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RESEARCH PAPER

Association of *CYP1A1*2C* Variant (Ile464Val Polymorphism) and Some Hematological Parameters with Acute Myeloid Leukemia (AML) and Chronic Myeloid Leukemia (CML) in Erbil-Iraq.

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A B S T R A C T:

Acute Myeloid Leukemia (AML) and Chronic Myeloid Leukemia (CML) are life-threatening hematological neoplasms characterized by an uncontrolled proliferation of myeloid progenitors. The phase I metabolism response, which metabolizes xenobiotics and endogenous and exogenous DNA-reactive chemical compounds that may cause genotoxicity and raise the risk of AML and CML, is catalyzed by the cytochrome P450 (CYP) enzyme. The current study is aimed to identify the frequency of *CYP1A1*2C* polymorphism in AML, and CML patients. Also, compare some hematological parameters in AML and CML to determine the role of allele variants as a risk factor for developing leukemia. Blood samples were collected from 100 (50 AML and 50 CML) patients and 30 controls of both sexes at different age groups. Samples were analyzed for the prevalence of *CYP1A1*2C* polymorphism, and the results showed that Ile/Ile (AA)- Wild**,** Ile/Val (AG)- Hetero, and Val/Val (GG)- Homo mutant genotypes were not significantly elevated in the AML and CML group compared to the control group. However, statistically significant differences were found between the patient's group concerning WBC and Hb estimation, and the frequency distribution of AA- genotypes in both AML and CML patients showed a significant difference. Despite the fact that individuals in the different genotypes have the same probability to develop AML and CML. During treatment genotypes for both AML and CML patients should be considered of patients with the GG genotype who have lower WBC and Hb, especially in AML which may increase disease severity.

KEY WORDS: *CYP1A1*2C*; AML; CML; Cytochrome P-450 Enzyme; Polymorphism.
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1. INTRODUCTION :

Acute myeloid leukemia (AML) and CML are stem cell-derived malignancies. Depending on the kind and stage of the disease, the molecular characteristics, and the response to treatment, the course and prognosis differ amongst patients (Estey and Döhner, 2006, Hehlmann et al., 2007, Smith et al., 2011, Marcucci et al., 2011).

AML is a condition marked by clonal proliferation that develops from progenitors or stem cells with primitive hematopoietic functions. Abnormal differentiation of myeloid cells affects a high level of immature malignant cells (myeloblast) accumulated in the peripheral blood and bone marrow and fewer differentiated red blood cells, white blood cells, and platelets (Khwaja et al., 2016).

About 30% of adult leukemia are CML, acquired hematopoietic stem cell diseases defined by increased production of immature granulocytes (blasts), which build up in the bone marrow and obstruct the formation of healthy blood cells (Guilhot et al., 2007). CML signs include bone marrow hyper-cellularity, anemia, splenomegaly, and leukocytosis (Guilhot et al., 2007). The number of blast cells in the blood and bone marrow, and the severity of the symptoms, are used to distinguish between the three stages of CML: the chronic phase, the accelerated phase, and the blast phase. Chromosome translocation leading to the creation of the Philadelphia (Ph) chromosome, which in turn causes the BCR-ABL fusion gene, is seen in 95% of CML cases (Guilhot et al., 2007).

The important phase-I XME aryl hydrocarbon hydroxylase is encoded by the

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polymorphic gene known as cytochrome P450, family 1, subfamily A, polypeptide 1 (CYP1A1), specifically, the *CYP1A1* gene controls the enzyme cytochrome P450 (Amine et al., 2022), which is also involved in drug metabolism and the activation of several exogenous pro-carcinogens into highly reactive electrophilic carcinogenic molecules (Indulski and Lutz, 2000). If the DNA repair system is not present, these electrophiles can bind to DNA and produce adducts that can cause mutations in proto-oncogenes and tumor suppressor genes, starting the process of carcinogenesis. In light of this, CYP1A1 may contribute significantly to both the etiology of malignancies and the response to cancer therapy (Oyama et al., 2004, Rooseboom et al., 2004). The cytochrome P450 family includes CYP1A1, which is in charge of oxidatively biotransforming most medicines, other lipophilic xenobiotics, as well as endogenous steroids, cholesterol, and fatty acids (Rendic and Guengerich, 2021, Zanger and Schwab, 2013).

