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Genetic Polymorphisms of DPYD in Patients with Breast Cancer on Capecitabine Therapy

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Abstract

Objectives: Breast cancer is the primary cause of death in Iraqi women aged 30–54 years. The study examined the relationship between (G > A) (rs3918290) and (rs55886062, T > G) DPYD gene polymorphisms, their haplotypes, and capecitabine serum concentrations in postmenopausal Iraqi women with breast cancer breast cancer in postmenopausal women during the capecitabine chemotherapy.

Methods: The study included 200 women: 100 apparently health (45–75 years old) and 100 with breast cancer (40–70 years old). This study, conducted between July and October 2022 at the oncology center at Imam al-Hussain medical city in Kerbala, Iraq, plasma levels of Capacetabine and 5FU were measured in breast cancer patients who had been taking capecitabine for at least three months. All participants gave informed consent.

Results: Capecitabine, and 5FU concentrations in breast cancer patients differed significantly. As the results showed, Capecitabine, and 5FU had a significantly higher concentration of them in patients with the TT allele than in those with the CC and CT alleles for the polymorphism (IVS14 + 1G > A) (rs3918290) and in patients with DPYD*13 (rs55886062) with the CC allele rather than the AA and AC alleles. Mutant allele carriers had increased Capecitabine concentrations ($P < 0.001$).

Conclusion: Ca15.3, serum calcium, and estradiol all exist in bodily serum, making them a potentially useful novel diagnostic biomarker for patients with breast cancer due to their high levels of stability, as well as the biological properties of tumors, such as serum calcium and estrogen.

Keywords: Ca15.3, breast neoplasms, estradiol, capecitabine, polymorphism

Introduction

Breast cancer is the most common cancer among women in developed countries, accounting for 23% of all cancers, and represents the most important location of cancer in women between 20 and 79 years of age. In men, breast cancer can also be present, although it only represents 1% of all diagnosed breast cancers.[1] In Iraq, it forms 22.3% of all malignant tumor and 37% of the registered female cancers with a sharp increase in the incidence of this tumor in younger age group.[2] The number of newly diagnosed female breast cancer (FBC) cases increased from 870.2 thousand to 1937.6 thousand, with the age-standardized incidence rate significantly increased from 39.2/100,000 to 45.9/100,000.[3]

Breast cancer, is a complex disease caused by common changes in the population in a number of genes, in combination with environmental factors. The identification and characterization of these common changes have been studies many years ago. They are a case-control association studies design with a selection of 33 candidate genes, 19 of them involved in DNA repair functions, and 14 genes with functions in the cell cycle control, genotyping a total of 169 SNPs in 547 cases of breast cancer.[4]

Chemotherapy in advancement of chemotherapy may be recommended if the potential benefit outweighs the risk after a thorough evaluation of the patient's health (age, menstrual status, blood test results, vital organs' function, comorbidities, etc.), tumor characteristics (histological type, tumor grade, lymph node status, HER2 and hormone receptor status, lymphovascular invation), and potential treatment strategies.[5]

Capecitabine is a one-of-a-kind treatment that is specific to the S phase of the cell cycle. It is an antimetabolic fluoropyrimidine deoxynucleoside carbamate and is administered orally.[6] The most probable place for the thymidine phosphorylase (dThdPase)-catalyzed conversion of capecitabine into 5-fluorouracil (5-FU) to take place in vivo is in tissues that are already carrying malignancies.[7] Dihydropyrimidine dehydrogenase is an enzyme that helps break down uracil and thymine when they are no longer needed in the cell, and it is encoded by the DPYD gene. Pyrimidines like uracil and thymine are a class of nucleotides. The nucleotide base is the fundamental unit of all nucleic acids, including DNA, RNA, and the energy-transfer molecules ATP and GTP.[8] The study aimed to detect the genetic polymorphism of DPYD2 *A (rs3918290) and DPYD *13 (rs55886062) in participated breast cancer women and to investigate the effect of genetic polymorphism on Capecitabine efficiency.

