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Association between Cytochrome P450 Genetic Polymorphism and Hematological Toxicity of Diffuse Large B-Cell Lymphoma Patients Treated with R-CHOP Protocol

Mohamed H Roshdy[a]*, Rania Hassan Mohamed[a], Lobna R. Ezz ElArab[b], Nour M Abd El-Kader[a], Magdy M Mohamed[a] and Menha Swellam[c]

[a] *Biochemistry Department, Faculty of Science, Ain Shams University, Cairo(11566), Egypt*
[b] *Clinical Oncology Department, Faculty of Medicine, Ain Shams University, Cairo(11566), Egypt*
[c] *Biochemistry Department, Biotechnology Research Institute, High Throughput Molecular and Genetic Laboratory, Central Laboratories Network and the Centers of Excellence, National Research Centre, Dokki, Giza(12622), Egypt.*

Abstract Response and toxicity monitored during the treatment cycles of Diffuse Large B-cell Lymphoma (DLBCL) enable the physician to take the decision about the best treatment protocol. These differences in outcomes are related mainly to age, physiological status and genetic factors. Toxicity is a frequent outcome that may be developed from accumulation of the drug itself or its metabolites. Current study aimed to study the correlation between the polymorphism in two families of cytochrome P450 system (CYP2C19 and CYP3A4) genes and the incidence of treatment toxicity in DLBCL patients. The genotypes of 2 SNPs for 98 patients were studied (rs4986893 and rs2740574). The rs2740574 of CYP3A4 is in significant association with the incidence of hematological toxicity. The C allele of rs2740574 were found to be more frequent with patients showing hematological toxicity. The disease-free survival (DFS) period was longer with TT genotype of rs2740574 than CC genotype. The current study suggests that CYP3A4 variants analysed might evaluate the incidence of hematological toxicity, hence predicting the most appropriate treatment protocol.

Keywords: DLBCL; hematological toxicity; CYP3A4; CYP2C19; Cytochrome P450 system

1. Introduction

Diffuse large B-cell lymphoma (DLBCL) classified as non-Hodgkin lymphoma (NHL), which is a highly heterogeneous disease at clinical, genetic and morphological levels [1]. DLBCL represents 30 % – 40 % of all adult NHLs and NHL incidence rate in Egypt was 5.9 % [2, 3]. The standard treatment protocol is a combination chemotherapy regimen R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone), other treatment strategies are involved such as R-GemOx (rituximab, gemcitabine, oxaliplatin), R-GDP (rituximab, gemcitabine, dexamethasone, carboplatin), R-CEPP (rituximab, cyclophosphamide, etoposide, prednisone, procarbazine) and RCEOP (rituximab, cyclophosphamide, etoposide, vincristine, prednisone) [4, 5]. Each treatment cycle in the standard R-CHOP regimens usually includes cyclophosphamide (750 mg/m2), doxorubicin (50 mg/m2), vincristine (1.4 mg/m2) with a maximal total dose less than 2 mg on day one, rituximab (375 mg/m2) on day 0 and oral prednisone (100 mg/m2) on day 1 through day 5 [4]. This treatment cycle is repeated every 21 days. Patients have complete remission (CR) received two additional cycles, while those having partial remission (PR) underwent another response assessment after 6 more cycles of treatment, and those with a further response underwent an additional 8 cycles. Patients with stable disease (SD) or progressive disease (PD) were switched to second-line therapy after 4-cycle R-CHOP according to guidelines set by the National Comprehensive Cancer Network for NHL [6]. The pharmacokinetics and pharmacodynamics of these drugs affect the treatment efficacy and side effects. The drugs and other xenobiotics are pass through metabolism by one of cytochrome P450 enzyme family or other enzymes [7, 8]. The cytochrome P450 system is divided into 18 families and 44 subfamilies

