Prevalence of dihydropyridine dehydrogenase (DPD) deficiency in cancer patients in Senegal

Abstract

Introduction:

Cancers are a real public health problem. Their prevalence varies greatly among the world population and many therapeutic strategies, such as chemotherapy, have been put in place and most chemotherapy protocols are based on products containing fluoropyrimidines. However, the use of the suggested products can cause side effects in patients who are deficient in dihydropyrimidine dehydrogenase enzyme, which plays an important role in the metabolism of fluoropyrimidine products. Our study was conducted in this context to determine the activity of this enzyme in a population with cancer.

Methods:

This is a prospective study that involved 103 cancer patients. Blood samples were taken on EDTA tubes and uracil (U) and dihydrouracil (UH2) were measured using HPLC.

Results:

In our study population, women were the most affected, representing 83% of patients and a sex ratio of 0.19. 47% of women had breast cancer, 25% had cervical cancer, 6% had ovarian cancer, 3% had rectal cancer, and 1% had other types of cancers such as vulvar, bladder, stomach, pelvic colon, chest, throat, and cheek/eye and cavirum. For the UH2/U ratio, the mean value was 12.6 ± 0.48 with a standard deviation of 4.91 ± 0.95. DPD activity was normal in 33% of our patients, while 66.9% had a partial deficit and 0.1% had a total deficit. The 40 to 50 and 50 to 60 age groups had high deficiency in this enzyme (21.3% and 20.3% respectively), with a total deficiency observed specifically in the 40 to 50 age group.

Conclusion

The results of our study highlighted the importance of screening for DPD deficiency prior to fluoropyrimidine administration. Screening makes it possible to identify patients at risk and to adapt chemotherapy doses, accordingly, thus reducing the incidence of severe and potentially fatal toxicities.

Keywords: Cancer, Dihyropyrimidine dehydrogenase, fluoropirimdines

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Introduction

Cancers are a set of pathologies of various forms and consequences. Their prevalence varies greatly in the world population. They are a major cause of death, with around 10 million deaths in 2020, or almost one in ten deaths [1]. To address this public health issue, many therapeutic strategies such as chemotherapy have been put in place over time. Chemotherapy consists of administering one or more agents to fight tumor cells. It is entirely adapted to each patient, depending on the characteristics of the tumor to be treated [2]. Within that process, fluoropyrimidines (5-fluorouracil, capecitabine) are the most widely used molecules since they are used in the composition of nearly 60% of protocols and in the treatment of nearly half of cancers: colorectum, esophagus, stomach, breast, upper aerodigestive tract. Like most anticancer agents, these molecules have a narrow therapeutic index and many toxicities, sometimes severe, are reported. These toxic effects are due to overexposure to the drug, linked to a wide inter-individual variability in metabolism [3].

5-FU is eliminated mainly after catabolism, mainly in the liver. Its metabolism depends mainly on the activity of an enzyme that is dihydropyrimidine dehydrogenase (DPD), a major enzyme in catabolism. Indeed, it allows a reduction of the molecule to 5-fluoro-5,6-dihydrouracil (FUH2). It is also responsible for the transformation of natural pyrimidine bases (uracil and thymine) into their dihydrogenated derivatives (dihydrouracil [UH2] and dihydrothymine [TH2]).

Thus, patients with a deficiency in the activity of this enzyme have a risk of overexposure and therefore of acute, early and serious toxicity with fluoropyrimidines [4]. In fact, this DPD deficiency should be screened before the first course for each patient in order to avoid standard-dose induced overdose. This screening can be achieved by determining the activity of the enzyme [5]. It is in this context that we decided to conduct this study with the general objective of the study; To assess DPD activity by assay of uracil and dihydrouracil in cancer patients.

Methodology

Collection of epidemiological data

For each patient, the surname, first name, date of birth and date of last chemotherapy were noted for those who have already started. The date of the last chemotherapy helps to avoid interference from the product.

Sample collection

They were carried out just before chemotherapy or a few days after the last session (to avoid interference from the product); between 8 a.m. and 12 p.m., on EDTA tube. All samples were centrifuged at 4000 revolutions per minute for 8 minutes.

Dosage

It was performed after the extraction of U and UH2, performed after the preparation of the stock solutions (calibration solutions, internal standard solution). Then the U and UH2 solutions were qualified before using them to perform controls or ranges. Each of these solutions is injected 3 times consecutively and the results are recorded in the qualification tables.