T6235C (*2A), A4889G (*2C), and C4887A (*4) are three single nucleotide polymorphisms (SNP) of CYP1A1 that have been investigated concerning various malignancies. *CYP1A1*2C* SNP A4889G (rs1048943; exon 7) (Fertrin et al., 2002, Beutler et al., 1998a) results in the protein's position 462 being changed from Isoleucine (Ile) to Val (Val), which increases the protein's hydrophobicity and catalytic activity by twofold. The higher frequency of AG in controls indicates that AG may play a protective role against developing CML and AML. In addition, patients with AG genotype showed a good therapeutic response toward Imatinib therapy (Lakkireddy et al., 2015). Presently, there is a lack of research on the *CYP1A1*2C* Variant (Ile464Val Polymorphism) according to AML and CML. Therefore, the present research is designed to evaluate the role of allele variations as a risk factor for developing leukemia by identifying the frequency of *CYP1A1*2C* polymorphism in AML and CML patients and comparing WBC and Hb estimations.

2.MATERIALS AND METHODS

The blood samples from patients were taken from Nanakali Hospital for Blood Diseases in Erbil city, Kurdistan region, Iraq from the period of September 2021 to November 2021. Patients and control participants were provided with a special questionnaire form, which includes some fields of information to be filled in about the patient (name, age, smoking, address, gender, genetic diseases, and job). Additionally, medical records gathered from the hospital with the help of doctors and staff, as well as direct interviews with patients and their families, were used to complete the questionnaire. All patients signed the informed consent and ethics application form and were accepted Human research ethics committee (HREC) of the Department of Biology, College of Science, Salahaddin University-Erbil. In the current study, genomic DNA extractions, PCR running, and gel electrophoresis were conducted at the Salahaddin University Research Center (SURC). This study was performed on 130 individuals, 50 patients diagnosed with AML, 50 patients diagnosed with CML, and 30 healthy individuals.

Two ml of blood was collected in EDTA tubes for the detection of white blood cells (WBC) and Hemoglobin (Hb) levels in AML and CML patients by using the Hematology analyzer Complete Blood Count instrument (Medonic-Sweden). 300 µl of blood in EDTA tubes was added to the microcentrifuge tube for genomic DNA extraction. Genomic DNA extraction was done using a solution kit (Jena Bioscience, Germany). PCR analysis was used to detect *CYP1A1* gene SNP by Alpha-cycler 96 well. A total 20 µl volume of PCR master mix reaction composed of 2µl (<200 ng) of template, 10 µl of 2X GoTaqGreen PreMix (Promega, USA), and 1µl of each forward and reverse primers of *CYP1A1* gene Table 1. Then the mixture was completed by adding 6 µl of *DNase*-free water (Lakkireddy et al., 2015).

The target gene was amplified using the PCR technique, thermal cycling conditions are shown in Table 2.

Data were statistically analyzed using GraphPad Prism 8.3.0. The chi-square test was used to show the genotype distribution in patients and healthy participants corresponding to the risk of CYP1A1 genotypes and the incidence of AML and CML. For comparison of WBC count in AML and CML patients according to the genotypes, one way ANOVA (Kruskal Wallis test) was performed. Mann-Whitney test was carried out for

comparison of each genotype between AML and CML patients.

Table 1: List of nucleotide primers used to amplify DNA and find *CYP1A1*2C* polymorphism sites.

Primer name	Gene	Sequence (5'—3')	Amplicon length (bp)
F1P1A1A F2P-1A1G RP-1A1.1	CYP1A1	F1P-1A1A; 5´GAAGTGTATCGGTGAGACCA-3´ F2P-1A1G; 5´-GAAGTGTATCGGTGAGACCG-3´ RP-1A1.1 5´-GTAGACAGAGTCTAGGCCTCA-3´	210 bp

Table 2: The PCR cycle's steps for *CYP1A1*2C.*

PCR Step	Duration	Temperature	
Initial Denaturation	3 minutes	94.0 °	
Denaturation	30 seconds	94.0°	30 cycles
Annealing	45 seconds	65.0 °	
Extension	1 minute	72.0 °	
Final extension	10 minutes	72.0°	

3.RESULTS:

3.1Age

The mean age of the AML patients was 47.29±4.150 in females and 41.58±4.326 in males. The mean age of the CML patients was 47.38±1.584 in females and 38.81±2.614 in males. While the mean age of the control group was 34.00 ±3.155 in females and 48.94 ±4.628 in males.