Materials and Methods

The study was approved by medical ethics committee of Iraqi ministry of health the study on postmenopausal women with breast cancer who were treated at Al-Hussein Medical Oncology Hospital and Kerbala from July to December 2022.

Hospital consultants detected the condition using diagnostic criteria. The study included 100 patients (average age 45–74 years) and 100 apparently healthy controls (age 40–70 years). Clinical, mammographic, and histological findings were used to diagnose patients, who received capecitabine chemotherapy after early detection. The serological study used ELISA, while the molecular study used allele specific-PCR. Five ml of peripheral blood was drawn from each patient, then divided into three gel tubes for serology and two ml remaining

of serum was saved in EDTA tubes for genotyping by detection of genetic polymorphism of DPYD2 *A (rs3918290) and DPYD *13 (rs55886062) in postmenopausal breast cancer women.

Inclusion Criteria

Postmenopusal women > 45 years, just get only capecitabine chemotherapy in their course of treatment

Exclusion Criteria

Women who started Capecitabine with adjuvant chemotherapy or radiation therapy were excluded. Women taking DPD inducers or inhibitors like 5-fluorouracil, Dihydrofluorouracil, Tegafur, Gimeracil, or ethynyluracil were excluded. The study excluded patients with gastrointestinal diseases or surgery. On the other hand each patient was asked if she had used any drugs that may interfere with Capecitabine metabolism or DPYD dehydrogenase activity during blood sample collection.

Genotyping for DPYD Dehydrogenase Polymorphisms Detection

For single-nucleotide polymorphisms (SNPs) genotyping, allele-specific amplification based on polymerase chain reaction (PCR) has seen widespread used in our study. On the other hand, the isolation of genomic DNA from whole blood that is compatible with PCR is typically used according to manufacture company (Addbio/korea). Primer 3 plus generates template-specific primer pairs, and the specificity testing programme searches for primer-target matches using BLAST [BLAST: Basic Local Alignment Search Tool (nih.gov)] for DPYD polymorphisms. Lyophilized primers were dissolved with a certain volume of nuclease free water according to instruction of manufacture to give concentration of 100 pmol/µl (represent a stock solution) represent the volumes of nuclease free water added to each primer to obtain 100 pmol/µl (Tables 1 and 2).

PCR collection tubes was prepared with a total volume of 20 µl in premix of PCR tubes (AccuPower® PCR PreMix/korea). The reaction components is described as 2 µl of Forward primer, 2 µl of Reverse primer, 4 µl of DNA template and 12 µl of distal water and PCR amplification program is described as initial denaturation at 95˚C for 3 minute 35 cycles consists of denaturation at 95˚C for 30 sec, annealing at 60˚C for 45 sec and extension at 72˚C for 30 sec and final extension at 72˚C for 5 minute.

DNA gel electrophoresis for detected PCR products as following 3 µl of loading buffer and 5 µl of product were loaded on 1.5% agarose gel (1.5 g/100 ml 1X TBE support) and ran at 100 volt for 35 min. Ethidium bromide (0.5 µg/ml) recolored the gel. DNA Bands were photographed on UV trans illuminator and then DNA ladder (100–1500 bp) is used to measure band molecular size.

Table 1. **Primers sequences of DPYD*2A (IVS14 + 1G > A) (rs3918290) genetic polymorphism**

Allele specific	Primer sequence (5'->3')	Product size
Reverse allele C	5-CTAAAGGCTGACTTTCCAGAACCCC-3	411 bp
Reverse allele T	5-CTAAAGGCTGACTTTCCAGAACCCT-3	
Forward Common	5-GATATGCTGCTTCTGCCTCAGGT-3	

Table 2. **Primers sequences of DPYD*13 (rs55886062, 1679T > G) genetic polymorphism**

