropyrimidine catabolism, it plays an important role in the synthesis of ß-Alanine in eutherian. In both animals and people, DPD has been found to have a circadian clock. PMNC-DPD activity is also known to fluctuate throughout the day and has been linked to the changes of 5-FU concentrations in the blood when given intravenously. Several studies have discovered that

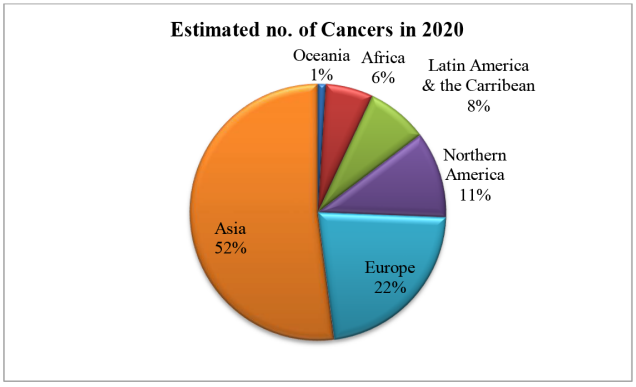

Figure 1: Scenario of the cancer in the year 2020.[6]

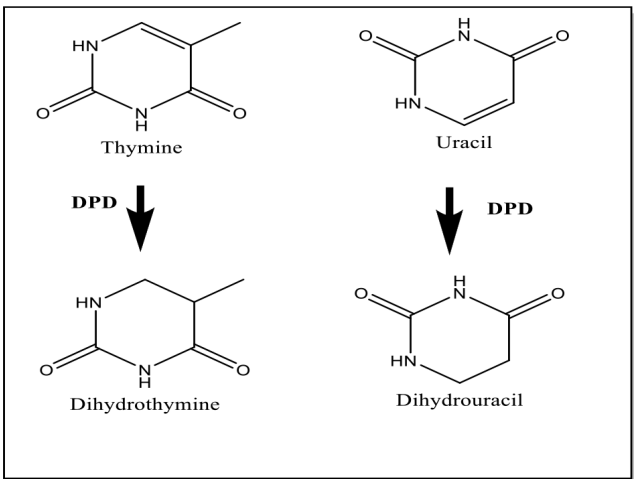

Figure 2: Thymine and Uracil gets metabolized by DPD.[55]

DPD has varying expression throughout tumor growth, which explains the difference in tumor pharmacological responsiveness to 5-FU. Even when the degree of thymidylate synthase expression was too low, patients with elevated DPD expression showed resistance to the 5-FU medication. Thymidylate synthase is responsible for the formation of DNA. Several investigations have revealed a link between malignant cell's DPD levels and anticancer drug resistance, whereas alcoholism and smoking have been observed to reduce 5-FU potential.[4,10]

5-Fluorouracil Drug

It is an important chemotherapeutic agent for solid tumor cancer.[11] For many years, it's been utilized to treat solid organ tumors. Heidelberger *et al.* 1957's research on 5-FU-induced tumor cell death was a game-changer in Oncology.[12] The 5-fluorouracil drug is been listed in the WHO (World Health Organization) Model List of Essential Medicines.[13] The bioavailability of 5-fluorouracil is unpredictable. After oral absorption, the drug is poorly absorbed. 5-fluorouracil drugs are mostly given in the parental dosage form.[14] The first-pass metabolism affects the bioavailability of the drug after oral delivery, due to the presence of the DPD enzyme in the liver more than 80% of the 5-fluorouracil drug is metabolized by the DPD enzyme.[9] It is a heterocyclic aromatic compound (Figure 3) and is similar to the structure of pyrimidine in DNA and RNA. Because its structure is similar to RNA and DNA it can easily get incorporated with the metabolism of nucleosides leading to the death of cancer cells.[15] In cancer treatment, 5-fluorouracil drug is mostly administered for colorectal cancer. 5-fluorouracil has an anticancer impact by causing RNA destruction, which is followed by DNA destruction, and lastly inhibiting the cell cycle.[11,16]

Structure of 5-Fluorouracil Drug

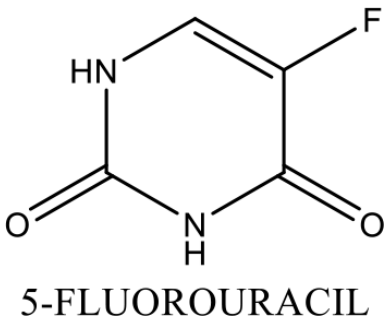

Figure 3: Chemical structure of 5-FU.[16]

Metabolism of 5-Fluorouracil Drug

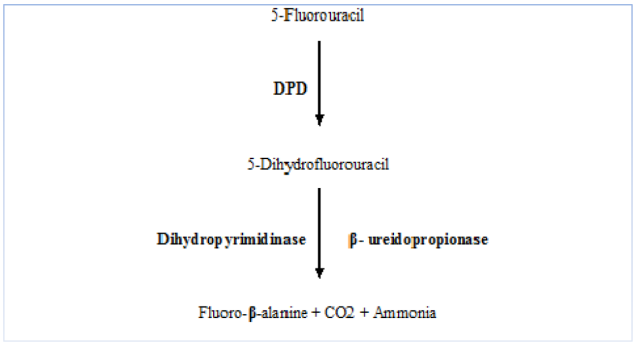

Figure 4: Flowchart representing the catabolism of 5-fuorouracil drug.