3.2Smokers and non-smokers

As shown in Table 5, among AML patients, 13 of them were smokers, and 37 of them were non-smokers. While among CML patients, 9 of them were smokers and 41 of them were non-smokers. The number of smokers and non-smokers in the control group was 8 and 22 respectively.

*3.3CYP1A1*2C* Genotyping Analysis

*CYP1A1*2C* gene polymorphism was genotyped by identifying both the wild-type Ile allele (210 bp DNA fragment with the 1A1A1/1A1.1 primers) and the mutant Val allele (210 DNA fragment with 1A1G/1A1.1 primers) (Figure.1). Table 3 displays the genotype distribution of the *CYP1A1*2C* polymorphism in AML, CML, and control persons. The distributions of the CYP1A1 Ile/Ile wild type, Ile/Val heterozygous, and Val/Val homo mutant genotypes in our study population did not differ between patients and controls, indicating that these genotypes were not associated with an increased risk of AML and CML. The distributions of the CYP1A1 Ile/Ile wild type, Ile/Val heterozygous, and Val/Val homo mutant genotypes in our study population did not differ between patients and controls, indicating that these genotypes were not associated with an increased risk of AML and CML. In addition, the frequency distribution of the A and G alleles differed among patients and healthy individuals. The (A) allele frequency was high in all participants compared to the (G) allele table 4.