*Corresponding author e-mail: mohamed_elmasry@sci.asu.edu.eg (Mohamed H Roshdy),
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consisting of 57 genes [9]. The CYP1, CYP2, and CYP3 families are most frequently involved in oxidative drug metabolism [9, 10]. During cancer treatment, the drug efficacy and toxicity may differ from one individual to another. These variations may be due to genetic factors or non-genetic factors such as ethnicity, food type, co-medication, physiological status that are affected by age, sex, co-morbidities and environmental factors, such as smoking. Also, liver and kidney conditions play a role [9, 10]. Pharmacogenetic screening and drug-specific phenotyping studies before the start of anticancer treatment help to expect the treatment outcomes [10]. Toxicity may be developed from accumulation of the drug itself or its metabolites. For example the cyclophosphamide has two metabolic pathways; the major pathway is 4-hydroxylation [11] catalyzed by the enzymes CYP2B6, CYP3A5, CYP2C19, CYP2C9, CYP2C8 and CYP2A6 to finally produce acrolein and phosphamide mustard, which has therapeutic [5, 12] and toxic effects including hematological toxicity such as neutropenia, anemia, thrombocytopenia and cardiac toxicity [13]. Polymorphism in genes expressing the Cytochrome P450 enzymes is common [14]. In the current study authors focus on the polymorphism of two genes CYP2C19 and CYP3A4 through evaluating a single nucleotide polymorphisms (SNP) for each gene and their correlation to the risk of hematological toxicities resulting from the used therapeutic protocol. The studied SNP for CYP2C19 is rs4986893 and the SNP of CYP3A4 is rs2740574.

2. Materials and methods

2.1 Patients. The study protocol was approved by the ethical committee of National Research Center, Cairo, Egypt and 98 patients were provided by Eldemerdash Hospital, Ain Shams University, Cairo, Egypt between the period from October 2018 till June 2023, and all patients provided written informed consent. Inclusion criteria for enrolled patients were those DLBCL patients confirmed by histopathology and (positron emission tomography) PET scan with age not less than 18 years old. The patients had Eastern Cooperative Oncology Group (ECOG) performance status of 1-3. All cases were Egyptian patients diagnosed with DLBCL and received CHOP regimen with or without rituximab and radiotherapy. Any patient with any other tumor, pregnant female patients, any contraindication to R-CHOP or patients with severe co-morbidities were excluded.

2.2 Sample processing and DNA extraction. Exactly, 0.2 ml of EDTA-blood from selected patients was used to purify DNA using the Thermo Scientific GeneJET Whole Blood Genomic DNA Purification Mini Kit (Cat no. # Thermo Scientific K0781, USA) and the manufacturer's instructions were followed.

Genotyping. The genotypes were determined for rs4986893 (C_27861809_10), and rs2740574 (C_1837671_50) by TaqMan SNP genotyping assay (Applied Biosystems; Catalog number: 4362691) and TaqMan genotyping master mix (Applied Biosystems; Catalog number: 4371353, USA). The Taqman assay was performed using Stratagene Real-time PCR system (Max3005P QPCR system, Stratagene, Agilent biotechnology, USA). Real-time PCR test for each SNP was done by adding 5 µl DNA to 10 µl of master mix and 1 µl of 20 X genotyping assay in 20 µl total reaction volume. The run was performed for 40 cycles of 2 steps; denaturation at 95^{o}C for 15 seconds and annealing-extension for 60 seconds at 60^{o}C, after initial holding phase for 10 minutes at 95^{o}C.

2.3 Traetment strategy. Ann Arbor staging system was used to identify the stage of each patient along with PET/CT scan. Patients with stage I and II received 3-6 cycles of R-CHOP with or without involved site radiotherapy to the initial bulky sites. Patients with stage III and IV received 6 cycles chemo-immunotherapy with involved site radiotherapy to initial bulky sites and extra-nodal sites. Lugano classification response criteria [15] were used for defining the treatment outcomes.

2.4 Statistical analysis. The software SPSS 24.0 was used to calculate the allele and genotype frequencies. Deviations from Hardy-Weinberg equilibrium (HWE) were tested by chi-square goodness of fit test. The association between the genotypes and hematological toxicity was done by multivariate logistic regression with odd ratios (OR) adjusted for age and gender with 95 % confidence intervals (95 % CI). Linkage disequilibrium (LD) in correlation with the hematological toxicity of treatment was examined using Haploview 4.2 software (MIT/Harvard Broad Institute, Cambridge, USA). Statistical significance was identified at $p \leq 0.05$; after Bonferroni correction the values are considered significant at $p \leq 0.025$. Kaplan-Meier method was used to construct the progression free survival and overall survival curves of their relationship with the different time intervals and the collected prognostic and predictive factors and compared via log-rank tests.