It has been stated that the metabolism of 5-fluorouracil (Figure 5) includes the catabolism of the drug into inactive metabolites (80-85%) by the DPD enzyme. And only 1-3% of the drug converts into an active form by anabolic action.[56] Figure 4 represents the catabolism of 5-FU drug.[57] The drug's metabolism is usually divided into three phases: phase-1 (modification), phase-2 (conjugation), and phase-3 (elimination via bile or urine). The medication undergoes oxidation, reduction, and hydrolysis during the modification phase, which is carried out by the enzyme Cytochrome P450. Glutathione S-transferases (GSTs) and uridine diphosphate glucuronosyltransferases (UGTs) are enzymes that activate and inactivate the medication during the conjugation phase. The enzymes thiopurine S-methyltransferase (TPMT) and dihydropyrimidine dehydrogenase (DPD) are involved in the anabolism and catabolism of purine and pyrimidine analogues, and they are also noted for having a lot of polymorphism in their encoding genes, which influences their activity.[17]

Mechanism of the Enzyme after Oral Administration

5-Fluorouracil Anabolism

The medication is converted into fluorouridine triphosphate (FUTP), an active metabolite that replaces

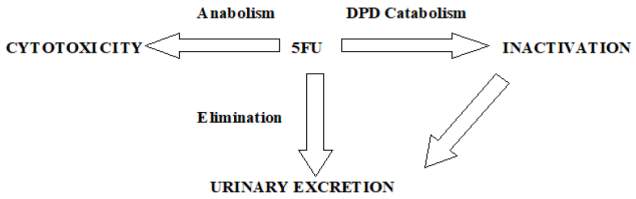

Figure 5: Mechanism of the DPD enzyme after oral administration of the 5-FU drug.[56]

uridine triphosphate in RNA. Fluorodeoxyuridine triphosphate (FDUTP) is also incorporated into DNA instead of deoxythymidine triphosphate. Finally, the ternary complex's thymidylate synthase (TS) activity is inhibited by flurodeoxyuridine monophosphate (FDUMP). This affects the process of RNA and DNA function leading to cell expiration.[18]

5-Fluorouracil Catabolism

The medication is converted into 5,6-dihydro-5-fluorouracil (DHFU), which is then converted into α-fluoro-ß-ureido-propionic acid and α-fluoro-ß-alanine during catabolism (FBAL). Within 24 hr, 60-90 percent of the medication is eliminated as FBAL in the urine.[18] In *in vivo*, the injected 5-FU has been found to have a half-life of only 10-20 min.[19] In the below-given Figure, Cassandra White *et al.* have mentioned the metabolic pathway of fluoropyrimidine drug (Figure 6).[20]

Hence, the cytotoxic effects of 5-FU are most likely mediated directly through anabolic pathways. The catabolic route also plays a role, as dihydropyrimidine dehydrogenase (DPD), which is found in the catabolic pathway's first and as a rate-limiting step, catabolizes more than 80% of the 5-FU administered. The anabolism and catabolism of 5-FU drug by the DPD enzyme is shown in Figure 5. DPD regulates the quantity of 5-FU attainable for anabolism, and so plays a vital role in 5-FU bioavailability.[21,56] Figure 6 indicates the inactivation of Uracil and 5-FU by DPD and Beta Ureidopropionase to yield the final metabolite product.[23] 5-Fluorouracil drug metabolism is very varied within the patients as high as 50%. Sex plays a major role in the variation with continuous evidence indicating that women are having lower 5-FU clearance. DPD has a wide range of expression and fluctuates in activity throughout the day. Furthermore, DPD deficiency (full or partial) is seen in 3% to 8% of the population, owing to DPYD polymorphisms. Lastly, DPD levels are 15% lower in women, which might be a clinically significant factor in the reduced clearance.[22]

Ureidopropionase Yeilding Beta-Alanine and Fluoro-beta Alanine as the Final Metabolites

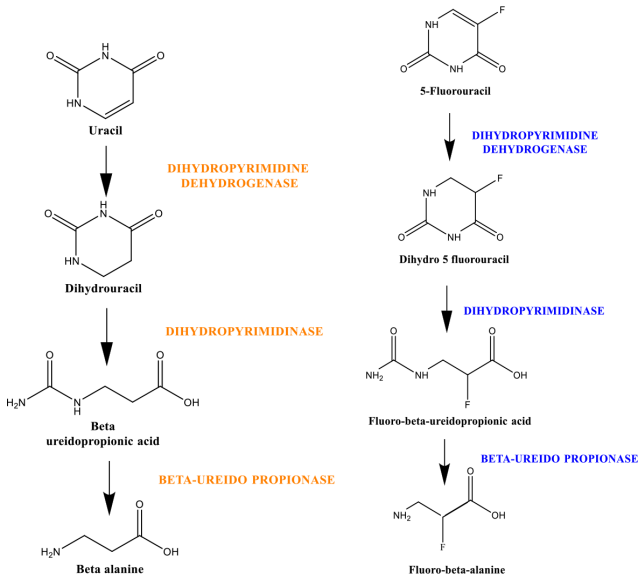

Figure 6: Pathway Indicating Uracil and 5-FU Inactivation by DPD and Beta-Ureidopropionase, Yielding Beta-Alanine and Fluoro-Beta Alanine as the Final Metabolites.[23]