Allele specific	Primer sequence (5'->3')	Product size
Forward allele T	5-AGCCACCAGCACATCAATGATT-3	400 bp
Forward allele G	5-AGCCACCAGCACATCAATGATG-3	
Forward common	5-TGTTCCGCACCAGCTCTGGAT-3	

Measurement of Drug Concentration in Patient Serum

Sample preparation and HPLC condition for measurement of Capacetabine concentration, the extraction tube received 0.1 mL of human plasma. Mixing 3 mL of ethylacetate/acetonitrile (4:1, v/v) followed. Freeze-centrifuged at 3500 rpm. Then the nitrogen stream evaporated the organic layer in a glass tube. Dissolving the dry residue in 200 uL of 50% MeOH, centrifuging at 3500 rpm, and transferring to an autosampler vial. HPLC injected a 100 uL sample aliquot. 0.1% formic acid: MeOH (45:55 v/v) was the mobile phase. Mobile phase flow was 1.1 mL/min. The detector was UV-Vis at 305 nm and the column was C18-ODS (25 cm × 4.6 mm).[9]

On the other hand, Sample preparation and HPLC condition for measurement of 5 FU concentration, $AgNO_3$ (20%, 600 µL) was added to human serum (1.0 mL) and vortexed for 3 min before standing for 5 min. NaCl (20%, 700 µL) was added and vortexing continued for 3 min. After 12 min at 13,000 rpm, the supernatant (0.5 mL) was diluted with water to 1 mL in a centrifuge tube and filtered over a 0.22-µm membrane. Analysis employed a German HPLC type SYKAM with UV detector. A 250 centimetre, 4.6 µm C18-ODS column separated. The column temperature remained 25°C. 5 mM KH_2PO_4 solution (pH = 6.0) and methanol (96 : 4) at 1 mL/min were used to determine the standards and samples. 100 µL was injected at 254 nm.[10]

Statistical Analysis

The Statistical Analysis System-SAS (2012) program was used to study the effect of different factor in study parameters. Mean ± standard error, Anova test and *t* test used to significant compare between means in this study. Alleles genotyping were presented as a percentage frequencies, and significant differences between their distributions in breast cancer patients and controls, were assessed by two-tailed Fisher's exact probability (P) test.

Results

The studied population included 100 female patients with breast cancer. Participants' average age at study entry was 55.36 ± 10.85 years (range: 45–65). Eighty six percent were married and only (14%) single. There was a 62% disparity between the women who had a family history of Breast Cancer

and those who didn't (38%) among those who were diagnosed. Cancer patients who had the disease on their left sides numbered 33%, while those on their right sides numbered 67%. It is also recorded that 94% of patients have already had surgery, 79% of patients already have radiation therapy, and 91% of patients have had chemotherapy (Table 3).

Results of Amplification Reaction "DPYD Polymorphism" (rs3918290)

The amplification of SNPs of DPYD gene: rs3918290 was shown in Figure 1. The presence of PCR bands with identity sizes in the agarose gel indicated the genotype of the samples as positive result. Each reaction in different SNPs and genotypes are shown in detail in Table 4. The PCR amplifications, fragment size 400 bp indicated that patient have specific alleles, it was required the use of two separate tubes for the amplification of wild-type and variant-type allele.

The frequency and percentage of rs3918290 genotype that detected in the breast cancer patients are shown in Table 4. The most frequent genotype in 100 breast cancer patients recruited in this study was the Wild type (CC) with frequency and percentage 58 and 58% respectively, while the heterozygote type (CT) represent the lowest frequent type with frequency and percentage of 28 and 28% respectively. The mutation type of rs3918290, which carry TT genotype have been identified in frequency and percentage of 14 and 14% respectively.

