RESEARCH ARTICLE

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Association of Cytidine Deaminase Polymorphism with Capecitabine Effectiveness in Breast Cancer Patients

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Abstract

Objective: Cancer is caused by abnormal growth and disruption of homeostatic mechanisms. Breast cancer is a major health problem and the second leading cause of death in women. Capecitabine is a prodrug of 5-fluorouracil, which is a non-cytotoxic agent and is used to treat advanced or metastatic breast cancer. Cytidine deaminase (CDA) plays an important role in the activation of capecitabine by acetylating 5-deoxycytidine in the liver, which ultimately leads to the formation of 5-fluorouracil. In this study, we aimed to investigate the relationship between cytidine deaminase polymorphism and capecitabine efficacy in breast cancer patients. **Methods:** One hundred breast cancer patients aged 45 to 75 years were included in this study, all of whom received capecitabine as monotherapy. The serum levels of CA15.3, calcium, and estradiol E2 in breast cancer patients were investigated. In addition, the relationship of these markers with cytidine deaminase polymorphism was investigated in order to show the association of cytidine deaminase polymorphism with capecitabine efficacy in breast cancer patients. **Result:** The findings of this study showed that there is a statistically significant relationship between CDA enzyme polymorphisms (rs2072671 and rs532545) with estradiol and CA15.3 serum levels and a non-significant relationship with calcium level. Furthermore, patients who participated were highly polymorphic and heterozygotes genotypes had the highest frequency in both rs2072671 and rs532545 polymorphisms. **Conclusion:** The results obtained from the present study identified different genetic polymorphisms in the gene encoding the CDA enzyme. Overall, our results clearly showed direct evidence for the association of cytidine deaminase polymorphism with the efficacy of capecitabine in breast cancer patients, which could be useful in reducing side effects and improving drug response to capecitabine.

Keywords: Cytidine deaminase- capecitabine- breast cancer- polymorphism

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Introduction

Breast cancer is a major health problem and the second leading cause of death in women (Wilkinson and Gathani, 2022). Breast cancer is a type of metastatic cancer that can spread to other organs such as the lung, liver, bone, and brain. Causes of breast cancer include genetic, environmental, dietary, lifestyle, and hormonal factors (Łukasiewicz et al., 2021). Capecitabine was specifically designed for oral administration, to deliver 5-fluorouracil (5-FU) to the tumor site and to avoid systemic 5-FU exposure. Oral capecitabine is highly effective for the management of breast and colorectal cancer (Kaklamani and Gradishar, 2003; Shankar et al., 2015). By using a three-step enzymatic cascade, the innovative oral fluoropyrimidine carbamate capecitabine (Xeloda) preferentially produces 5-FU in tumor tissue. The prodrug capecitabine is quickly and completely digested after being absorbed from the gastrointestinal tract in its intact form (Reigner et al., 2003). It undergoes a three-step enzymatic cascade to become 5-FU. It is first converted to 5′-deoxy-5-fluorocytidine (5′-DFCR) by hepatic carboxyl esterase. This is then changed to 5′ deoxy-5-fluorouridine (5′ DFUR) by the enzyme cytidine deaminase. Thymidine phosphorylase (TP) is an enzyme that converts 5′ DFUR into 5-FU in both tumor and healthy tissues (Schellens, 2007; Varma et al., 2019).

The CDA gene, which has four exons, is found on the first pair of chromosomes and codes for the human CDA enzyme (1p36.2-p35). The active site of CDA is composed of four identical subunits, each of which must contain a zinc atom. Although the liver and placenta are where it is mostly produced, mature neutrophils also contain significant amounts of CDA (Cambi et al., 1998). Cytidine and 2-deoxycytidine are physiologically deaminated into uridine and 2-deoxyuridine, 65, respectively, by

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this enzyme. Due to the significant inter-individual variation in CDA's period of action, it is of great interest (Serdjebi et al., 2015) . In terms of CDA, the promoter region mutations CDA c.-451C>T (rs532545) and CDA c.-33delC (rs3215400, also known as c.-943del/insC) have been linked to altered enzyme activity. Those who carry the CDA T-allele c.-451C>T showed increased CDA enzymatic activity and a higher incidence of grade 2 diarrhea. In these patients, the likelihood of grade 3 HFS was similarly elevated. Decreased mRNA expression was caused by CDA c.-33delC, which is closely related to CDA c.-451C>T, and carriers of the C-insertion variant had a lower chance of grade 3 HFS. The enzymatic activity of CDA in serum has been recommended as a reasonable biomarker for the metabolic conversion rate of capecitabine in addition to genotyping analysis of CDA. An ultra-metabolizing condition was linked to higher capecitabine efficacy or extreme toxicity (Carpi et al., 2013; de With et al., 2023).