The FDA Drug Label for Fluorouracil: Warning DPD Deficiency (2020)

In 2020, (Table 1) FDA (Food and Drug Administration) approves a 5-Fluorouracil warning label for DPD Deficiency.[24]

5-Fluorouracil Drug Toxicity in Patients with DPD Deficiency

The 5-Fluorouracil drug is the standard drug for use in cancer treatment since 1962. The first 5-fluorouracil drug toxicity case was reported in 1985 and it was observed that it occurred due to partial or deficiency of the DPD enzyme. In 1985, the patient was diagnosed with familial pyrimidinemia, which resulted in serious, almost deadly, toxicity after treatment with 5-FU, and it was also proposed that a genetic impairment in pyrimidine catabolism may be the cause of fluoropyrimidine-associated toxicity. In Japan also sixteen death reports were noted due to deficiency of DPD in cancer patients.[17,25] After oral administration of the 5-fluorouracil, only 1-5% of the drug is transformed into cytotoxic metabolites and nearly 80% of the drug is metabolized into a non-cytotoxic metabolite. Fluorodeoxyuridine monophosphate (FdUMP), fluorodeoxyuridine triphosphate (FdUTP), and fluorouridine triphosphate are cytotoxic metabolites, but 5-fluoro-5,6-dihydrouracil (DHFU) is non-cytotoxic.[12] The activity of DPD determines the levels of cytotoxic metabolites.[25] The therapeutic index of Fluorouracil drug is limited, and there is a danger that a considerable number of patients would have severe toxicity. Even at conventional dosages, over 30% of patients have grade 3 or higher toxicity, such as myelosuppression, gastrointestinal issues (most often oral mucositis and diarrhea), and hand-foot syndrome. Nearly 1% of patients died due to Fluorouracil drug-related toxicity. DPD enzyme insufficiency is thought to be responsible for 39–61 percent of severe 5-FU toxicity, which usually appears within the first 1–2 cycles of therapy.[20]

Table 1: FDA approved label of 5-FU drug.[24]

Phenotype	Fluorouracil
DPD deficient	People with low or no dihydropyrimidine dehydrogenase (DPD) activity are more likely to experience severe or deadly adverse effects. In patients exhibiting acute early-onset or extremely severe toxicity, which may signify a near complete or entire lack of DPD action, withhold or permanently cease fluorouracil or its oral tablet form Capecitabine. In those without DPD activity, no fluorouracil dosage has been demonstrated to be safe.

Symptoms of DPD Deficiency

The disorders present range from symptomless to dreadful neurological manifestation that includes:

- Intellectual disability
- Seizures
- Microcephaly
- Mucositis
- Granulocytopenia
- Neuropathy
- Autistic behavior
- Eye Abnormalities.[8]

Common Side Effects of 5-Fluorouracil Drug

- Nausea
- Vomiting
- Alopecia
- Anorexia
- Elevated liver Enzymes
- Hyperuricemia.[13]

DPYD Gene

DPD enzyme is encoded by the gene DPYD. There are many DPYD variants associated with DPD enzyme activity. It is known as *1 if no variant is determined for the DPD enzymes. In this case, it is said to have normal enzyme activity. If any variant haplotypes are present, their allele names with a star (*) are used to identify them. If a patient has two copies of the DPYD allele, he or she is considered a normal metabolizer. Many of

the variations that have been studied have been found to have reduced enzyme activity has been associated to severe 5-FU and has been considered as a cause of toxicity, but only four variants out of many variants were consistently linked to having a reduction in DPD activity and promotion in fluoropyrimidine toxicity, with 3-grade toxicity as per the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) related to 5-FU in mice.

The four variant genes are:

- DPYD*2A single nucleotide polymorphism (SNP) (c.1905+ 1G>A)
- DPYD*13 SNP (c.1679T>G)
- SNP c.2846A>T
- HapB3 (a novel haplotype – hapB3, consists of several variations).

According to current statistics, the combination of these variations is a significant contributor to the development of adverse events, accounting for at least 20% of the recorded instances of severe toxicities connected to 5-FU. Current evidence suggests that the combination of these variations is a significant contributor to the development of adverse events, responsible for at least 20% of the documented instances of severe toxicities associated with 5-FU.[26]

Population Study

An approach to determine the deficiency of enzyme DPD is by determining the uracil concentration or its byproduct dihydrouracil in peripheral blood mononuclear cells. The ratio of the uracil and dihydrouracil corresponds with the DPD activity in the PBMCs. Determining only the uracil concentration can also be done. But doing the phenotyping study of DPD and genotyping study of DPYD to detect the DPD activity will provide the best strategy to know the DPD deficiency in patients.[27]

DPYD Genotyping

Detecting DPD enzyme activity is done through DPYD genotyping.[28] DHPLC performance liquid chromatography (denaturing high-resolution chromatography) can be used to screen and genotype known and undiscovered homozygous and heterozygous sequence variations. The temperature-modulation heteroduplex production of dsDNA (Anti-double stranded deoxyribonucleic acid) with mismatched base pairs is used in DHPLC, a DNA screening technology. A mixed mixture of reference and mutant DNA samples is denaturized and amplified to produce heteroduplex DNA. The heteroduplexes were then resolved using a DHPLC method that avoided the need of acrylamide gels, radioactivity, or chemical denaturants. DHPLC is capable of detecting all DPYD sequence variations. It was confirmed using DNA sequencing and the dideoxynucleotide chain termination method.[29]