Results of Amplification Reaction "DPYD Polymorphism" (rs55886062)

The amplification of SNPs of DPYD gene: rs55886062 was shown in in Figure 2, The presence of PCR bands with identity sizes in the agarose gel indicated the genotype of the samples as positive result. Each reaction in different SNPs and genotypes are shown in detail in Table 5. The PCR amplifications, fragment size 410 bp indicated that patient have specific alleles, it was required the use of two separate tubes for the amplification of wild-type and variant-type allele.

The frequency and percentage of rs55886062 genotype that detected in the breast cancer patients are shown in Table 5.

Table 3. **Patients' demographic groups and breast cancer's unique features**

Characters		Percentage
Age (Years)		55.36 ± 10.85
Duration of disease (Years)		4.31 ± 1.22
Duration of capecitabine (Years)		3.29 ± 1.95
Family history (%)	Yes	62%
	No	38%
Marital status (%)	Married	86%
	Single	14%
Lymph node involvement (%)	Yes	39%
	No	61%
Breast cancer side (%)	Left breast	33%
	Right breast	67%
History of breast cancer chemotherapy (%)	Yes	92%
	No	8%
History of breast cancer surgery (%)	Yes	90%
	No	10%
History of breast cancer radiotherapy (%)	Yes	88%
	No	12%

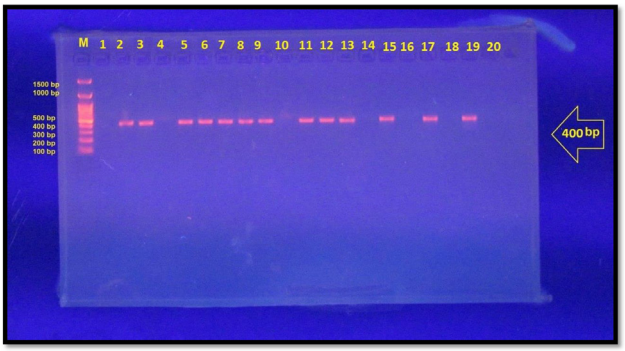

Fig. 1 **PCR amplification of rs3918290 gene showed: Line M: Represented DNA marker (ladder) 100–1500 bp, Line 1,2; : Represented TT genotype (Mutation), Lines 5, 6, 7, 8;11,12: Represented CT genotype (Hetrozygoite) were showed in 400 bp, Lines 3,4; 9,10;13,14; 15,16;17,18;19,20 : Represented CC genotype (wild) were shown in 400 bp.**

Table 4. **Distribution of genotype and allele frequency of DPYD (rs3918290) polymorphism**

DPYD (rs3918290)	Genotype	Patients $n = 100$ n (%)	(X^2)	HWE
Genotype frequency	CC	58 (58%)	(9.3364)	0.001
	C/T	28 (28%)		
	TT	14 (14%)		
Allele frequency	C	72 (72%)	(13.5)	0.001
	T	28 (28%)		

HWE; Hardy weinberg equlibrium.

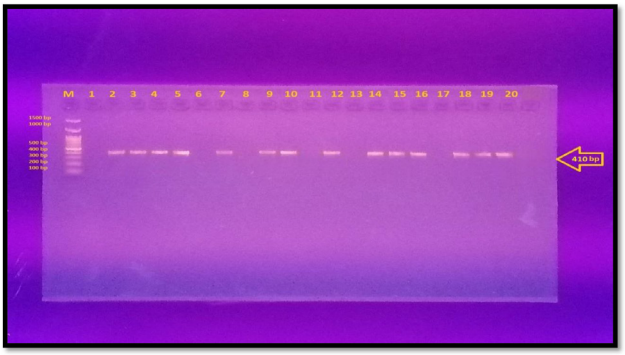

Fig. 2 **PCR amplification of rs55886062 gene showed: Line M: Represented DNA marker (ladder) 100–1500 bp, Line 1,2;11,12;13,14;17,18 : Represented CC genotype (Mutation), Lines 3, 3;9,10;15,16;19,20: Represented CT genotype (Hetrozygoite) were showed in 410 bp, Lines 5,6;7,8 : Represented AA genotype (wild) were shown in 410 bp.**

The most frequent genotype in 100 breast cancer patients recruited in this study was the mutant type (CC) with frequency and percentage 7 and 7% respectively, while the heterozygote type (CA) represent the lowest frequent type with frequency and percentage of 40 and 40% respectively. The wild type of rs55886062, which carry AA genotype have been identified in frequency and percentage of 53 and 53% respectively.