3. Result

3.1 Clinical and biochemical characteristics of DLBCL patients. A total of 56 female and 42 male DLBCL patients were included in the study, among them 28 patients out of 98 (28.57 %) were perform hematological toxicities. The clinical and biochemical characteristics of all patients are shown

in Table (1). About 74 patients were less than 60 years old which represents 75.5 % of total patients and the other 24.5 % (24 patients) were more than 60 years old. The Age-cut of 60 years old is one of the determinants that is identified to calculate International Prognostic Index (IPI), IPI was zero for 16 patients (16.3 %), IPI was 1 for 50 patients (51 %), IPI score of 2 was found in 28 patients (28.6 %) and finally IPI score for 3 was found within 4 patients (4.1 %). The ECOG status of selected patients was between 1-3 represented as 65.3 %, 32.7 % and 2 %; respectively. Fifty-one patients had no extranodal involvement (52 %) and a total of 47 patients showed one (43.9 %), two (2 %) or four (2 %) extranodal organs Only 39 patients showed B-symptoms and only 56 patients showed elevated LDH levels in their blood. The initial stage of patients was stage I in 51 % of patients and stages II, III and IV represents 49 %.

3.2 Genotype distribution and allele frequency.

For 98 patients divided into two groups (Group A: No hematological toxicity (70 patients), Group B: Hematological toxicity (28 patients)), no significant deviation of genotype frequencies for CYPs from HWE as shown in Table 2.

3.2.1 CYP2C19 (rs4986893):

Both groups (A and B) represent only 2 genotypes GG and GA. The total number of patients who showed hematological toxicity was 28 patients among them 20 patients represented GG genotype (71.4 %) and 8 patients represented GA genotype (28.6 %). The G allele frequency was 85.7 % and the A allele frequency was 14.3 %. The total number of patients who received the treatment and did not show hematological toxicity was 70 patients, the GG genotype represented 48 patients (68.6 %) and the GA genotype represented 22 patients (31.4 %) with an allele frequency of 84.3 % for the G allele and 19.7 % for A allele. The allelic distribution among both groups of patients did not show any significance ($p = 0.802$).

3.2.2 CYP3A4 rs2740574:

The C allele had a significantly ($p= 0.015$) higher percentage in patients who showed hematological toxicity (50 %) than those had no hematological toxicity (31.4 %) and consequently, more CC genotype (28.6 %) among patients who showed hematological toxicity ($p=0.007$).

The 3 genotypes were represented in group B, the TT genotype represented 8 patients (28.6 %), CT genotype represented 12 patients (42.9 %) and the CC genotype represented 8 patients (28.6 %). While in group A, only 4 genotypes were represented. The TT genotype represented 30 patients (42.9 %) and the CT genotype represented 36 patients (51.4 %). The C allele showed a higher frequency (50 %) among patients in group B than its frequency among patients in group A (31.4 %).

Table 1: Clinical and biochemical characteristics of DLBCL patients included in the study (N= 98)

Variable	Number (percentile)
Age	
≤ 60	74 (75.5%)
> 60	24 (24.5%)
Gender	
Male	42 (42.9 %)
Female	56 (57.1)
Co-morbidity	
Yes	45 (45.9 %)
No	53 (54.1 %)
LDH[(a)]	
High	56 (57.1 %)
Normal	42 (42.9 %)
B-symptoms	
Yes	39 (39.8 %)
No	59 (60.2 %)
ECOG status[(b)]	
1	64 (65.3 %)
2	32 (32.7 %)
3	2 (2%)
Number of extranodal	
Zero	51 (52 %)
1	43 (43.9%)
2	2 (2 %)
4	2 (2%)
Initial stage	
I	50 (51 %)
II	16 (16.3 %)
III	22 (22.4 %)
IV	10 (10.2 %)
IPI[©]	
0	16 (16.3%)
1	50 (51%)
2	28 (28.6 %)
3	4 (4.1%)

a: lactate dehydrogenase, b:eastern cooperative oncology group, c: international prognostic index

3.3 Association of hematological toxicity resulted from treatment with genetic variants.

Regarding rs2740574, the best models were the homozygous codominance model (OR = 12.497, 95 % CI, P = 0.007) and the recessive model (OR= 7.968, 95 % CI, P= 0.005) and allelic model (OR =

2.345, 95 % CI, P = 0.016). The multivariate logisitic regression for rs4986893 of CYP2C19 was not significant for predicting the hematological toxicity.