DPD Phenotyping

DPD phenotyping is done to measure the actual DPD enzyme activity.[28] When compared to DPYD genotyping; the DPD phenotyping method is greatly sensitive in determining DPD deficiency. DPD phenotyping involves four main tests which include the determination of the activity of the DPD enzyme in PBMCs, Endogenous uracil and dihydrouracil level, Uracil breath test, and Uracil test dose.[12] Estimating DPD movement in PBM is one of the standard methods. The uracil test dosage is administering uracil and then dihydrouracil to the patient, followed by determining uracil pharmacokinetics from plasma at various time intervals. The highest quality level in phenotyping strategies is the degree of PBM action corresponding with the hepatic level. The pretreated endogenous grouping of dihydrouracil and uracil from plasma was investigated to ensure DPD's enzymatic action. A link was found between the fraction of UH2/Uracil, 5-FU clearance, and treatment-related toxicity. Age affects pharmacokinetics because of the physiological changes that come with it. For example, as we become older, hepatic perfusion decreases, and we have fewer leeway's for a few drugs to pass via the major hepatic area. Drug digestion is further hampered by the presence of liver malignancies and chemotherapy-induced steatohepatitis, which may necessitate a change in uracil digestion.[25]

Gene Activity Score

Gene activity score (AS) is used to ascribe the DPD phenotype, the score is computed as the sum of the two DPYD variants with the smallest variant activity value and is developed on the DPYD allele functioning. Table 2 represents the gene activity score of the DPD phenotypes.[26]

EMA Recommendation on DPD Testing before Initiation of the Treatment with Fluorouracil Drug in Cancer Patients

Before using the 5-fluorouracil medication, the European Medicines Agency (EMA) suggests that the patients be checked for DPD enzyme deficiency to prevent the life-threatening issues due to increased toxicity of the drug. Patients who have partial DPD enzyme deficiency can be administered the drug 5-fluorouracil but it should be of

Table 2: DPYD allele's activity value and its functional ability.[26]

Sl. No	Allele	Activity value	Functional status of allele
1.	Reference	1	Regular function
2.	c.1905+1G>A (*2A)	0	No function
3.	c.1129-5923C>G, c.1236G>A (HapB3)	0.5	Reduced function
4.	c.2846A>T	0.5	Reduced function
5.	c.1627A>G (*5)	1	Regular function
6.	c.85T>C (*9A)	1	Regular function

low dose than the normal starting dose and if any side effects were observed, the drug should be completely stopped. Patients who have complete DPD deficiency should not be administered with the drug 5-fluorouracil. For conditions like actinic keratosis, warts the fluorouracil drug can be given because in these conditions the drug gets absorbed through the skin only at a very low dose since they are topically applied. Hence they recommend that pre-testing for DPD enzyme is mandatory before starting the treatment with fluorouracil drug. It can be achieved by calculating the concentration of uracil in the blood or by looking over for any mutations present in the DPD gene. Even Therapeutic Drug Monitoring (TDM) can also assist in improving the patient's conditions while receiving fluorouracil medication.[30]

Effect of Food on Dehydrogenase Enzyme Activity

It has been reported that by determining the levels of the dehydrogenase enzyme marker – uracil and dihydrouracil, the activity of the DPD enzyme can be determined for individualizing the anticancer activity of fluoropyrimidine. The level of the DPD markers in plasma level is higher during the fasting state and becomes low after food intake. The sampling for fluoropyrimidine drug should be done after overnight fasting from 8 am to 9 am to prevent the bias caused by food and circadian rhythm effect. The food given to the subjects involved in the trial was high fat and high caloric content with the guidance of the US FDA. This type of food was given due to the high content of RNA, as uracil is one of the bases of RNA. It was determined that the uridine level which is a precursor of uracil is decreased after the food intake whereas increased in the fasting state. In the fasting state, the uridine biosynthetic pathway is dominated by adipose tissue. Uridine stability was also found to be closely regulated by the enzyme uridine phosphorylase, which converts uridine to uracil. As a result, uracil activity is high during fasting.[31]

Diagnostic Test

Due to the availability of some diagnostic tests, it has become easier to predict the patient who is in danger of treatment with noxious 5-fluorouracil because of a deficiency of the DPD enzyme. It is impossible to diagnose DPD deficiency by physical examination of the patient, because of the absence of symptoms before the administration of 5-fluorouracil drugs.[32]

• Uracil Oral Loading Dose

In this method, the uracil drug is used to examine the activity of DPD by measuring the uracil and its metabolite in the plasma. A high-performance liquid chromatography (HPLC) method was used to determine the uracil and dihydrouracil concentration after an oral dose of uracil.[33] Patients were selected and distributed into two groups, which included sufferers having DPD activity in PBMC <5nmol mg^{-1} *h-1 [deficient subjects] and DPD activity in PBMC ≥ 5nmol mg-1*h-1 [normal subjects]. All the subjects were administered orally with U 500 mg as a loading dose and then their blood sample was collected. Then, using the HPLC technique, the concentration of the uracil loading dosage and its metabolites dihydrouracil were determined. The concentrations of thymine, dihydrothymine, DHU (dihydrouracil), N-carbamoyl-beta-amino isobutyric acid, and N-carbamoyl-beta-alanine in the plasma of all subjects were determined using reversed-phase HPLC and electrospray tandem mass spectroscopy prior to the intake of the uracil loading dose. For the deficient subjects after the intake of the uracil loading dose, the blood sample was collected and was undergone for the genetic analysis of the DPYD gene. All 23 coding exons and surrounding intronic regions were amplified using the polymerase chain reaction. A pharmacokinetic study was also conducted on all the subjects who indicated that the V_{max} value for both groups was different. The normal subjects group showed the highest value whereas the deficient subject group showed the lowest value. It also indicated that the concentration of pyrimidine metabolites was high in the deficient patient due to the absence of the enzyme responsible for metabolism. Hence this method can be used as a diagnosis for patients who are in danger of severe toxicity.[34]