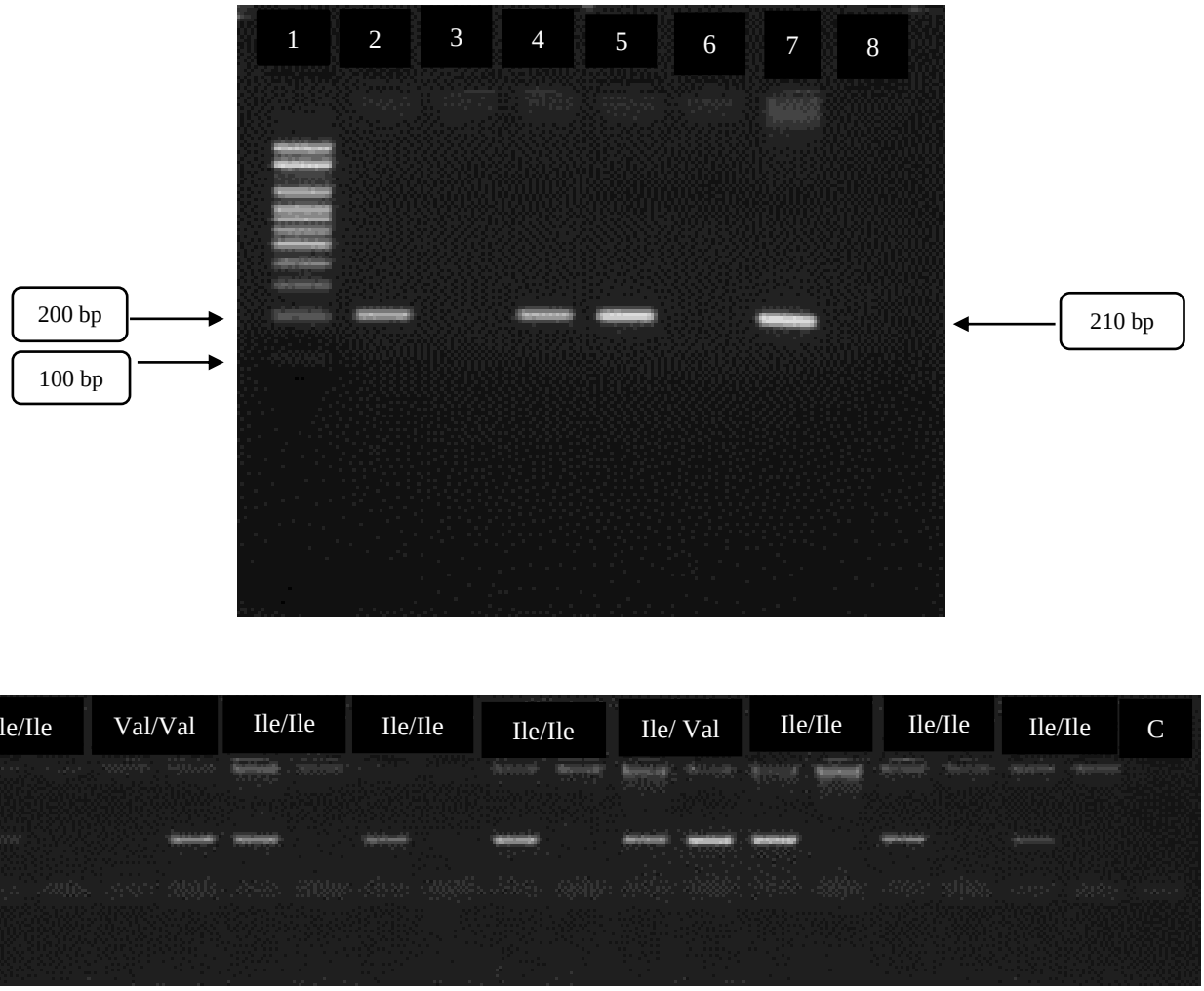

Figure 1: A. Utilizing allele-specific primers, *CYP1A1*2C* AML patient PCR products were electrophoresed on a gel. The 100 bp DNA ladder is seen in lane 1. Ile/Ile homozygous wild-type individuals are represented by lanes 2 and 3, Ile/Val heterozygous individuals are represented by lanes 4 and 5, and the Val/Val homozygous mutant genotype is represented by lanes 6 and 7. Lane 8 displays the adverse control. **B.** Shows CML patient PCR products and lane C represents the negative control.

Table 3: Genotype distribution of *CYP1A1*2C* polymorphism in 50 AML, 50 CML patients, and 30 controls.

Genotype	Ile/Ile (AA)-Wild	Ile/Val (AG)-Hetero	Val/Val (GG)-Homo mutant	P value
AML Patients	40	9	1	0.383 (ns)
CONTROL	25	3	2	
CML Patients	40	8	2	0.676 (ns)
CONTROL	25	3	2	

ns: non-significant

Table 4: Allele frequencies of *CYP1A1*2C* polymorphism in AML, CML, and controls.

Allele - frequency	A	G
AML Patients	89 (89%)	11 (11%)
CML Patients	88 (88%)	12 (12%)
Control	53 (88.3%)	7 (11.6%)

According to our present study, our analysis of smokers and non-smokers revealed that most smokers and non-smokers with AML and CML displayed an AA genotype. In addition, there were no appreciable variations in the distribution of CYP1A1 Ile/Ile wild type, Ile/Val heterozygous, and Val/Val (GG) homo mutant genotypes in the study population between patients and controls. The frequencies of smoking in AML and CML patients are shown in Table 5.

Table 5: The comparison of smokers and non-smokers according to genotype frequency in AML, CML, and control groups.

		Ile/Ile (AA)- Wild No.	Ile/Val (AG)- Hetero No.	Val/Val (GG)- Homo mutant No.	P value
AML (50 patients)	Smoker	10	2	1	0.2307 (ns)
	Non-smoker	30	7	0	
CML (50 patients)	Smoker	8	1	0	0.7007 (ns)
	Non-smoker	32	7	2	
CONTROLS (30)	Smoker	7	0	1	0.4338 (ns)
	Non-smoker	18	3	1	

3.4. WBC and Hb Estimation

The normal range for WBC count is between 3,500 to 10,000 WBCs per microliter (3.5 to 10.0 × 10^9/L). Analysis of the frequency distribution of the AA genotype in patients with AML and CML showed a significant difference (*P* value=< 0.001) in which CML patients with the AA genotype had a higher WBC count which was 6.4 (1.6-46.8) compared to AML patients which were 3.3 (0.3-22.9). Moreover, a comparison of WBC count according to genotype among CML patients showed a significant difference (*P* value=0.0386) in which patients with AA and AG genotypes revealed significantly higher WBC count compare to patients with GG genotype, 6.4, 6, 4.05, respectively.

However, the frequency distribution of the AG and GG genotypes showed no significant difference between AML and CML patients. In addition, there is a non-significant difference in the comparison of WBC counts according to genotype among AML patients. The frequencies

of WBC count in AML and CML patients are shown in Table 6.

The normal range for Hb estimation is between 11.5 – 16.5 grams per deciliter (g/dL). Our investigation of Hb estimation concerning the frequency distribution of the genotypes AA, AG, and GG mostly did not reveal any discernible difference. Nonetheless, the frequency distribution of AA genotypes in both AML and CML patients showed a significant difference (*P* value=0.0034) in which CML patients with the AA genotype had higher Hb levels (11.9) compared to AML patients. The frequencies of Hb estimation in AML and CML patients are shown in Table 7.