Association between Capacetabine and 5FU Concentration and DPYD Genotype (rs3918290) in Patient Postmenopausal Women have Breast Cancer

Table 6 showed that there were clear significant differences in each of the following concentrations of Capecitabine and 5FU in women with breast cancer, As the results recorded a significant higher concentration of them was recorded in each of Capecitabine and 5FU in patients with the TT allele rather than in patients with the CC and CT alleles in patient with DPYD (rs3918290) characteristics,

Association between Capacetabine and 5FU Concentration and DPYD Genotype (rs55886062) in Patient Postmenopausal Women have Breast Cancer

Table 7 showed that there were clear significant differences in each of the following concentrations of Capecitabine and 5FU in women with breast cancer, As the results recorded a significant higher concentration of them was recorded in each of Capecitabine and 5FU in patients with the CC allele rather than in patients with the AA and AC alleles in patient with DPYD*13 (rs55886062) characteristics.

Table 5. **Shows the genetic basis of rs55886062 gene polymorphisms**

DPYD (rs55886062)	Genotype	Patients *n* = 100 *n (%)*	(X^2) HWE	HWE
Genotype Frequency	AA	53 (53%)	**(9.3364)**	0.001
	C/A	40 (40%)		
	CC	7 (7%)		
Allele Frequency	A	73 (773%)	**(13.5)** 0.001	0.001
	C	27 (27%)		

Discussion

Capecitabine is an anti metabolite, a class of chemotherapeutic agent. Capecitabine is converted in the body to fluorouracil, which is used in a variety of chemotherapy treatments. The process of DNA replication and repair is halted, making and repairing DNA is essential for cancer cell proliferation.[11] Chemotherapy with the oral fluorouracil prodrug capecitabine has been found to be efficacious for breast cancer, yet, some of studies was usefulness in treating breast tumors is still up for debate. After anthracycline and taxane failure, capecitabine is typically used as a second-line chemotherapy option for patients with metastatic breast cancer.[12]

One hundred breast cancer patients were included in the study. 55.36 ± 10.85 was the average age of participants, 86% was married and 14% were all single people among those who were diagnosed with breast cancer, the percentage of women who reported a family history of the disease was 62%.

Women with a family history of breast cancer have a higher risk of developing the disease, as shown by several studies employing a wide variety of approaches to study. However, the severity of this risk varies not just by the individual's age but also by the specifics of their family history, the type of related involved, the age at which the related acquired breast cancer, and the number of relative influenced.[13]

100 female breast cancer patients were studied. At the start of the trial, the average age of the participants was 55.36 ± 10.85 years (range: 45–65). When breast cancer is found in the lymph nodes, it means the disease has spread from the main tumour and is at least stage two. Lymph nodes are an important part of the staging process and help determine which treatments are most likely to succeed.[14]

Female medical surgery are rising due to indifference and failure to see a professional. Due to the lack of knowledge about mastology services in programmes, a high percentage of patients are unnecessarily referred to mastology services, which creates opportunity problems for patients with breast cancer or benign breast disease who need a specialist.[15]

Iraqi Muslim women have socio-ethical, theological, and cultural misconceptions concerning breast cancer and healthy practises.[16]

Table 6. **Mean ± SD of tumor markers with genotype frequencies of DPYD (rs3918290)**