• Activity of DPD in Peripheral Bloods Mononuclear Cells

In this method, the action of the DPD enzymes can be checked in cancer patients before and as well as during the treatment with 5-fluorouracil. The assay method was opted for determining the PBM-DPD activity. As

the DPD enzyme is synthesized in the liver, liver DPD activity was also determined in the cancer patient and the normal population. The liver is one of the major sites of fluorouracil metabolism, determination of liver DPD activity in patients was not found to be practical because the number of fresh human liver samples was very limited and so the study was conducted by determining the baseline of human liver DPD by using frozen liver tissues from normal donors. This study indicated that the activity of the liver DPD was decreased in a cancer patient with the DPD deficiency initially identified by PBM DPD and in normal patients; there was no significant decrease in the enzyme. Hence this method was reported to be a sensitive, accurate, and precise method.[35]

- **Rapid UPLC-UV Method**

This method is an Ultra Performance Liquid Chromatography-Ultraviolet (UPLC-UV) which determines the level of uracil and dihydrouracil level in the plasma as an agent to determine the DPD enzyme activity. It takes only less than 12 min to give the result of the test. The UPLC used is the H-CLASS Acquity UPLC system. Since DPD converts uracil into dihydrouracil, this method is easy, rapid, and inexpensive to detect the ratio of uracil and dihydrouracil. This method is an advanced technique of HPLC because in HPLC it needs a huge volume of samples and also increased time to detect. In plasma, it measures the uracil level in a range of 5-500ng/mL and dihydrouracil level in a range of 40-500ng/mL without any chromatographic interference. This method has been useful in detecting non-deficient patients (uracil level will be < 16ng/mL) and deficient patients (uracil level will be >16ng/mL). Hence this method is an inexpensive, reliable, and durable technique.[36]

- **Uracil Breath Test**

This technique is based on the determination of CO_2 present in the exhaled breath after ingesting 2-13C-Uracil to determine the pyrimidine catabolism.[37] This test involves the measurement of CO_2 released during the degradation of 13C-Uracil by the DPD enzyme.[28] In this method, in the morning a test dose of the aqueous solution of uracil is administered before the treatment of 5-fluorouracil therapy and the patients should be in a fasting state. Before the administration of uracil to the patients, patient breath samples were collected in a breath bag to denote the baseline. Next after the uracil ingestion, the breath samples were again collected in 100mL breath bags. The estimation of the CO_2 concentration present in the patient's breath after ingesting uracil is determined by an IR (Infrared rays) spectrophotometer.[38] In IR spectrophotometer, the amount of $^{13}CO_2$ and $^{12}CO_2$ is determined and $^{13}CO_2$ is indicated as delta over baseline ratio (DOB) and it indicates the shift in the ratio of the $^{13}CO_2$ /$^{12}CO_2$ after and before the administration of uracil.[39] The results were produced by checking the concentration of the $^{13}CO_2$ in the breath samples after 50 min of administration of uracil. Patients who have DOB50 greater than or equal to 128.9 DOB are said to have normal activity of the DPD enzyme whereas patients with DOB50 lesser than 128.9 DOB are categorized as DPD deficient.[38]

Screening for DPD in the Clinical Setting

While certain diagnostic tests have been created by scientists, the risk of fluoropyrimidine-induced toxicity is a significant drawback; the genomic viewpoint appears to be preferable for indirect DPD evaluation.

The following procedure can opt for the patients who take 5-fluorouracil for their chemotherapy:

For patients who have been never treated with 5-fluorouracil drugs

- Initially screen for the IVS14+1G>A.
- The occurrence of homozygous or heterozygous polymers appears.
- If homozygous occurs for IVS14+1G>A, Stop the therapy.
- If heterozygous occurs for IVS14+1G>A, go for dose adjustment.
- Suppose results occur negative after initial screening, then treat with standard dose.
- If toxicity occurs, screen for additional polymers, and if suppose toxicity does not occurs then continue the therapy.
- If additional polymers result in heterozygous or homozygous.
- Go for dose adjustment if possible.
- Measure the activity of DPD.
- Finally, go for therapeutic dose adjustment based on DPD activity or clearance value.[40]

For patients who have been treated with 5 fluorouracil drugs and produced toxicity

- Initially screen for multiple variants.
- If homozygous occurs for IVS14+1G>A, do not give the treatment.
- Occurrence of IVS14+1G>A heterozygous OR hetero or homozygous for additional variations.
- Then go for empirical dose adjustment if possible.