Serum levels of cancer antigen 15-3 (CA15-3) are monitored periodically in breast cancer patients throughout the course of treatment (Duffy et al., 2010). CA15-3 may be useful in estimating the extent to which breast cancer has spread. An elevated level of CA15-3 would suggest the presence of metastases, particularly bone metastases. Patients with bone metastases may find it helpful to examine CA15-3 as a prognostic factor (Abed et al., 2020). However, no research has linked CA15-3, a tumor marker, to CDA enzyme genetic variation.

Many studies have examined the correlation between rising serum estrogen levels and the onset of breast cancer (Saha Roy and Vadlamudi, 2012). Most human breast cancers begin as estrogen-dependent, suggesting a role for estrogen signaling and the estrogen receptor (ER) in cancer growth. Hormone receptor-positive tumor cells have estrogen or progesterone receptors (Chakravarty et al., 2010). Hypercalcemia is a common consequence of breast cancer and a major source of morbidity and mortality from the disease. Patients with multiple skeletal metastases are especially at risk. However, in a sizeable subset of patients, elevated calcium levels develop even in the bones and joints illness (DeMauro and Wysolmerski, 2005; Erdogan and Cicin, 2014). When serum calcium levels are over 10.5 mg/dl and albumin levels are under 4 g/dl, a condition known as hypercalcemia develops (Hadisuwarno et al., 2021). When chemotherapy is given to patients with hypercalcemia due to breast or lung cancer, the calcium levels decrease, most likely due to a decrease in PTHrP level (Hassan et al., 2012).

The pro-drug capecitabine is activated by 3 enzymatic activities to -DFCR and subsequently -DFUR. Thymidine phosphorylase (TP), the final enzyme in the process of converting thymidine to 5-FU, is specific to tumors and is selectively upregulated by capecitabine. In contrast, 5-FU is inactivated by dihydropyrimidine dehydrogenase (DPYD) (Innocenti et al., 2020). Different polymorphisms (P) of the enzymes involved in the metabolism of capecitabine may be responsible for the varying efficacy and toxicity in patients receiving the drug (Roberto et al., 2017). Overall, in this study, we aimed to investigate the serum levels of CA15.3, calcium, and estradiol E2 in breast cancer patients and the association of CDA polymorphism with capecitabine effectiveness in breast cancer patients.

Materials and Methods

This study was done at Imam Hussein Medical/ Oncology Center in Karbala province. The study is cross cross-sectional observation study that begins from 1 July 2022 to 1 January 2023. The study's protocol was approved by the Pharmacy College's Scientific and Ethical Committee at Karbala University, and all participants signed a consent form indicating that they understood the purpose and nature of the research. One hundred breast cancer patients, ages 45 to 75, were enrolled in this trial, all of whom were receiving capecitabine as monotherapy.

Inclusion criteria

The inclusion criteria involved women aged from 45 to 75 years and with adequate organ function taking capecitabine as monotherapy for treating breast cancer

Exclusion criteria

Exclusion criteria included pregnancy or lactation, serious infection, and women taking capecitabine as combination or adjuvant therapy with another chemotherapy.

Sample collection and analysis

After obtaining permission from the Scientific and Ethical Committee of the Faculty of Pharmacy / Karbala University, 5 ml of blood samples were taken from patients, 2 ml were placed in an EDTA tube for genetic analysis and 3 ml were placed in a gel tube, and then at a speed of 4000 rpm for centrifuged for 10 minutes for biochemical analysis such as calcium, estradiol E2 and tumor marker CA 15.3.

Primer's design

The primers used in this study were designed by Oligo7 software. Table 1-1 shows forward primer and reverse primer sequence used for CDA K27Q genotyping (rs2072671).

Statistical analysis

All experiments were performed in three independent experiments. The data of this research was analyzed with SPSS software and using a t-test. The variable mean value and standard division (SD) value were collected from patients with p-value less than 0.05 which means the data is statically significant.