The prepared analysis ranges were injected into the high-performance liquid chromatography (HPLC) device. The preparation of the range of analysis was carried out following the following steps:

- Prepare the Bench
- Distribute samples and controls
- Add 50 µL of UH2C13 ammonium sulfate to each tube.
- Vortex the tubes for one minute to dissolve the ammonium sulfate.
- Under a fume hood, add 2.5 mL of extraction solvent to each tube and cap for 12 minutes.
- Shake for 10 minutes on the rotary stirrers and centrifuge the tubes at 4000 rpm for 15 minutes at 4°C.
- recover 2 mL of the supernatant in the new glass tubes and evaporate the supernatant to recover the dry extract
- After evaporation, take the dry extract back with 150 µl of solvent.
- transfer the entire sample volume to other numbered tubes, then centrifuge at 13000 rpm for 10 minutes at 4°C.
- Then move on to the plate setting step

Technical validation of patient outcomes

It was carried out according to the following measurement limits

- For uracil: 2.5 to 200 ng/ml with a reference value < 16 ng/ml
- For dihydrouracil: 5 to 400 ng/ml with a UH2 reference value > 10 ng/ml

Interpretation of uracil (U) concentration

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- U < 16 ng/mL: absence of DPD deficiency
- 16 < U <150 ng/mL: presence of partial DPD deficiency
- U >150 ng/mL: presence of a complete DPD deficiency

Interpretation of the complementary analysis of the UH2/U ratio:

- UH2/U >13: no DPD deficit
- 1< UH2/U <10: presence of a partial DPD deficiency
- UH2/U < 1 presence of complete DPD deficiency

Interpretation of Phenotyping Test Results

- If U <16 ng/ml and UH2/U>13: the phenotyping test is in favor of normal DPD activity
- If 16 ng/ml < U< 50 ng/ml and 10< UH2/U<13: the phenotyping test is in favor of a partial DPD deficiency.

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Results

Our study showed that the most affected age group was 40 to 50 years old, with 30 patients, representing 27% of cases. The mean age of patients was 49.77 years ±2.46 years.

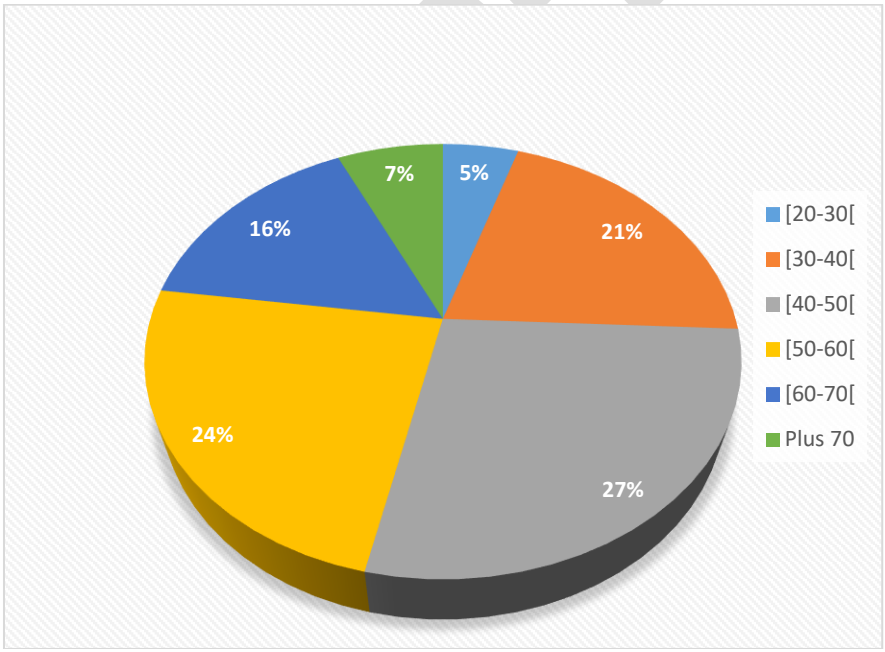

Figure 1: Age distribution of the population

Regarding the distribution according to sex, out of 103 patients, 86 were females and 17 were males, (83%) and (17%) respectively with a ratio of 0.19