- Measure the activity of DPD.
- Finally, go for therapeutic dose adjustment based on DPD activity or clearance value.[40]

Oral 5-Fluorouracil Drugs

Oral 5-fluorouracil drugs are classified into two groups i.e. 5-fluorouracil prodrug and a combination of the 5-fluorouracil drug with a dihydropyrimidine dehydrogenase inhibitor. Prodrugs have increased bioavailability and pharmacokinetic properties than normal 5-FU due to the attachment of the pyrimidine ring with a fluorine atom at position 5.[41]

Since many cancer patients have been reported to produce severe toxicities due to the administration of 5-fluorouracil drugs because of DPD enzyme deficiency, the idea of 5-fluorouracil prodrugs was brought to improve the quality of patient's life. Prodrug differs from their standard drugs by chemical alterations. They are designed to initially be protected from the first-pass metabolism and they get converted to their active 5-fluorouracil drug by the enzymes in the liver or the tumor cells. In tumor cells, the exposure of the drug will be for a longer duration but at a low concentration when compared to the intravenous bolus. This technique will be able to decrease the adverse effect of the drug as well as the overall cost of the treatment.[42]

• Capecitabine

Capecitabine has been authorized by the FDA for use as a first-line therapy in people with metastatic colorectal cancer who want a single-agent fluoropyrimidine treatment. The drug is also licensed for use as a single agent in patients with metastatic breast cancer who have previously failed anthracycline-based treatment and are resistant to both anthracycline and paclitaxel-based chemotherapy. Or for whom even further treatment with anthracyclines is not recommended, as well as in combo with Docetaxel after the collapse of prior Anthracycline-based chemotherapy.[42] Capecitabine (CAP), also known as Xeloda® (N4 -pentyloxycarbonyl-5'-deoxy-5-fluorocytidine), is an anticancer drug that is consumed directly and has a higher possibility of causing malignancies. It was produced in the 1990s by Japanese scientists. To prevent the toxicity of 5'd5-FUrd (5'deoxy-5-fluorouridine), scientists devised an oral formulation. Following oral administration, CAP passes through the gastrointestinal barrier intact and is rapidly and nearly totally absorbed. In a three-stage method involving numerous enzymes, it is transformed to 5-FU. Hepatic carboxyl esterase converts it to 5'-deoxy5-fluorocytidine (5'-dFCR) in the first step. Cytidine deaminase, which is mostly found in liver and tumor tissues, deaminates 5'-dFCR to 5'd5-FUrd. Finally, 5'd5-FUrd is converted to 5-FU by triphosphate enzyme that has greater activity in tumors than in normal tissues. As a result, tumors manufacture an excessive amount of 5-FU, making them less vulnerable to living tissue.[43]

• Tegafur

It's another 5-fluorouracil drugs prodrug. Tegafur (1-(tetrahydro-2-furanyl)-5-fluorouracil) is a lipophilic medication with a 10 hr serum ß-half-life and a volume of distribution similar to total body water. 5-FU is cleaved by hepatic cytochrome P 450-dependent microsomal enzymes, derived from tegafur and gradually released into serum. Hiller is a researcher at the Institute of Organic Synthesis and Tegafur was created in 1967 by the Latvian Academy of Sciences in Riga. Following that, it was put through a series of experimental and clinical trials. Tegafur is commonly used to treat a number of conditions in Europe, United States and Japan. Due to its high lipophilicity and large distribution volume, it is extensively distributed in all tissues, including tumor tissue. As a result, Tegafur activation is potential within the tumor cell. Its most typically used to treat cancer of the intestine.[44,45]

• Carmofur

Carmofur is an anticancer medication that is often used to treat colorectal cancer. It's a fluorouracil derivative that goes by the name 1-hexylcrbomoyl 5-fluorouracil. This medicine is also looking very promising in the fight against cancers of the breast, bladder, and stomach. It is a vigorous inhibitor of acid ceramidase leading to the death of cancer cells.[46] Acid ceramidase inhibition can stop or limit the cancer cells by ceramide-induced apoptosis.[47] The two intracellular subunits of cysteine amidase of human acid ceramidase catalyze the hydrolysis of ceramides into sphingosine and free fatty acids. Ceramides promote cell cycle arrest and death, whereas sphingosine-1 phosphate (S1P) is a direct product of sphingosine that promotes growth, angiogenesis, cell survival, and cell proliferation as these are all important aspects of cell biology. In many cancers such as brain, colorectal, and breast cancers, acid ceramidase levels get increased. Carmofur drug shows its anticancer effect by inhibition of acid ceramidase. Melanoma cells with the Acid ceramidase gene deleted have been demonstrated to prevent the development of cancer-initiating cells.[48]

ENILURACIL - the inhibitor of DPD enzymes [A cautious agent while giving to partial DPD deficient patients]

Eniluracil drug produces decreased anti-tumor effect

by acting as an inhibitor. The 5-fluorouracil drug gets catabolized and inhibited by the eniluracil drug. This drug is an inactivator of DPD enzymes. Eniluracil drug is also known as ethynyl uracil manufactured by Glaxo Wellcome, USA. It is an analog of uracil which will inhibit the DPD enzyme irreversibly thereby increasing the 5-FU drug's bioavailability.[49]