Characteristic DPYD (rs3918290)	Mean ± SD CC *n* = 58	Mean ± SD CT *n* = 28	Mean ± SD TT *n* = 14	*P*
Capecitabine Concentration ng/ml	27.32 ± 6.49	36.87 ± 5.39	42.22 ± 5.19	0.028 S
5FU Concentration ng/ml	259.33 ± 14.36	301.77 ± 21.77	341.53 ± 16.21	0.019 S

Table 7. **Mean ± SD of tumor markers with genotype frequencies of DPYD (rs55886062)**

Characteristic DPYD*13 (rs55886062)	Mean ± SD AA *n* = 53	Mean ± SD AC *n* = 40	Mean ± SD CC *n* = 7	*P*
Capecitabine Concentration ng/ml	26.15 ± 3.95	30.23 ± 4.34	38.31 ± 6.38	0.047 S
5FU Concentration ng/ml	277.4 ± 14.21	283.77 ± 23.98	375.92 ± 18.59	0.038 S

Left and right breast cancer in Iraqi women is studied. Some countries offer novel cancer registry and hospital case series. According to a study, right-sided breast cancer is more common in women. This study found that right-sided breast cancer was more common than left-sided breast cancer in other countries.[17]

Eighty-six percent of women with breast cancer were married, while 14% were single.[18] Despite conflicting findings, no systematic study has been done on the relationship between marital status and this malignant disease.[19]

This study showed how capacitabain chemotherapy was used to diagnose and treat people with breast symptoms or signs. Suspected patients are not diagnosed. It also aims to standardise mammary cancer diagnosis and treatment.

Ninety-one percent of postmenopausal women had received capecitabine chemotherapy for 3.29 ± 1.95 years, and breast cancer cells grow and spread abnormally fast. Chemotherapy targets fast-dividing cells. Chemotherapy medicines can damage healthy cells, especially fast dividing ones.[20]

The DPYD gene SNP rs3918290 is amplified, a positive results for the samples' genotype were shown by the appearance of PCR bands of same sizes on the agarose gel. Table 2 displays the detailed responses for each SNP and genotype combination, patients were found to have particular alleles based on the results of polymerase chain reaction (PCR) amplifications, with fragment sizes of 400 bp.

In this study of 100 breast cancer patients, the mutant type (CC) was shown to be the most common genotype (frequency = 58, 58%), whereas the heterozygote type (CT) was found to be the least common genotype (frequency = 28, 28%). rs3918290 TT genotype mutations have been shown to occur at a frequency and percentage of 14% and 14%, respectively.

The study found these alleles out of hardy Weinberg equilibrium ($P < 0.05$), In addition to mutations and "natural selection, nonrandom mating, genetic drift, and gene flow" are also capable of upsetting the Hardy-Weinberg equilibrium. For instance, mutations introduce novel alleles into a population, which shifts the balance of existing allele frequencies.[21]

SNP rs55886062 in the DPYD gene was observed to be amplified, Positive results for the samples' genotype were shown by the appearance of PCR bands of same sizes on the agarose gel. It was displays the detailed responses for each SNP and genotype combination. Patient had specific alleles as shown by PCR amplifications of fragments of 410 bp in size; amplification of wild-type and variant-type alleles required use of two different tubes.

The mutant type (CC) was the most common genotype among the 100 breast cancer patients recruited for this investigation, with a frequency and percentage of 7 and 7%, respectively, while the heterozygote type (CA) was the least common, with a frequency and percentage of 40 and 40%, respectively. The percentage of the AA genotype found in the rs3918290 wild type are 53%.

There were clear significant differences in each of the following concentrations of Ca15.3, Capecitabine and 5FU in women with breast cancer, As the results recorded a significant higher concentration of them was recorded in each of Ca15.3, Capecitabine and 5FU in patients with the TT allele rather than in patients with the CC and CT alleles in patient with DPYD (rs3918290) characteristics. The result was agreement with (Olivera et al., 2019)[22] who was found The T allele of rs3918290 is assigned no function by CPIC. Patients with the CC genotype may have increased activity of DPYD as compared to patients with the CT or TT genotype. However, conflicting evidence has been reported. Other genetic and clinical factors may also influence catalytic activity of DPYD.