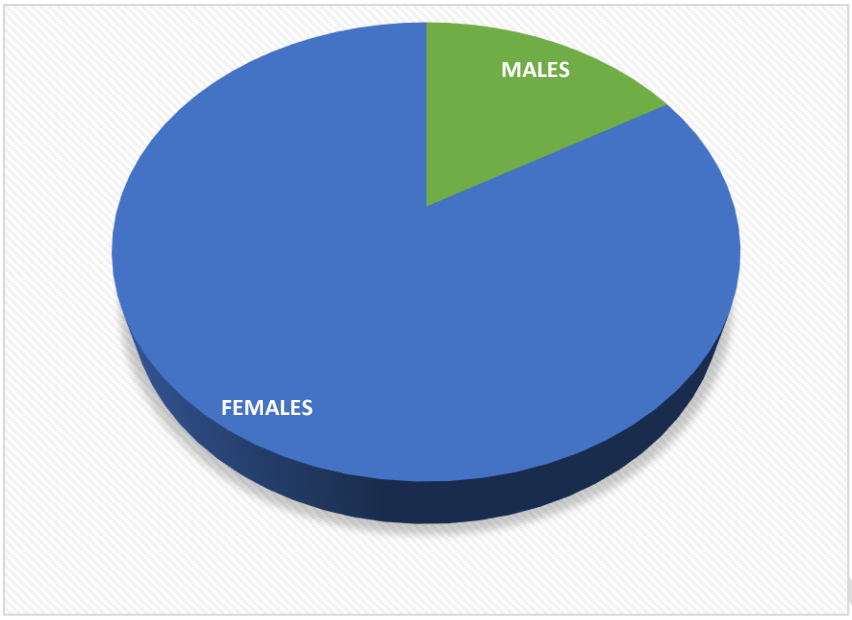

Figure 2: Gender distribution of the population

Our sample was mainly composed of female patients (83%). 47% had breast cancer, 25% had cervical cancer, 6% had ovarian cancer, 3% had rectal cancer, and 1% had various other cancers such as bladder, stomach, vulva, pelvic colon, chest, throat, cheek/eye, and cavirum

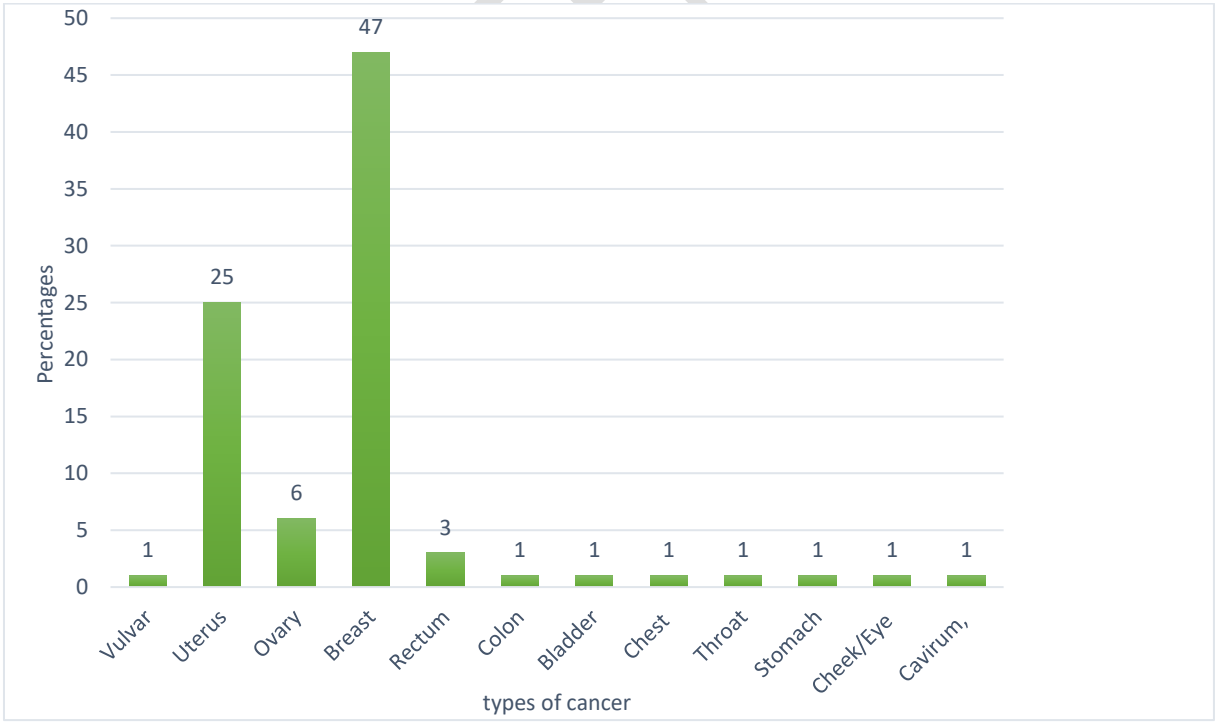

Figure 3: Distribution of cancer types in the female population

Laboratory data showed that, on average, the measured uracil concentration in patients was 17.44 ± 1.65 ng/ml with a standard deviation of 16.64 ± 3.23 ng/ml. For dihydrouracil, the mean