GUIDELINES RECOMMENDING ADJUSTMENT OF THE DOSE

In recap (Table 3), patients starting 5-fluorouracil or capecitabine should follow the recommendation as follows

i. Patients with a gene activity score of 0 should avoid 5-fluorouracil and capecitabine, both systemically and topically.
ii. It is suggested that patients with a gene activity score of 1 or 1.5 begin treatment with half the normal dosage of 5-fluorouracil or capecitabine.
iii. Toxicity can be used to guide further dosage titration. If genotyping findings are unable to PHENO, for example owing to many detected variations, it is recommended that the DPD enzyme activity be determined in order to calculate an initial starting dosage.
iv. For patients starting tegafur, a gene activity score of 0, 1, or 1.5 indicates that tegafur should be avoided; if this is not feasible, start with a low dosage and titrate the dose based on tolerability. If genotyping findings are unable to PHENO, for example owing to many detected variations, it is recommended that the DPD enzyme activity be determined in order to calculate an initial starting dosage.
v. For 5-FU, capecitabine, or tegafur, a gene activity score of 2 does not result in a dose adaptation recommendation.[28]

URIDINE TRIACETATE: ANTIDOTE

Uridine Triacetate was considered as an antidote for fluorouracil toxicity by the FDA (Food and Drug Administration) on May 1, 2009. In 2015 December, uridine triacetate was approved by the FDA to indicate for the patients with life-threatening or early-onset illnesses of 5-fluorouracil or its prodrug toxicities (Table 4). The frequency of uridine triacetate is between three hours till four days after the patient encountered an overdose. Since this drug is lipophilic, it readily crosses the gastrointestinal tract and gets metabolized into uridine and triacetate in which the uridine will treat the toxicity by inhibiting the incorporation of FUTP (fluorouridine triphosphate) into RNA. Uridine triacetate drug comes under the brand name Vistogard, whose active ingredient is uridine triacetate belonging to pyrimidine analog. It's an acetylated uridine prodrug that

Table 3: Fluoropyrimidines: CPIC (Clinical Pharmacogenetics Implementation Consortium) Recommended Dosage.[28]

Phenotype	Effects on Phenotypic Measurements	Activity Score	Dosing Recommendations	Grouping of Recommendations'
DPYD normal metabolizer	Fluoropyrimidine toxicity risk is "normal," and DPD activity is normal.	2	There is no need to alter the dose or course of treatment based on genetics. Use the dose and administration suggested on the package.	STRONG
DPYD intermediate metabolizer	Fluoropyrimidine treatment results in decreased DPD activity (leukocyte DPD activity at 30-70 percent of that of the general population) and an elevated risk for serious or even deadly drug toxicity.	1–1.5	Individuals with homozygous c.[2846A>T];[2846A>T] genotype may require a >50% decrease in beginning dosage. This should be followed by dose titration based on clinical judgment (and preferably therapeutic drug monitoring).	Activity Score 1: STRONG Activity Score 1.5: MODERATE
DPYD poor metabolizer	Complete deficiency of DPD and increased danger of catastrophic or extremely severe drug toxicity while using fluoropyrimidine medications	0.5	Avoid utilizing 5-fluorouracil or regimens based on 5-fluorouracil prodrugs. If other drugs are not deemed to be an appropriate treatment choice based on clinical guidance, 5-fluorouracil should be provided at significantly lower dosages with early medication management.	Activity score 0.5-0: STRONG
		0	Avoid using 5-fluorouracil or regimens based on 5-fluorouracil prodrugs.	

Table 4: Treatment of Severe Fluoropyrimidine Toxicity and Evaluation for Genetic Risk.[53]

1.	FDA Approval	For severe fluoropyrimidine toxicity in 2015
2.	Population	Adult and Pediatric Patients
3.	Indications	Severe Cardiac or Central nervous system toxicities Severe Gastrointestinal toxicity or neutropenia
4.	Side effects	Vomiting (10%), Nausea (5%), Diarrhoea (13%)
5.	Dose	10 g orally every 6 hr for 20 doses
6.	Timing	Within 96 hr of fluoropyrimidine administration

prevents fluorouracil-induced cell death and damage. It comes in a 250g container in an orange-flavored powder and should be taken along with food followed by a glass of water. The side effects associated with the use of uridine triphosphate are noted to produce nausea and vomiting in a small percentage of people because of the bitter taste of the drug. An antiemetic drug can be given to prevent emesis if the patient had. Suppose the patient vomited within the two hours of taking the drug, in such case the drug has to be administered again within 15 min of vomiting. Thus, the Uridine triacetate drug is considered an emergency treatment for adults as well as for pediatric patients undergone fluorouracil toxicity within 96 hr of the fluorouracil administration.[50-53]

Practice Suggestions for DPD Deficiency and Fluorouracil Toxicity

Early treatment of 5-FU-induced toxicity necessitates thorough patient assessment and education. Table 5 indicates the tests that must be conducted for 5-FU toxicity associated with DPD deficiency. Advanced oncologists and oncology nurses should extensively educate patients and their caregivers on the typical and unpleasant side effects of 5-FU-based therapy, as well as when it might be vital to call their healthcare provider right away.[54]

CONCLUSION

Adverse drug reactions should be of major concern in today's modern healthcare system. Many patients die due to the adverse reaction of the drugs in their treatment and it accounts for nearly 100,000 deaths per year. Till today, based on prior data, people with DPD activity less than 70% of that seen in the general population may be more likely to acquire severe 5-FU-related adverse effects. Likewise, it has been found that the toxicity experienced by individuals with severe DPD deficit seems to be much

Table 5: Tests that are conducted for 5-FU toxicity associated with DPD deficiency.[54]