There was displays a statistically significant differences between the following Capecitabine and 5FU concentrations in breast cancer patients: Patients with the CC allele of DPYD*13 (rs55886062) had significantly greater concentrations of Capecitabine and 5FU concentration. The result was agreement with (Lunenburg et al., 2020)[23] who was found The C allele of this variant is assigned a no function allele by CPIC. Patients with the AA genotype and cancer who are treated with fluorouracil, a fluoropyrimidine-based chemotherapy, may have decreased, but not absent, risk of drug toxicity as compared to patients with the AC or CC genotype. However, conflicting evidence has been reported. Other genetic and clinical factors may also influence risk of drug toxicity.

Conclusion

Different frequencies of the (G > A) (rs3918290) and of (rs55886062, T > G) polymorphisms of the DPYD gene homozygous wild, homozygous mutant, and heterozygous genotype were discovered using Allele specififc-PCR in Iraqi breast cancer women who were treated with Capacetabine.

Conflict of Interest

None.

References

1. Parkins, K. M., Dubois, V. P., Hamilton, A. M., Makela, A. V., Ronald, J. A., & Foster, P. J. (2018). Multimodality cellular and molecular imaging of concomitant tumour enhancement in a syngeneic mouse model of breast cancer metastasis. Scientific Reports, 8(1), 8930.
2. Alwan, N. A. (2016). Breast cancer among Iraqi women: Preliminary findings from a regional comparative Breast Cancer Research Project. Journal of Global Oncology, 2(5), 255.
3. Chen, Z., Xu, L., Shi, W., Zeng, F., Zhuo, R., Hao, X., & Fan, P. (2020). Trends of female and male breast cancer incidence at the global, regional, and national levels, 1990–2017. Breast Cancer Research and Treatment, 180, 481–490.
4. Rasool, A., Bunterngchit, C., Tiejian, L., Islam, M. R., Qu, Q., & Jiang, Q. (2022). Improved machine learning-based predictive models for breast cancer diagnosis. International Journal of Environmental Research and Public Health, 19(6), 3211.
5. Moo, T. A., Sanford, R., Dang, C., & Morrow, M. (2018). Overview of breast cancer therapy. PET Clinics, 13(3), 339–354.
6. Alqahtani, S., Alzaidi, R., Alsultan, A., Asiri, A., Asiri, Y., & Alsaleh, K. (2022). Clinical pharmacokinetics of capecitabine and its metabolites in colorectal cancer patients. Saudi Pharmaceutical Journal, 30(5), 527–531.
7. Jurczyk, M., Król, M., Midro, A., Kurnik-Łucka, M., Poniatowski, A., & Gil, K. (2021). Cardiotoxicity of fluoropyrimidines: Epidemiology, mechanisms, diagnosis, and management. Journal of Clinical Medicine, 10(19), 4426.
8. Sharma, V., Gupta, S. K., & Verma, M. (2019). Dihydropyrimidine dehydrogenase in the metabolism of the anticancer drugs. Cancer Chemotherapy and Pharmacology, 84(6), 1157–1166.