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measured in these patients was 177.95 ± 5.62 ng/ml, with a standard deviation of 57.75 ± 11.01 ng/ml. And for the ratio of dihydrouracil to uracil, we found a mean of 12.6 ± 0.48 with a standard deviation of 4.91 ± 0.95.

Table I: Mean and standard deviation of phenotypic parameters

DPD Phenotyping	Average ± Standard Deviation
URACIL	17.44 ± 1.65 ng/ml
UH2	177.95 ± 5.62 ng/ml
UH2/U	12.6 ± 0.48

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The DPD status of the study population indicates that 34 patients had no DPD deficiency, 68 patients had partial deficiency and 1 patient with total deficiency.

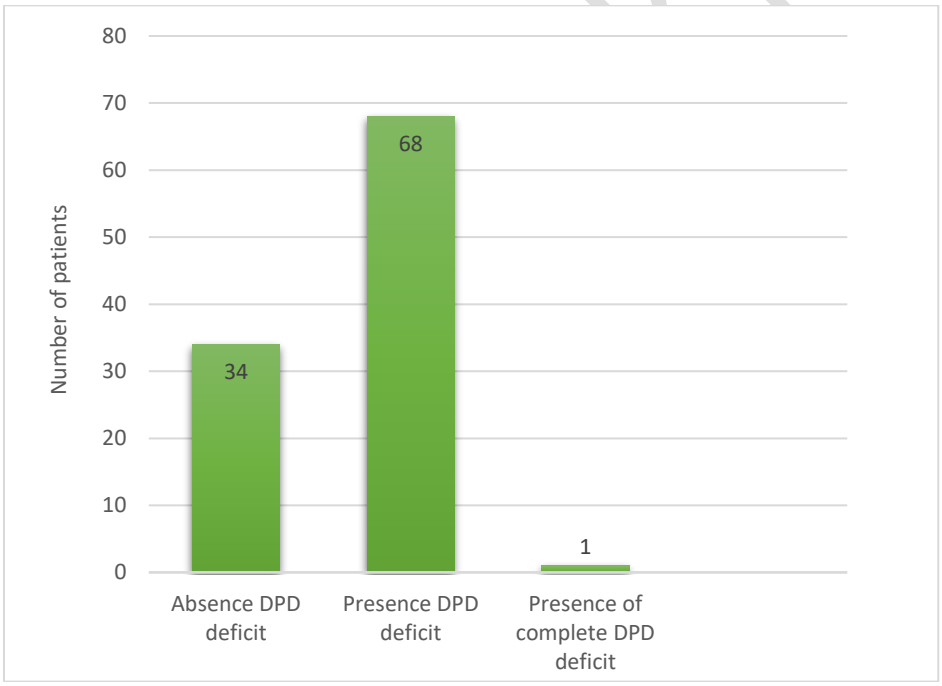

Figure 4 : DPD status of the study population

Following this DPD status, we found that in the male group, 4 patients had no DPD deficiency, and 13 patients had a partial deficiency. On the other hand, for women, 30 patients had no deficit, 55 patients had a partial deficit, and 1 patient had a total deficit.