Results

Table 2 shows the demographic data for the individuals who participated in this study. The age range at breast cancer diagnosis was 45-75 years, with a mean of 55.136; and the body mass index was 26-32; individuals who had a family history of breast cancer were about 62%; while the ratio of married women was 86%, whereas unmarried women were just 14%; And the percentage of women with

Table 1-1. Primer Sequences used for CDA K27Q Genotyping (rs2072671).

Primer	Sequence	Tm (°C)
(A) Forward (common)	5′-CCTTCCAGTAGCGTGGCACCAC-3	71.82
(B)Reverse (K*-specific)	5′GACTGTAGGGGCAGTAGGCTGACTT-3′	68.36

Table 1-2: Primer Sequences Used for CDA rs532545 (451 A>G).

Primer	Sequence	Tm (°C)
(A) Forward (common)	F: GAA GGG CTG AGG CTG AAA	73
(B) Reverse	R: TGG GCT AGG GCA AAG AGA	65.36
(C) Reverse	CTG CAG CTT GTT CAT GCC TCC TGC CT	70

Table 2. The Demographic Data of the Participated Individuals in This Study

Age of patients with breast cancer		Percentage
Marital status	Married	86%
	Single	14%
Family history	Positive	62%
	Negative	38%
location of breast cancer	Left	33%
	Right	67%
Receptor status	Estrogen receptor positive	94%
	Progesterone receptor positive	6%
Lymph node involvement	Yes	39%
	No	61%
Radiation therapy	Yes	88%
	No	12%
Surgery	Yes	90%;
	No	10%
Hand foot syndrome	Yes	53%
	No	47%

left side breast cancer was about 33%, while on the right side it was about 67%. The percentage of patients who had surgery (mastectomy, lumpectomy with or without lymph node removal) was 90%; the percentage of individuals who had estrogen receptor-positive was about 94%, and only 6% were progesterone positive; and 39% of patients in this study had lymph node involvement; The proportion of females who had radiation therapy was 88%, whereas 12% of females did not receive radiation therapy; and the percentage of patients who took chemotherapy was 92%, whereas 8% didn't take chemotherapy, and the percentage of patients suffering from hand-foot syndrome, one of the serious side effects of capecitabine, was 53%.

CA15.3 tumor marker concentration in breast cancer patients with cytidine deaminase enzyme polymorphism

The CA15.3 levels and the cytidine deaminase enzyme polymorphism are shown in Table 3. The majority of patients (around 45%) have tumor marker levels that are higher than the normal range (less than 30 U/mL).

Regarding rs532545, the mutant (GG) has a CA15.3

Table 3. Mean and Standard Division of CA 15.3 for each Group of SNIPS rs532545and rs2072671 Small Letter Alphabetic Represent Correlation between each Genotype.

SNIP	Genotype	Frequency	Mean ±SD	*P value
rs2072671	AA (Homozygote, wild type)	30	93.09± 30.57 d	0.027
	AC (Heterozygote type)	48	65.89± 13.97 b	
	CC (homozygote, mutant type)	22	117.77± 36.41 a	
rs532545	AA (Homozygote, wild type)	40	71.83± 21.22 b	0.046
	AG (Heterozygote type)	48	106.76± 31.33 a	
	GG (homozygote, mutant type)	12	66.08± 19.02 b	

* p value if a-0.05< significant p>0.05 non-significant difference

Table 4. Mean and Standard Division of Estradiol Level for each Group of SNIPS rs532545and rs2072671, Small Letter Alphabetic Represent Correlation between each Genotype

SNIP	Genotype	Frequency Frequency	Mean ±SD	P value
rs2072671	AA (Homozygote, wild type)	30	51.89± 10.42 a	0.057
	AC (Heterozygote type)	48	53.98± 10.96a	
	CC (homozygote, mutant type)	22	41.01± 10.78b	
rs532545	AA (Homozygote, wild type)	40	74.43± 14.86a	0.044
	AG (Heterozygote type)	48	33.08± 3.72b	
	GG (homozygote, mutant type)	12	37.98± 3b	

p value if a-0.05< significant p>0.05 non-significant difference

Table 5. Mean and Standard Division of Calcium Level for each Group of SNIPS rs532545 and rs2072671 and, Small Letter Alphabetic Represent Correlation between each Genotype.