9. Piórkowska, E., Kaza, M., Fitatiuk, J., Szlaska, I., Pawiński, T., & Rudzki, P. J. (2014). Rapid and simplified HPLC-UV method with on-line wavelengths switching for determination of capecitabine in human plasma. Die Pharmazie-An International Journal of Pharmaceutical Sciences, 69(7), 500–505.
10. Zhu, L., Shen, G. J., Ding, S. Q., & Hua, X. I. N. (2012). Determination of 5-fluorouracil in 5-fluorouracil injection and human serum by HPLC. Journal of Food and Drug Analysis, 20(4), 15.
11. Murthy, R. K., Loi, S., Okines, A., Paplomata, E., Hamilton, E., Hurvitz, S. A., ... & Winer, E. P. (2020). Tucatinib, trastuzumab, and capecitabine for HER2-positive metastatic breast cancer. New England Journal of Medicine, 382(7), 597–609.
12. Ayala-Aguilera, C. C., Valero, T., Lorente-Macías, Á., Baillache, D. J., Croke, S., & Unciti-Broceta, A. (2021). Small molecule kinase inhibitor drugs (1995–2021): Medical indication, pharmacology, and synthesis. Journal of Medicinal Chemistry, 65(2), 1047–1131.
13. Niehoff, N. M., Nichols, H. B., Zhao, S., White, A. J., & Sandler, D. P. (2019). Adult physical activity and breast cancer risk in women with a family history of breast cancer. Cancer Epidemiology, Biomarkers & Prevention, 28(1), 51–58.
14. Han, L., Zhu, Y., Liu, Z., Yu, T., He, C., Jiang, W., ... & Luo, Y. (2019). Radiomic nomogram for prediction of axillary lymph node metastasis in breast cancer. European Radiology, 29, 3820–3829.
15. Calì Cassi, L., Vanni, G., Petrella, G., Orsaria, P., Pistolese, C., Lo Russo, G., ... & Buonomo, O. (2016). Comparative study of oncoplastic versus non-oncoplastic breast conserving surgery in a group of 211 breast cancer patients. European Review for Medical and Pharmacological Sciences, 20(14), 2950–2954.
16. Moey, S. F., Sowtali, S. N., Mohamad Ismail, M. F., Hashi, A. A., & Che Mohamed, N. (2022). Cultural, Religious and Socio-Ethical Misconceptions among Muslim Women towards Breast Cancer Screening: A Systematic Review. Asian Pacific Journal of Cancer Prevention, 23(12), 3971–3982.
17. Koto, M. Z., Becker, J. H. R., Mokone-Fatunla, D. H., Mundawarara, S., & Bondo, M. (2019). Laterality of breast cancer at Dr George Mukhari academic hospital. South African Journal of Surgery, 57(3), 55–61.
18. Kruk, J. (2012). Self-reported psychological stress and the risk of breast cancer: A case-control study. Stress, 15(2), 162–171.
19. Melchior, M., Goldberg, M., Krieger, N., Kawachi, I., Menvielle, G., Zins, M., & Berkman, L. F. (2005). Occupational class, occupational mobility and cancer incidence among middle-aged men and women: A prospective study of the French GAZEL cohort. Cancer Causes & Control, 16, 515–524.
20. Hassan, M. S. U., Ansari, J., Spooner, D., & Hussain, S. A. (2010). Chemotherapy for breast cancer. Oncology Reports, 24(5), 1121–1131.
21. Hu, N., Si, Y., Yue, J., Sun, T., Wang, X., Jia, Z., ... & Yuan, P. (2021). Anlotinib has good efficacy and low toxicity: A phase II study of anlotinib in pre-treated HER-2 negative metastatic breast cancer. Cancer Biology & Medicine, 18(3), 849.
22. Oliveira, M., Saura, C., Nuciforo, P., Calvo, I., Andersen, J., Passos-Coelho, J. L., ... & Isakoff, S. J. (2019). FAIRLANE, a double-blind placebo-controlled randomized phase II trial of neoadjuvant ipatasertib plus paclitaxel for early triple-negative breast cancer. Annals of Oncology, 30(8), 1289–1297.
23. Lunenburg CA, van der Wouden CH, Nijenhuis M, Crommentuijn-van Rhenen MH, de Boer-Veger NJ, Buunk AM, Houwink EJ, Mulder H, Rongen GA, van Schaik RH, van der Weide J. Dutch Pharmacogenetics Working Group (DPWG) guideline for the gene–drug interaction of DPYD and fluoropyrimidines. European Journal of Human Genetics. 2020 Apr;28(4):508-17.