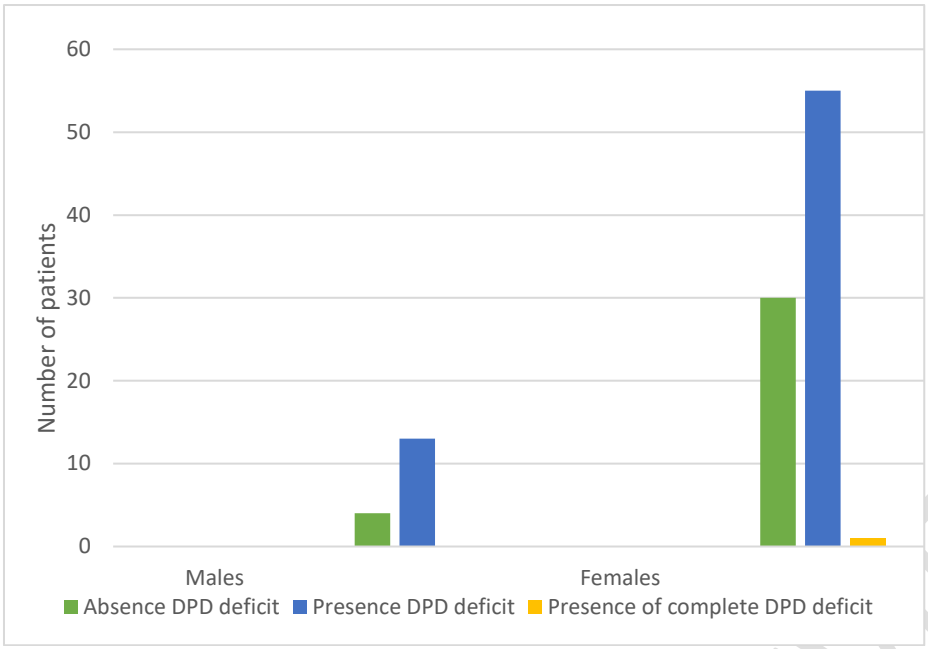

Figure 5 : DPD status by gender

Based on age, the age groups of 40 to 50 and 50 to 60 showed a very high presence of DPD deficit, with proportions of 21.3% and 20.3% respectively. In addition, a total deficit was observed specifically in the 40 to 50 age group.