Sl. No	Tests	Description
1.	5-FU Toxicity	Detects the targeted mutations in the DPYD and TYMS gene.
2.	DPD 5-FU Toxicity	Detects the wild type or mutant type IVS14+1G>A mutation.
3.	DPYD Gene Mutation Analysis	Detects the IVS14+1G>A mutation.
4.	DPD enzyme Assay	Tests the IVS14+1G>A mutation.
5.	Theraguide testing	Comprehensive test that offers a full sequencing of DPYD as well as the analysis of the TYMS gene.

greater than that experienced by people with moderate DPD deficiency. Furthermore, according to a recent population survey, 31 to 34% of patients treated with 5-FU had dose-limiting toxicity, and 40% to 50% of individuals experiencing 5-FU toxicity of grade 3 or 4 that were found to be substantially or severely DPD deficient. Diagnosis of DPD deficiency for the cancer patient is very important before initiating the chemotherapy. Hence cancer patients who have DPD deficiency should undergo an alternative regimen that includes non-fluoropyrimidine substances like Irinotecan, Oxaliplatin, and Raltitrexed which can be safely given to cancer patient having DPD deficiency. All cancer patients must undergo a test for the 4 most common genetic DPYD variant before starting the treatment with drugs containing 5-fluorouracil and the variants are DPYD*2A, DPYD*13, C.2846A>T polymorphism and B3 haplotype. It is possible to conduct a clinical trial to compare toxicity rates following dosage modification to detect the DPD deficiency. Since DPD deficiency has treatment only for the symptoms, there are some options available for managing patient toxicities after the administration of the 5-fluorouracil drug in DPD deficient patients. The first and most obvious action would be to stop the further administration of 5-fluorouracil or related drugs followed by supportive care. Dehydration and hypotension may be treated with fluid and electrolyte support. In case of severe drug toxicities, the elimination of the unreacted drug would be advantageous. This can be done by opting hemodialysis or hemoperfusion method. Administration of pyrimidine nucleoside quickly after 5-fluorouracil administration can overcome the block in thymidylate synthase by the 5-FU nucleotide fluorodeoxyuridine monophosphate (FdUMP). If an overdose results in a life-threatening condition after 96 hr of drug administration, uridine triacetate antidote can be given.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

DPD: Dihydropyrimidine dehydrogenase; **EMA:** European Medicine Agency; **UPLC:** Ultra Performance Liquid Chromatography; **UV:** Ultraviolet; **FDA:** Food and Drug Administration; **RNA:** Ribonucleic acid; **DNA:** Deoxyribonucleic acid; **DPYD:** Gene encoding DPD; **PBMCs:** Peripheral Blood Mononuclear cells; **SP:** Specificity protein; **WHO:** World Health Organization; **GSTs:** Glutathione S-transferases; **TPMT:** Thiopurine S-Methyltransferase; **FUTP:** Fluorouridine Triphosphate; **FDUTP:** Fluorodeoxyuridine triphosphate; **TS:** Thymidylate synthase; **FDUMP:** Fluorodeoxyuridine monophosphate; **DHFU:** Dihydro Fluorouracil; **FBAL:** Fluoro Beta Alanine; **NCI CT CAE:** National Cancer Institute Common Terminology Criteria for Adverse Events; **SNP:** Single Nucleotide Polymorphism; **DHPLC:** Denaturing high resolution chromatography; **dsDNA:** Anti-double stranded deoxyribonucleic acid; **AS:** Activity Score; **TDM:** Therapeutic drug monitoring; **CAP:** Capecitabine; **5'd5-FUrd:** 5'deoxy 5-fluorouridne; **5'dFCR:** 5'deoxy 5-fluorocytidine; **S1P:** Sphingosine-1 phosphate; **DOB:** Delta over base **CPIC:** Clinical Pharmacogenetics Implementation Consortium.

SUMMARY

Dihydropyrimidine dehydrogenase deficiency is an autosomal recessive disorder. DPD enzyme is responsible for the metabolism of the 5-fluorouracil drug. Fluorouracil drug is an anticancer drug for solid cancer tumors and dihydropyrimidine dehydrogenase enzyme is required for the metabolism of the 5-fluorouracil drug. Patients having DPD deficiency, when get administered with 5-fluorouracil drug, leads to drug toxicity due to increased concentration of drug in the body. Symptoms of DPD deficiency include seizures, intellectual disability, vomiting, mucositis, hyperuricemia, etc. By determining the DPYD genotyping and DPYD phenotyping, it will provide the best strategy to know the DPD deficiency in patients. To prevent life-threatening issues, EMA (European Medicine Agency) has suggested that patients should be treated for DPD enzyme deficiency prior to the administration of the 5-fluorouracil drug. The level of dehydrogenase markers is also affected by the presence of food i.e., fasting and in a non-fasting state. Prodrugs of the 5-fluorouracil drug are Tegafur and Capecitabine. In the clinical setting, genomic approaches appear to be a superior indirect assessment for screening DPD deficiency. Other than this there are several diagnostic tests such as Uracil oral loading dose, Uracil Breath test, Rapid UPLC-UV method, and DPD activity in mononuclear cells from the peripheral circulation. On May 1, 2009, FDA has considered Uridine Triacetate as an antidote for patients having early-onset or severe life-threatening toxicities. Hence early treatment of 5-FU-induced toxicity necessitates thorough patient assessment and education.

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