SNIP	Genotype	Frequency	Mean ±SD	P value
rs2072671	AA (Homozygote, wild type)	30	8.75± 0.12 a	0.247
	AC (Heterozygote type)	48	9.11± 0.15 a	
	CC (homozygote, mutant type)	22	8.99± 0.08 a	
rs532545	AA (Homozygote, wild type)	40	9.09± 0.16 a	0.481
	AG (Heterozygote type)	48	8.93± 0.12 a	
	GG (homozygote, mutant type)	12	8.82± 0.1 a	

* p value if a-0.05< significant p>0.05 non-significant difference

Table 6. Percentage and Frequency of Cytidine Deaminase Enzyme SNIP (rs532545) Genotype in Breast Cancer Patients

SNIP	Genotype	Frequency	Percentage
rs532545	AA (Homozygote, wild type)	40	40%
	AG (Heterozygote type)	42	42%
	GG (homozygote, mutant type)	18	18%
	Sum	100	100%

Table 7. The Percentage and Frequency of Cytidine Deaminase Enzyme SNIP (rs2072671) Genotype in BreastCcancer Patients

SNIP	Genotype	Frequency	Percentage
rs2072671	AA (Homozygote, wild type)	30	30%
	AC (Heterozygote type)	48	48%
	CC (homozygote, mutant type)	22	22%
	Sum	100	100%

(mean +/- SD) of (66.08± 19.02), which exceeds the normal range, followed by the heterozygous (AG) with a value of (106.76± 31.33), and the wild type (AA) with a value of (71.83± 21.22), and there is no significance between heterozygous with mutant, heterozygous with homozygous, and mutant with homozygous, respectively.

Concerning rs2072671, the mutant (CC) has a CA15.3 (mean +/- SD) of (117.77± 36.41) which exceeds the normal range, followed by the heterozygous (AC) with a value of (65.89± 13.97) and the wild type (AA) with a value of (93.09± 30.57), and there is no significant difference between the three groups.

Estradiol level in breast cancer patients with cytidine deaminase enzyme polymorphism

Table 4 illustrates the estradiol levels of breast cancer patients. Postmenopausal women often have estradiol levels between 10 and 50 pg/ml.

Specifically, for rs2072671, the mutant (CC) has an elevated estradiol level (mean +/- SD) of (41.01 ± 10.78), the heterozygous (AC) has a value of (53.98 ± 10.96), and the wild type (AA) has a value of (51.89 ± 10.42), with a statistically significant difference between the three groups.

Concerning rs532545, the mutant (GG) has an elevated estradiol level (mean +/- SD) of (37.98 ± 3), the heterozygous (AG) has a value of (33.08 ± 3.72), and the wild type (AA) has a value of (74.43 ± 14.86), with significant differences between the heterozygous and mutant and homozygous genotypes.

Calcium level in breast cancer patients with cytidine deaminase enzyme polymorphism

Table 5 illustrates the calcium levels of this study's breast cancer patients who also possessed a CDA enzyme polymorphism. Postmenopausal women often have estradiol levels between 8.4 - 11.5 mg/dl. Relating to rs532545, the mutant (GG) has a calcium level (mean +/- SD) of (8.82 ± 0.1), followed by the heterozygous (AG) with a value of (8.93 ± 0.12), and the wild type (AA) with a value of (9.09 ± 0.16); there is no significance between heterozygous with mutant, heterozygous with homozygous, and mutant with homozygous.

Regarding rs2072671, the mutant (CC) has a CA15.3 (mean +/- SD) of (8.99± 0.08), followed by the heterozygous (AC) with a value of (8.99± 0.08) and the wild type (AA) with a value of (8.75 ± 0.12), and there is no significance difference between the three groups.

The genetic poly morphism of cytidine deaminase enzyme in breast cancer patients

The genetic diversity of rs532545 (451 A>G) in patients with breast cancer

Table 6 shows the percentage and number of SNIP rs532545 genotypes found in patients with breast cancer on capecitabine. The most common genotype among the 100 breast cancer patients enrolled in this study was

(AG) heterozygous with 42 numbers of patients and 42% percentage; while the wild type was homozygous with frequent numbers of 40 and a percentage of 40%, and the mutant type, which has a lower frequency and percentage, was 18% percentage and frequently 18.

The genetic diversity of rs2072671 in breast cancer patients

Table 7 shows the percentage and number of SNIP rs2072671 genotypes found in the patients with breast cancer on capecitabine. The most common genotype among the 100 breast cancer patients enrolled in this study was (AC) heterozygous, with 48 numbers of patients and 48% percentage, while the wild type was homozygous (AA), with frequent 30 and a percentage of 30%, and the mutant type (CC), which has a lower frequency and percentage, was 22% and frequently 22.