Table 6: Comparison of WBC count in AML and CML patients according to the genotypes and comparison of each genotype between AML and CML.

	AML	CML	P-Value
AA Median (range)	3.3 (0.3-22.9)	6.4 (1.6-46.8)	< 0.001
AG Median (range)	4.8 (0.4-10)	6.0 (3.5-9.8)	0.4079
GG Median (range)	2.7 (2.7-2.7)	4.05 (3.9-4.2)	0.1210
P-Value	0.3617	0.0386*	

Table 7: Comparison of Hb count in AML and CML patients according to the genotypes and comparison of each genotype between AML and CML.

	AML	CML	P-Value
AA Median (range)	10.1 (5.7-15.1)	11.9 (7.0-16.8)	0.0034**
AG Median (range)	9.0 (7.8-15.1)	12.7 (7.4-14.3)	0.3026
GG Median (range)	8.6 (8.6-8.6)	12.1 (11.9-12.4)	0.0773
P-Value	0.7854	0.9530	

All data are represented as Median (minimum-maximum).

4.DISCUSSION

Leukemia is a hematopoietic malignancy and has become more common recently, with each passing year seeing an increase. Among the 9.7 million deaths due to malignant tumors, 300,000 deaths are due to leukemia (3.2%) (Shin et al., 2016). Aggressive cancer called AML affects 3.5 individuals per 100,000 annually, age-adjusted, and affects 15-20 adults per 100,000 beyond age 60 (Dores et al., 2012). Myeloproliferative disease CML is caused by the BCR-ABL1 fusion gene or reciprocal chromosome translocation t(9; 22)(q34;q11) (Rostami et al., 2019).

The enzyme activity and/or indelibility rise in the presence of the CYP1A1 Val allele (Fertrin et al., 2002, Beutler et al., 1998b). Due to enhanced enzyme activity, when a person with the CYP1A1 Val allele is exposed to carcinogens, the carcinogenic effect of the carcinogens may be stronger. Therefore, DNA adducts building up in the cell lead to various mutations when the CYP1A1 Val allele is present. This situation may end up being the reason why human cells develop cancer (Beutler et al., 1998b).

To determine the relationship between *CYP1A1*2C* polymorphism and the onset of AML and CML, *CYP1A1*2C* polymorphism was examined in this study's patients with AML, CML, and healthy controls. Three genotypes *CYP1A1*2C* (AA) wild type, *CYP1A1*2C* (AG) heterozygous, and *CYP1A1*2C* (GG) homozygous variant genotype as found in both the

patients and the control group. The AML, CML, and control groups did not differ in the frequency in a statistically significant manner. Our findings are consistent with those found in the meta-analysis (Lu et al., 2015) that originate no association between A2455G and the risk of AML among Caucasians and Asians. Dissimilar to our findings, a study (Pelloso et al., 2013) found that *CYP1A1*2C* polymorphisms were more frequent in the control group than in the AML group. A study (Razmkhah et al., 2011) people with the CYP1A1 Ile/Val genotype did not have a higher risk of developing CML compared to the controls. According to these publications, it is important to note that due to differences and not differences in population demographics and other contributing factors like the environment, life style, a relationship between a certain polymorphism and cancer in one community may not have the same relevance in another one.

Regarding the smoker and non-smoker, noted that the majority of the smoker and non-smoker patients with both AML and CML reflected the AA genotype in the smoker and non-smoking population. However, found no significant differences between patients and controls in the distribution of CYP1A1 Ile/Ile wild type, Ile/Val heterozygous, and Val/Val (GG)-homo mutant genotypes.

According to our study results, WBC in AA genotypes showed a significant difference between AML and CML (p-value= < 0.001), also a comparison of WBC based on genotypes of CML patients showed a significant difference. Although the Hb level in AA, AG, and GG genotypes did not show any significant difference, while Hb in AA genotypes in AML and CML showed a significant difference.

5.CONCLUSIONS

This study exhibited three genotypes (AA wild type, AG heterozygous, and GG homozygous) in both patients and the control group in Erbil City. Most patients and controls have no smoking habit especially males, without any other genetic disease. This is suggesting that smokers are not more likely to develop AML and CML. WBC and Hb estimation, in AA genotype in both AML and CML patients, revealed a significant difference.

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Conflict of Interest

None declared.

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