3.4 Linkage disequilibrium (LD) analysis.

Pairwise LD estimates obtained for the four gene polymorphisms of CYP2C19 and CYP3A4 in the study population are depicted. The results showed that there was no linkage disequilibrium between rs2740574 and rs4986893.

3.5 Survival data.

Only homozygous codominant model of rs2740574 showed significance with disease free survival (DFS) rate (p = 0.012). Patients carry CC genotype relapsed after a mean period of 32 months; while the patients carry TT genotype showed longer disease free period of a mean of 54 months (Figure 1).

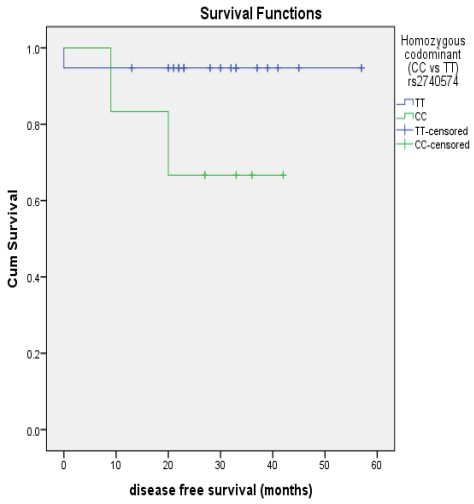

Figure (1): Kaplan-Meier DFS curve according to hematological toxicity for homozygous codominant model of rs2740574

4. Dsicussion:

Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma (NHL) worldwide, and represents about 30%–40% of all adult NHLs. The standard treatment protocol for DLBCL is rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone on a 21-day schedule (R-CHOP- 21) for six cycles [4]. The individual genetic variations in drug metabolizing enzymes (DME) and transporters influence the efficacy and toxicity of drugs. Among the enzymes that are involved in drug metabolism is cytochrome P450 family [10]. Pharmacokinetics and pharmacodynamics of the prescribed drugs are affected by unique genetic makeup of drug-metabolizing genes and drug transporters [9]. One of the side effects of R-CHOP treatment metabolites is the hematological toxicity caused primarily due to myelosupression [12]. The metabolites of cyclophosphamide, doxorubicin and vincristine may play a role in this phenotypic toxicity [11]. The current study aimed to evaluate the association between occurrence of hematological toxicity and polymorphism in CYP3A4 and CYP2C19 genes. The SNP rs2740574 (upstream variant) of CYP3A4 is related to reduced enzyme activity and in turn reduced rate of metabolism of cyclophosphamide and other CYP3A4-metabolizable drugs. The low rate of metabolism makes the produced amounts of the toxic metabolites, such as 4-hydroxycyclophosphamide is low and consequently lowers toxicity [16]. The findings of our results reported a correlation between the hematological toxicity and C allele of rs2740574. The rs2740574 showed increased risk of toxicity for C allele than T allele with 3 logistic regression models. This result is inconsistent with the study of Song Yao et al., (2010), they found no significant relationship between the clinical outcomes and rs2740574. The authors attributed these findings to redundancy in drug metabolism genes and in other words another gene involved in the metabolic pathway may neutralize the effect of genetic variations [17]. Based on genetic variation only, the studied SNP of CYP3A4 should be linked to reduced possibility of hematological toxicity, but according to the results of the current study, the SNP was higher in frequency in patients suffering from hematological toxicity and this may be due to any other factor such as the induction of CYP3A4 gene by prednisone [4]. This may lead us to the suggestion to limit prednisone use when hematological toxicity appears. The toxicity may be also due to another reason as found in the study of Xu et al. (2019), the study found that some medications such as doxorubicin may be metabolized in the tumor cells itself besides its metabolism within the liver [18] and this may increases the toxic metabolites of such medications. Vincristine, which is mainly metabolized by CYP3A5 and to a lesser extent by CYP3A4 may be also the cause of hematological toxicity [19].