Discussion

The most common form of cancer in women, breast cancer, has many causes, including genetics, family history, age, and lifestyle choices (Abbas et al., 2020; El Haidari et al., 2020). The demographic information of the patients who took part in this investigation is displayed in Table 2. Women in this study had a mean age of 55.136 years. Some women develop breast cancer at a younger age (below 40 years), but this only accounts for a small percentage of the overall incidence. However, because these cases are combined with more aggressive subtypes, it was anticipated that they would be diagnosed at a more advanced stage, which would result in a worse prognosis. This finding may be because early menarche will cause early progression of the breast and thus early exposure to estrogen, which results in an increase in the risk of developing breast cancer. On the other hand, older age at menarche will result in a lower risk of breast cancer in premenopausal females (Azamjah et al., 2019).

A number of studies have found a link between a woman's marital status and her risk of developing breast cancer. Single women and women who had spent their entire lives without marriage had a significantly higher chance of developing breast cancer. On the other hand, a study that investigates the effect of marriage on the stage of cancer at diagnosis shows that single female cancer patients are more likely to develop the disease in more advanced stages than married cancer women (Yuan et al., 2021). Marriage can benefit female patients with gynecological cancer in several ways and improve their prognosis regarding disease progression (Li et al., 2020). The presence of a spouse or life partner in a woman's life has a beneficial effect on lifestyle choices and health behaviors. In addition, marriage reduces risky behaviors and exerts social control over behaviors such as maintaining a healthy diet and exercise routine (Al-Kelabi et al., 2022; Brewer et al., 2017; Hadi et al., 2023; Nindrea et al., 2017).

Based on the findings of this study, breast cancer patients with a positive family history were more likely to develop this disease than those with a negative family history, which is consistent with the research. A positive family history practically doubles a woman's chance of developing breast cancer in situ (Mukama et al., 2020).

Many studies have examined the correlation between rising serum estrogen levels and the onset of breast cancer (Russo and Russo, 2006). Most human breast cancers begin as estrogen-dependent, suggesting a role for estrogen signaling and the estrogen receptor (ER) in cancer growth. Hormone receptor-positive tumor cells have estrogen or progesterone receptors (Soewoto and Agustriani, 2023). Estrogens are thought to play a significant role in encouraging the proliferation of both normal and malignant breast epitheliums. Epidemiological studies have now proved what has been suspected for a long time: that they are breast carcinogens. Three main pathways are thought to be implicated in their carcinogenic effects: stimulation of cellular proliferation via their receptor-mediated hormone action, direct genotoxic effects via boosting mutation rates via a cytochrome P450-mediated metabolic activation, and production of aneuploidy (Stingl, 2011).

In this study estradiol level for patients who received capecitabine was (88.06 ± 15.33) in contrast to another study in Asian countries (Grau et al., 2008) which the mean ± St. Division was 61.51 ± 42.06. In our study, the estradiol level for SNIP rs2072671 for CC (homozygote, mutant type) mean and standard deviation was 41.01±10.78, whereas for AA (homozygote, wild type) it was 51.89±10.42, and for AC (heterozygote type) it was 53.98±10.96. There was no significant difference between them. The estradiol level for homozygote AA was the same as heterozygote AC. However, the mutation type of SNIP rs2072671 enzyme CDA, which is responsible for the conversion of capecitabine to 5-FU, lowers the estradiol level, which is in contrast with the study effect chemotherapy that lowers estradiol level (Grau et al., 2008).

Furthermore, the estradiol level for SNIP rs532545 for GG (homozygote, mutant type) mean and standard deviation was 37.98±3, whereas for AA (homozygote, wild type) it was 74.43±14.86, and for AG (heterozygote type) it was 33.08±3.72. For this SNIP, the estradiol level of homozygote was higher than heterozygote and mutant type. Serum levels of cancer antigen 15-3 (CA15-3) are monitored periodically in breast cancer patients throughout the course of treatment (Fejzić et al., 2015). CA15-3 may be useful in estimating the extent to which breast cancer has spread. An elevated level of CA15-3 would suggest the presence of metastases, particularly bone metastases. Patients with bone metastases may find it helpful to examine CA15-3 as a prognostic factor (Steinauer et al., 2014). However, no research has linked CA15-3, a tumor marker, to CDA enzyme genetic variation.