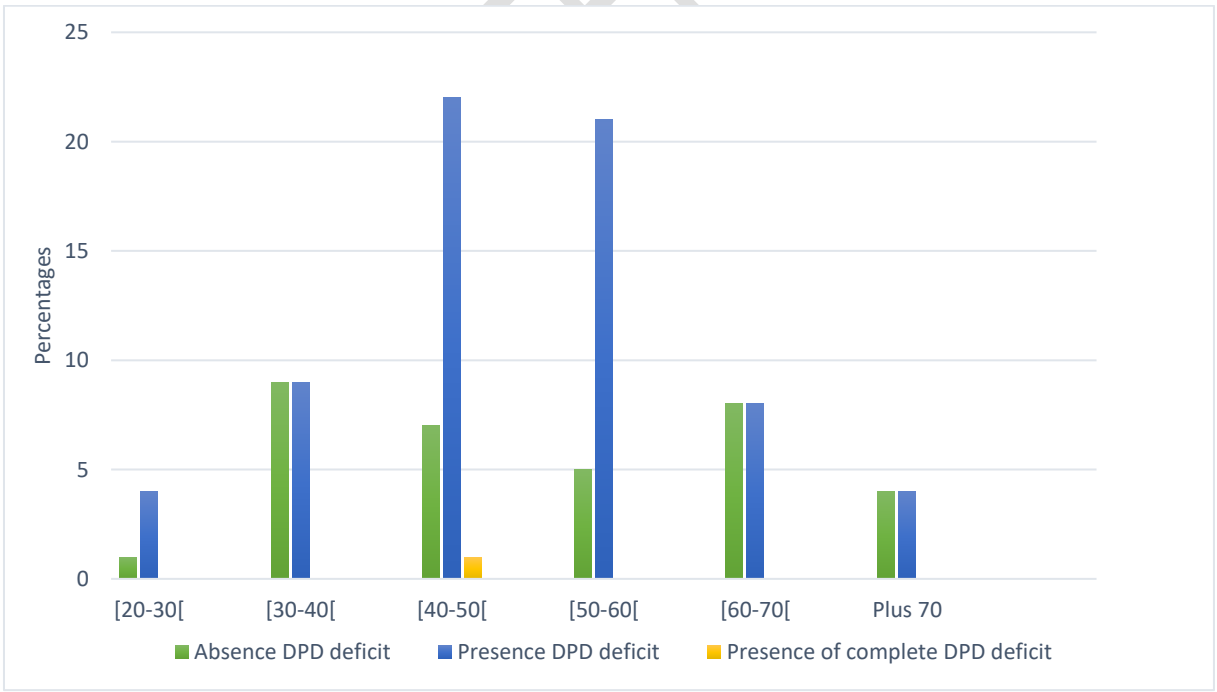

Figure 6 : DPD status by age

Discussion

DPD is the limiting enzyme for the metabolism of fluoropyrimidines. Its deficiency may lead to higher exposure to 5-FU and its cytotoxic metabolites, hence the interest of this study. The main objective was to determine the activity of DPD in patients with cancer. The study involved 103 patients, treated with fluoropyrimidine-based chemotherapy.
In our study population, women were the most affected, with 83% of patients and a sex ratio of 0.19. These results are close to those of the Global Cancer Observatory [6] which revealed a female predominance with a percentage of 64% in cancer patients. This result is also comparable to the one of Sidibe in Mali [7] and is similar to the data reported by some authors on the epidemiology of cancers in sub-Saharan Africa [8]. Hence the importance of targeting screening and prevention strategies specifically at the female population, which seems to be more affected by cancer.
Moreover, in this female population, 47% were affected by breast cancer, 25% by cervical cancer, 6% by ovarian cancer, 3% by rectal cancer and 1% for other types of cancers such as vulvar, bladder, stomach, pelvic colon, chest, throat, cheek/eye and cavirum. Similar information was found in the Globocan (2008) which indicates that the most common cancers in women are breast cancer (24.5%) and cervical cancer (21.3%) [9]. The same results are reported by other authors sub-Saharan Africa [10]. Similarly, the Caroline Calderon study carried out at the University of Limoges showed that the most common female cancer is breast cancer [11]. In the same perspective, studies carried out in Togo and Mali in 2009 showed proportions of 12.38% (N=210) and 13.24% (N=536) respectively [12] [13].

For the UH2/U ratio, the mean value was 12.6 ± 0.48 with a standard deviation of 4.91 ± 0.95. The lowest and highest values of this ratio were 0.2 and 28.1, respectively. Indeed, the mean uracil (U) was 17.44 ± 1.65 ng/ml with a standard deviation of 16.64 ± 3.23 ng/ml. The lowest and highest values for uracil were 5.8 ng/mL and 165.79 ng/mL. Compared to the results of Caroline Calderon, the mean UH2/U in the patients was 10.70 +/- 4.60 with the lowest value 0.74 and the highest value 24.61. For the same study, the mean uracil was 12.81 +/- 7.81 ng/mL, with the lowest value being 3.87 ng/mL and the maximum value being 62.38 ng/ml [11].
DPD activity was normal in 33% of our patients, while 66.9% had a partial deficit and 0.1% a total deficit. These results are close to those of M. R. Johnson and R. B. Diasi. In 103 cancer patients treated with 5-FU; 43% had a partial deficiency, compared to 57% with normal enzyme activity [14]. The study by Launay et al. showed how the DPD screening test, combined with an adapted dosage, could reduce the incidence of toxicities while maintaining optimal efficacy in patients treated with 5-FU [5].
According to our results, the percentage of patients with complete DPD deficiency was low. Several studies have shown that it is a rare event. Although the determination of DPD activity before treatment cannot be used as an indicator to adjust the dose strategy of 5-FU, the identification of a severe deficiency could justify starting treatment with a significantly reduced dose of 5-FU, or even using a therapeutic alternative [15].
Regarding the DPD deficit by sex, we found that four men have no DPD deficiency, while 13 have a partial DPD deficiency. On the other hand, for women, 30 have no deficit, 55 have a partial deficit and 1 have a total deficit. These results are close to those of SOHN et al. who reported a significant difference between the sexes in DPD activity in a Korean population and a higher average activity than that observed in the French and American population [16]. Hence the importance of considering inter-individual differences and geographical factors in the evaluation of DPD activity.
The present study shows that the groups of 40 to 50 and 50 to 60 age groups had high percentages of deficiency in this enzyme (21.3% and 20.3% respectively), with a total deficiency observed specifically in the 40 to 50 age group. Previous research such as the one carried out by Lu et al. and Etienne et al. reported variations in enzyme activity, depending on

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age, with a significant difference observed in people over 65 years of age compared to younger groups [17] [18]. This explains the importance of also taking age into account in DPD activity before the administration of 5-FU therapies.

Conclusion

The results of our study highlighted the importance of screening for DPD deficiency prior to the administration of PF. Screening makes it possible to identify patients at risk and to adapt chemotherapy, accordingly, thus reducing the incidence of severe and potentially fatal toxicities. The implementation of these screening tests before treatment could constitute a significant advance in the management of cancer patients, allowing for the personalization of chemotherapy doses and a reduction in the risk of toxicity.

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