Ethnicity, food type, co-medication, age, sex, and co-morbidities should be taken into consideration besides renal, hepatic health and the environmental factors that may affect the treatment outcomes [9, 10]. The studied SNP for CYP2C19 (rs4986893) showed a non-significant relationship with the risk of hematological toxicity and this is agree with the results obtained by Wenying et al., 2017 study [5].

Linkage disequilibrium analysis showed no LD between the two SNPs. Linkage disequilibrium occurs when genotypes at the two loci are dependent on each other while logarithm of the odds (LOD) is the probability of the inheritance of two gene loci as a unit indicating genetic linkage [20]. The survival data showed lower disease-free survival (DFS) with rs2740574; this result disagree with the result of Andrea et al., 2023 study carried out for infants not exceeding 18 months, the study found a significant correlation with lower overall survival but not

Table 2: Genotype distribution, allele frequencies and multivariant models of the studied SNPs in DLBCL in accordance to hematological toxicity

	Group (A)	Group (B)	p-value
rs4986893 (CYP2C19) GG GA AA	48 (68.6 %) 22 (31.4 %) 0 (0 %)	20 (71.4 %) 8 (28.6 %) 0 (0 %)	0.782
p-value HWE [(a)]	0.062	0.102	
Allelic frequencies G A	84.3 % 15.7 %	85.7 % 14.3 %	0.802
Logestic regression models	**OR^{+} (95% CI) [(b)]**		**p-value**
Homozygous codominant (AA vs GG)	----------		0.851
Heterozygous codominant (GA vs GG)	----------		0.482
Dominant (AA/GA vs GG)	----------		0.611
Recessive (AA vs GG/GA)	----------		0.325
Allelic model (A vs G)	----------		0.534
rs2740574 (CYP3A4) TT CT CC	30 (42.9%) 36 (51.4%) 4 (5.7 %)	8 (28.6 %) 12 (42.9 %) 8 (28.6 %)	0.007
p-value of HWE	0.053	0.153	
Allelic frequencies T C	68.6% 31.4 %	50 % 50 %	0.0125
Logestic regression models	**OR^{+} (95% CI)**		**p-value**
Homozygous codominance model (CC vs TT)	12.497 (2.011 – 77.659)		**0.007**
Heterozygous codominance model (CT vs TT)	1.334 (0.45 – 3.934)		0.601
Dominant model(CC/CT vs TT)	2.042 (0.734 – 5.678)		0.171
Recessive model (CC vs CT/TT)	7.968 (1.846 – 34.391)		**0.005**
Allelic model (C vs T)	2.345 (1.173 – 4.688)		**0.016**

a: Hardey Weinberg equilibrium; b: adjusted odd ratio with age and sex with 95 % confidence interval

significant with DFS, the contradictory results may be due to the difference in age among the two studies [21]. The limitations of the current study are: 1- the small number of patients enrolled in the study, which may be attributed to the low rate of DLBCL patients admitted to El-Demerdash hospital during the period of sample collection. 2- the study was carried out on 2 SNPs of two genes from CYP450, which may be already affected by other SNPs uninvestigated in the current work.

5. Conclusion:

We concluded that the C allele of rs2740574 in CYP3A4 gene increases the risk of the hematological toxicity. Also, the current study indicated that the TT genotype of rs2740574 has a longer disease free survival period than CC genotype. The promising findings of the study suggest that the investigation of these variants might evaluate the incidence of hematological toxicity in DLBCL patients. Consequently, this study recommends analyzing the

SNP rs2740574 prior to the beginning of the treatment strategy. Based on our findings, more studies with higher number of participants and other types of cytochrome P450 system genetic variants are recommended to validate the effect of other SNPs on the treatment outcomes.

Competing interests: Authors declare that there are no competing interests.

Conflict of interest: Authors declare there are no competing interests.

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