Higher mean and St. Division were found in this study for the SNP rs2072671 mutant type compared to homozygotes and heterozygotes. All genetic polymorphisms that reduce CDA's ability to convert the prodrug capecitabine into the active drug 5-FU through acetylation in the liver reduce the medication's activity and its effect on tumor markers. According to the findings of the Grau study (Grau et al., 2008), a genetic variation of the CDA enzyme has an effect on the activity of

capecitabine and results in a poor prognosis for cancer.

Hypercalcemia is a common consequence of breast cancer and a major source of morbidity and mortality from the disease. Patients with multiple skeletal metastases are especially at risk. However, in a sizable subset of patients, elevated calcium levels develop even in the bones and joints illness (DeMauro and Wysolmerski, 2005). When serum calcium levels are over 10.5 mg/dl and albumin levels are under 4 g/dl, a condition known as hypercalcemia develops. When chemotherapy is given to patients with hypercalcemia due to breast or lung cancer, the calcium levels decrease, most likely due to a decrease in PTHrP levels (Hassan et al., 2012).

In the current study (Table 5) SNP rs2072671, homozygotes AA, heterozygotes AC, and mutant homozygotes CC were all given calcium levels that were within the normal range. Regarding SNP rs532545, the same scenario applies: all homozygotes are AA, heterozygotes are AG, and mutant homozygotes are GG, and all of them have calcium levels that are within the normal range. Which is to be expected the calcium level is kept within an extremely tight range, which is between 8.5 and 10.5 mg/dl (Saif, 2013).

Table 6 and Table 7 show the results of genotyping testing revealing the frequencies and percentages of CDA enzyme polymorphisms among the breast cancer patients of this study. Our results for SNP are consistent with what a study that indicated heterozygotes of type AC were more common than those of type AA or CC (mutant homozygotes), respectively. Xandra's research reveals that 239 individuals in Spain were genotyped using SNP rs2072671. Of those patients, 106 have heterozygotes AC, 37 have mutant homozygotes CC, and 96 have homozygotes AA. In contrast to the findings of a prior study (García-González et al., 2015), the current study on SNP rs532545 demonstrates that heterozygotes AG are more prevalent than homozygotes AA and mutant heterozygotes GG. Cohen studies have shown that for SNP rs532545, 47% of patients contain homozygotes AA, 42% of patients contain heterozygotes AG, and 11% of patients contain mutant homozygotes. These findings were based on the analysis of 181 patients. In this study, we investigated the markers associated with cancer, it is suggested that the rate of tumor growth in relation to the CDA polymorphism in patients receiving capecitabine is investigated in the next studies.

In conclusion, our results indicated the presence of a highly polymorphic cytosine deaminase (CDA) enzyme in breast cancer patients. The CDA enzyme was found to have different genotypes and variable frequencies in these women. Homozygotes and mutant homozygotes were less common compared to heterozygotes for two specific genetic variations of rs532545 and rs2072671. The levels of estradiol hormone and cancer antigen CA15.3 were found to be variable and correlated with the highly polymorphic CDA enzyme. This implies that the genetic variations in the CDA enzyme may be influencing the levels of these parameters, potentially affecting the progression or treatment response of breast cancer in these women. Overall, the findings suggest that the genetic polymorphism of the CDA enzyme may play a role in breast cancer prognosis and treatment response in breast cancer patients. Further research is warranted to better understand the implications of these genetic variations on the levels of hormone and antigen markers and to explore potential therapeutic strategies targeting the CDA enzyme.

Author Contribution Statement

Ali Amal Aldeen Majeed: Methodology, Investigation, Data curation, Original draft preparation; Karar Kadhim Mohsin: Methodology, Data curation, Original draft preparation; Hasanian Shakir Mahmood: Reviewing and Editing Raaid Fadhl Abbas: Writing- Reviewing and Editing; Ahmed Salih Sahib: Supervision, Conceptualization, Writing- Reviewing and Editing.

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Availability of data and materials

The data and materials that support the findings of this study are available from the corresponding author, upon reasonable request.

Ethical Approval

The study's protocol was approved by the Pharmacy College's Scientific and Ethical Committee at Karbala University

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Conflict of interest

The authors declare that they have no conflict of interests.

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